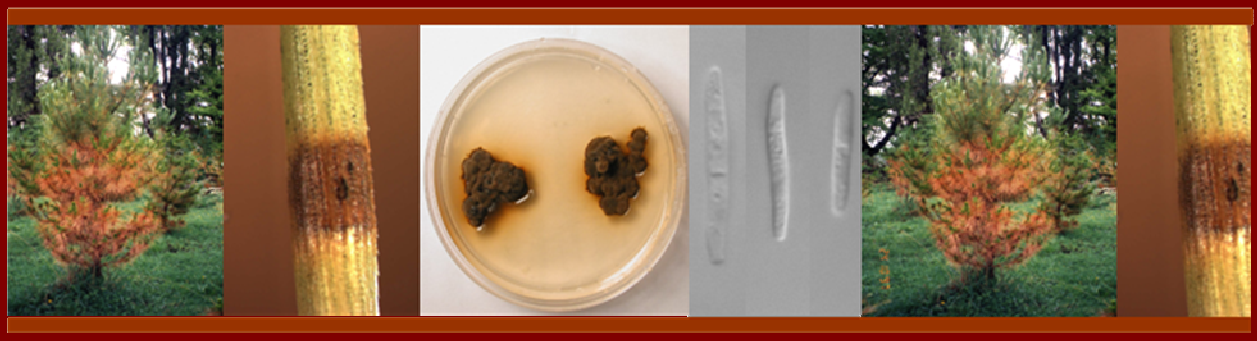


Chapter 5

Global population structure and diversity of the red band needle blight pathogen, *Dothistroma septosporum*, reflects anthropogenic activity

ABSTRACT

Dothistroma septosporum is a haploid fungal pathogen that causes a serious needle blight disease of pines, particularly on *Pinus radiata* in the Southern Hemisphere. During the course of the last decade, the pathogen has also resulted in unexpected epidemics in the non-native plantations in the Northern Hemisphere. Unusually, *D. septosporum* is hypothesized to be endemic to the Himalayas as well as Central America, and there is clearly a distinct lack of knowledge regarding the population biology or genetic diversity of the pathogen. The aim of this study was to determine the population diversity and structure of a collection of *D. septosporum* isolates from 14 countries on six continents using microsatellite markers. In the Northern Hemisphere countries where pines are native and two mating type genes are present, high genetic diversity was found. Most of the populations from Europe were randomly mating showing little differentiation and high gene flow. Similar results were obtained for the North American (U.S.A) and Asian (Bhutan) populations. Isolates from most of the Southern Hemisphere countries had low genetic diversity and were represented by a single mating type. However, populations from Africa, which have had longer histories of pine introductions, had relatively high genetic diversity and were randomly mating. The results are consistent with the expected Northern Hemisphere origin of *D. septosporum,* and the patterns of diversity reflect the movement of germplasm and expansion of pine planting in the Southern Hemisphere.

INTRODUCTION

Biological invaders are considered to be any plants, animals, invertebrates or micro-organisms that have established in a new area and that threaten, or have a detrimental effect on, the biodiversity, ecology or safety of the new environment (Pimental *el al.* 2000, Sakai *et al.* 2001, Allendorf & Lundquist 2003, Anderson *et al.* 2004). Biological invasions by fungal plant pathogens have had huge impacts in forest ecosystems in both their native and non-native ranges and have been recognised for many years (Elton 1958, Desprez-Loustau *et al.* 2007). Important examples include Chestnut blight caused by *Cryphonectria parasitica* (Murr.) Barr and Dutch elm disease caused by *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier. The former pathogen was introduced into North America from Asia around 1900 and devastated American chestnuts throughout their native range (Anagnostakis 1987). Likewise, Dutch elm disease has caused disastrous pandemics on elm trees in their native ranges of Europe and North America (Brasier *et al.* 1998, Brasier 2001). The devastation caused by these diseases has been on native trees in natural forests, but plantation forestry, based on non-native trees particularly in the tropics and Southern Hemisphere, has also been seriously damaged by diseases (Wingfield *et al.* 2001, Wingfield 2003). Amongst these, *Dothistroma septosporum* (Dorog.) M. Morelet, that causes Dothistroma needle blight (DNB), is by far the most important invasive pathogen of non-native pine species (Gibson 1972, Ivory 1987, Bradshaw 2004, Barnes *et al.* 2008a). This disease is characterised by successive needle defoliation, retarded wood growth and in severe cases, tree death (Gibson *et al.* 1964).

Two haploid fungal species cause DNB (Barnes *et al.* 2004). These morphologically similar fungi can be distinguished from each other based on spore size, but more effectively using DNA-based identification techniques (Barnes *et al.* 2004, Chapter 6). *Dothistroma pini* has a limited host range and is known in the North-Central USA, Russia, Ukraine (Barnes *et al.* 2004, Barnes *et al.* 2008a) and has most recently been reported from Hungary (Chapter 6). In contrast, *D. septosporum* has been known since 1911 when it was first described from *P. mugo* in Russia (Doroguine 1911). Since then, the pathogen has been reported in more than 45 countries world-wide (Ivory 1994, Bradshaw 2004), infecting over 70 different species of pine (Bednářová *et al.* 2006). DNB owes its notoriety to this species and the epidemics it has caused in plantations of non-native *Pinus* spp. in the Southern Hemisphere (Gibson 1972, Ivory 1987).

Given its importance, it is surprising how little is known regarding the origin of *D. septosporum.* In this regard, there are two hypotheses (Evans 1984, Ivory 1994). Based on its presence on native pines in the high, minimally disturbed cloud forests of Central America in the absence of epidemics, one view is that *D. septosporum* is native in that region (Evans 1984). The fact that the pathogen has also been found on indigenous pine in remote forests in the Himalayas prompted Ivory (1994) to suggest that it might also be native to these areas. Given the fact that *D. pini* and *D. septosporum* are almost impossible to discriminate from each other in the absence of DNA based techniques, reports such as those listed above, and hypotheses regarding the origin of these pathogens, are spurious at best. Recently, the presence of *D. septosporum* has, however, been confirmed from native blue pine (*P. wallichiana*) trees in the Himalayas, in the absence of epidemics (Barnes *et al.* 2008a) and this area of origin seems probable.

One of the main factors attributed to the increase in biological invasions is the expansion of global travel and trade that has promoted the introductions of plant pathogens into new areas, mainly via infected plant material (Richard & Lonsdale 2001, Rossman 2001, Wingfield *et al.* 2001). Alternatively, introductions into new environments can be due to natural events such as long distance dispersal via wind-blown spores (Brown & Hovmøller 2002, Stukenbrock *et al.* 2006). *Dothistroma septosporum* provides a good example of how both forms of dispersal have increased its presence world-wide.

All pines are exotic to the Southern Hemisphere and pathogens such as *D. septosporum* in this region would have to have been accidentally introduced, probably on plant material. The increase in air traffic and the establishment of commercial pine plantations, especially after World War II, is how *Dothistroma* was most likely introduced into the Southern Hemisphere (Gibson 1972).

Within and between countries trade in plant material would have provided sources of new infections in the Northern Hemisphere. For example, in 1999 and 2000, planting stocks of *P. nigra* and *P. mugo* respectively, that were infected with *D. septosporum,* were intercepted when they were transported into the Czech Republic via Hungary (Jankovský *et al.* 2004). Intercontinental spread of the pathogen has most likely been due to trade in plant germplasm and human activity. *D. septosporum* in New Zealand is speculated to have been introduced by forestry officials who travelled to East Africa during 1957 to observe DNB (Hirst 1997). The pathogen was discovered five years later causing disease in central North Island forests of

New Zealand (Gilmour 1967). Natural long distance dispersal of *D. septosporum* into Australia, from New Zealand, via spores in mist clouds blown over the Tasman Sea has been suggested (Edwards & Walker 1978). This view is supported due to the strict quarantine regulations in Australia, which would make it unlikely that an introduction via plant material had occurred (Edwards & Walker 1978, Bradshaw 2004).

A characteristic of a successful invasive species lies in its ability to establish itself in a new environment and then to spread to new areas (Sakai *et al.* 2001). Short distance spread of the spores of *D. septosporum*, either as asexual conidia or sexual ascospores is very effective (Gibson 1972, Bradshaw 2004). The ability of the pathogen to expand its range can be observed from the chronological records of first reports from countries from both the Northern and the Southern Hemisphere.

In the Southern Hemisphere, the first report of *D. septosporum* was from Africa (Zimbabwe) in 1940 where young *P. radiata* were severely damaged (Barnes 1970, Gibson 1972). In 1957, the pathogen was then found in Tanzania and within seven years, the associated disease had spread to all major plantations of *P. radiata* in Kenya, Malawi and Uganda (Gibson 1972). Similarly in Chile and New Zealand, where 92 % of the worlds *P. radiata* is grown, (Toro & Gessel 1999, Rogers 2002), *D. septosporum* has caused disease epidemics since 1957 (Gibson 1972) and 1963 (Gilmour 1967) respectively. The pathogen was only found in Australia much later, in 1975 (Edwards & Walker 1978).

In Europe, *D. septosporum* has successfully invaded countries and established itself during the last 50 years. During this time, the range of the pathogen has expanded and disease epidemics have emerged. In Serbia (Yugoslavia), for example, the pathogen has been known since 1955 (Krstić 1958) but by 1988 the infections had reached epidemic proportions on both native and exotic hosts (Karadzić 1988). From there, the disease spread northwards, and by 1969 it had entered the southern part of Hungary (Marinković & Stajković 1969, Karadzić 1989). By 1995 (Szabó 1997), the disease had spread into all *P. nigra* monoculture plantations, most of which had been established during a pine afforestation programme in the 1960's (ÁESZ 2002). Approximately two years later, the disease was recorded in the Southern tip of Slovakia, close to the border of Hungary. Today, it is found throughout Slovakia on both native and non-native pine species. Plantations of *P. nigra* for Christmas trees are the most severely affected and pines growing in stressed situations in both Hungary and Slovakia show signs of tree mortality.

Dothistroma septosporum has been present in many European countries where there has been no real cause for concern because damage was minimal and epidemics were never seen (Evans 1984). In the last two decades, however, *D. septosporum* has emerged as a threat to native, and particularly exotic, pine species planted within Europe and other Northern Hemisphere countries. The disease is prominent, for example, in epidemic proportions on susceptible, exotic plantations of *P. nigra* subsp. *laricio* (Corsican pine) in the U.K. (Brown *et al.* 2003) and France (Aumonier 2002). Likewise, in British Colombia, although native pine species are infected, non-native lodgepole (*P. contorta* var. *latifolia)* pine plantations have experienced severe epidemics with 60 – 100 % mortality (Parker & Collis 1966, Woods 2003, Woods *et al.* 2005). These new epidemics have largely been attributed to global climate change (Woods 2003) and as a result, the pathogen is now of international concern.

Although the ecology of *D. septosporum* has been well documented, little is known regarding the genetics of the pathogen, its mode of spread or the sources of new introductions. In a preliminary study of the mating type distribution in *D. septosporum,* Groenewald *et al.* (2007) revealed that only one mating type was present in New Zealand and Australia. This was consistent with the study conducted by Hirst (1997) in which several populations collected in New Zealand and screened with RAPD markers, showed that these populations were clonal. Both mating types were, however, observed in other parts of Africa, Europe and the Americas (Groenewald *et al.* 2007) indicating the possibility of sexual reproduction in these areas.

The aim of this study was to consider the diversity and genetic structure of *D. septosporum* populations from a world wide collection of isolates from 14 countries over six continents using 12 polymorphic microsatellite markers. More specifically, we considered whether the patterns in genetic diversity and structure of *D. septosporum* populations reflected the movement of its host from the Northern Hemisphere to the Southern Hemisphere and whether epidemic populations on non-native pines in both the Northern and Southern Hemisphere reflect recent introduction events. In addition we considered the degree of genetic differentiation and variation that exists between populations and groups of populations and estimated geneflow between geographic locations. The frequency and distribution of mating types in the countries was also investigated.

MATERIALS AND METHODS

Sampling, fungal isolation and DNA extraction

In this study, isolates of *D. septosporum* were obtained from a variety of *Pinus* spp. representing 14 different countries (referred to as populations) from six regions. These included Africa (South Africa and Kenya), Europe (Austria, Czech Republic, Hungary, Poland, Romania, Slovakia), Asia (Bhutan), North America (U.S.A.), South America (Chile and Ecuador) and Australasia (Australia and New Zealand) (Table 1). The main pine species from which collections were made included *P. nigra* from the Northern Hemisphere and *P. radiata* from the Southern Hemisphere. The sampling strategy from plantations included collecing a handful of diseased needles from every alternate tree randomly chosen along two or more transects. Each alternate tree was sampled to reduce the potential of obtaining clones. Samples from native trees, or those not in plantations, were randomly collected from infected trees. Samples were collected from one or several locations in a country (Table 1).

Isolations were made as described in Barnes *et al.* 2004, from a single conidiomata on a needle, per tree. Cultures were grown on 2 % malt extract agar (MEA, Biolab, Midrand, Johannesburg) at 20 °C. All cultures have been deposited and are maintained at the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Genomic DNA was extracted from each isolate using freeze-dried, ground mycelium, from 2- to 3-month-old cultures with the aid of the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Haplotype identification

The genotypes of isolates were determined at 12 microsatellite loci including Doth_E, Doth_F, Doth_G, Doth_I, Doth_J, Doth_K, Doth_L, Doth_M, Doth_O, Doth_DS1, Doth_DS2 described by Barnes *et al.* (2008b) and DCB2 of Ganley & Bradshaw (2001) (Figure 1). An internal diagnostic marker, Doth_A, was used to confirm the identity of the isolates as *D. septosporum* during allele scoring based on the fact that *D. septosporum* is monomorphic for the allele 124 while *D. pini* is monomorphic for the allele 114 at locus Doth_A (Barnes *et al.* 2008b).

Polymerase chain reactions (PCR) were carried out in 96-well plates. A total volume of 12.5 µl per well contained 5-10 ng DNA template, 0.06 U FastStart Taq DNA Polymerase (5 U/µl)

(Roche Diagnostics GmnH, Mannheim, Germany), 1x PCR buffer containing 2 mM $MgCl_2$, 0.25 mM of each dNTP, 100 nM of the forward and reverse primer, one of which was fluorescently labelled and 1 mM additional $MgCl_2$. The PCR conditions consisted of an initial denaturation step of 10 min followed by 10 cycles at 94 °C for 30 s, specified annealing temperature for 45 s (as described in Barnes *et al.* 2008b) and 72 °C for 1 min. A further 30 cycles were carried out using the same conditions above except that a 0.5 s increment was added to the elongation time. A final elongation time of 30 min at 60 °C was added to avoid the +A effect (Clark 1988, Magnuson *et al.* 1996) during genescan analyses. PCR amplicons were separated, with a 100 bp marker, on 2 % low electroendomosis agarose gels (Roche Diagnostics) stained with ethidium bromide and visually analysed under UV light (Vilber Lourmat, Omni-Science). PCR amplicons were not purified.

To facilitate multiplexing, PCR amplicons (for each individual) were combined according to the approximate size of the amplicons and type of fluorescent label attached to the primer. Samples were electrophorised on an ABI 3100 sequencer. Allele assignments were determined using ABI-Prism ® GENEMAPPER™ software version 3.0 (Applied Biosystems, Foster City, USA). Multilocus genotypes were obtained by combining the alleles present at all twelve loci for each isolate. All isolates having the same multilocus haplotype in a population were considered clones. Only one representative of each haplotype (clone-corrected) was retained for the analyses of each population. Clone-corrected datasets were thus used in all analyses unless otherwise stated.

Genetic data analyses

Genetic diversity

Allele frequencies were estimated for each SSR locus using the program POPGENE version 1.32 (http://www.ualberta.ca/fyeh/index.htm). Total number of alleles, unique alleles and expected heterozygosity, calculated as Nei's (1973) measure of gene diversity, were calculated for each population and region across all 12 loci.

In addition to calculating gene diversity, estimates of allelic richness were computed for each population and region in the program FSTAT for windows, version 2.9.3.2 (http://www.unil.ch/izea/softwares/fstat.html) (Goudet 2001). Unequal sample sizes were standardized, by rarefaction, to a uniform sample size of the smallest population (USA, $n = 7$) and region (Australasia, $n = 6$) as described by El Mousadik & Petit (1996). The clonal

fraction was calculated for each population by dividing the number of genotypes observed in the population by the total population size and subtracting this from one.

Measures of genotypic diversity (D) were quantified using non clone-corrected datasets in MULTILOCUS version 1.3 (Agapow & Burt 2001) as (n/n-1)(1- $\sum_i pi^2$) where *pi* is the frequency of the *i*th genotype and *n* is the number of individuals sampled. Here, the multilocus genotype of all possible pairs of individuals is compared and the proportion of pairs that are different is calculated. Populations that are completely clonal will score a value of 0 while those where all individuals have different multilocus genotypes will score 1.

The random associations of alleles among different loci (consistent with sexual reproduction), were tested by calculating the index of association (I_A) in the program MULTILOCUS version 1.3 (Agapow & Burt 2001). I_A, which is a measure of multilocus linkage disequilibrium (Maynard Smith *et al.* 1993), was calculated for all isolates in a population using clone-corrected data sets. The I_A was only calculated for those populations in which both mating types were present.

The hierarchical partitioning of molecular variation within and among populations and among regions was assessed with an AMOVA-test implemented GENALEX version 6.1 (Peakall & Smouse 2006) using the complete dataset. The significance was tested by 1000 permutations of the dataset. Only regions with more than two populations were included in analyses.

Population differentiation

The level of pair-wise genetic differentiation between populations was tested using Weir's θ (Weir 1996) for haploids, which is an estimate of Wright's F_{ST}, using the program MULTILOCUS. The observed θ was calculated and compared to values of 1000 artificial randomized datasets to test for the null hypothesis of no population differentiation (θ = 0). If θ = 1, no alleles are shared between the populations. Corresponding estimates of the number of migrants per generation was calculated as M=[(1/θ) - 1]/2 (Cockerham & Weir 1993).

Population structure

The program STRUCTURE version 2.2 (Pritchard *et al.* 2000, Falush *et al.* 2003) was used to determine the optimal number of populations (K) and to assign individuals to these distinct populations based on their genotype data and not by their geographic location. The program uses a Bayesian Monte Carlo Markov Chain (MCMC) clustering algorithm and the

simulations assumed a model of mixed ancestry and correlation of allele frequencies within clusters. Individuals are assigned to clusters to minimize Hardy-Weinberg disequilibrium and linkage disequilibrium between the loci within each cluster. The MCMC scheme was run for 100 000 iterations after a burn-in period of 10 000 and twenty simulations were performed for K ranging from one to 14 to verify the convergence of the Log likelihood values for each value of K. Delta K (ΔK), a statistic based on the rate of change in the log probability of data with respect to the number of clusters, was used to help interpret the real number of clusters (Evanno *et al.* 2005). After the optimal K was determined, a final parameter of 1 million MCMC replicates and a burn-in period of 100 000 was run for the assignment of individuals into K populations.

Mating type distribution

The mating genotypes (MAT1-1 and MAT1-2) were assayed using a set of primers defined by Groenewald *et al.* (2007) to amplify the mating-type idiomorphes of *D. septosporum*. Degenerate primers (Dot Mat1r 5'- TTGCCTGACCGGCTGCTGGTG-3' and Dot Mat2r 5'-CTGGTCGTGAAGTCCATCGTC-3') and species-specific primers (*D. septo* Mat2f 5'-GTGAGTGAACGCCGCACATGG-3' and *D. septo* Mat1f 5'-CGCAGTAAGTGATGCCCTGAC-3') were multiplexed in a single reaction. PCR reactions were carried out in 96-well micro titre plates with a total volume of 12.5 µl in each well consisting of: 10 ng - 20 ng DNA, 1x PCR buffer containing 2 mM $MgCl_2$, 0.5 mM $MgCl_2$, 0.12 mM of each dNTP, 200 nM of each of the four primers and 0.032 U FastStart Taq DNA Polymerase (5 U/µl) (Roche Diagnostics GmnH, Mannheim, Germany). Cycling conditions consisted of an initial denaturation of 5 min at 94 ^{o}C followed by 40 cycles at 94 ^{o}C for 20 sec, 65 ^{o}C for 20 sec, 72 ^{o}C for 40 sec and a final elongation of 7 min at 72 ^{o}C. To determine whether populations were deviating from the null hypothesis of a 1:1 ratio of mating types, a χ^2-test was applied to the frequencies of the two mating types.

RESULTS

Haplotype identification

A total of 471 (Table 1) isolates were successfully recovered from the infected pine needles. All isolates screened with the internal diagnostic marker, Doth_A, produced an allele size of 124, consistent with *D. septosporum,* except for 13 isolates from Hungary that produced an allele size of 119 (See Chapter 6). These isolates were excluded from further analyses. After clone correction, 240 unique haplotypes were obtained.

Genetic data analyses

Genetic diversity

From 12 microsatellite loci, a total of 130 alleles were produced ranging from three alleles at Locus_O to 24 alleles at Locus_L. The allele frequencies at each locus are recorded for all populations in Table 2. Other indices of variation are reported in Table 3 for each population (country) and region (continent).

Isolates from the USA had the highest percentage of unique alleles at 25 % followed by Austria (11.76 %) while RSA, Chile, Ecuador and New Zealand contained no unique alleles. Only Austria and Hungary showed 100 % polymorphism for the 12 loci. Expected heterozygosity ranged from 0.04 in New Zealand to 0.60 in Austria. Similarly, allelic richness, based on rarefaction to the smallest sample size, ranged from 4.32 to 1.78. On the continental scale, Europe contained the highest percentage of unique alleles at 42 % followed by North America at 25 % and South America at 15 %. Expected heterozygosity and allelic richness was highest in Europe (H = 0.58, A = 4.0) and lowest in South America (H = 0.24, A = 1.9) and Australasia (H = 0.12, A = 1.33).

Overall, the clonal fraction for the Northern Hemisphere countries was low (ave. 17 %) compared to the Southern Hemisphere (ave. 62 %). The Czech Republic and isolates from Bhutan contained no duplicate haplotypes while in New Zealand, the clonal fraction was 91 %. Genotypic diversities were higher in the Northern Hemisphere countries ranging from 0.833 to one. The genotypic diversity for isolates from the Southern Hemisphere ranged from 0.964 (Kenya) to 0.091 (New Zealand). Three haplotypes were shared between Chile and Ecuador and one between New Zealand and Australia.

AMOVA analyses showed that on a global scale, with the continents representing regions, most of the variation observed was distributed within populations (61 %), but a significant proportion of the variation was also attributed to differences among regions (32 %) (Table 5). Only 8 % of the variation was partitioned among populations within regions. AMOVA analyses on individual regions showed that for both the South American and Australasian groups, a high percentage of the variation was within populations (99 % and 96 % respectively) with no genetic differences observed among these populations. A large proportion of the variation within Africa was observed between Kenya and South Africa (33 %, Table 5) and, within Europe, although variation among populations was low (Φ-PT = 0.088), it was still significant for population structure.

Population differentiation

Theta values ranged from 0.004 (between Czech and Austria) to 0.704 (between USA and Australia) and were significant for 25 of the 91 population pairs indicating an absence of genetic differentiation between 12 of the populations sampled (Table 6). All populations paired with RSA and Kenya, from Africa, had high and significant pairwise theta values ($P < 0.05$) ranging from 0.283 (between Austria and Kenya) and 0.616 (between Australia and Kenya). The numbers of migrants per generation were substantial within the European populations (113 migrants between Austria and Czech). For the Southern Hemisphere countries, geneflow was indicated between Ecuador and Chile (76 migrants) and between New Zealand and Australia (12 migrants).

Population structure

STRUCTURE analyses revealed the highest posterior probability for five populations among all the isolates (Figure 2). Assignments of the individuals into these clusters closely resembled that of their geographic distribution (Figure 3). The Southern Hemisphere isolates were partitioned into two clusters; the majority of isolates from Africa (Kenya and RSA) formed one cluster and, validating the AMOVA results, the remaining isolates from Chile, Ecuador, New Zealand and Australia formed a second cluster. For the Northern Hemisphere, three additional clusters were observed. The isolates from USA and Bhutan were assigned to the same cluster while in Europe, a fair amount of the isolates from Romania, Hungary, Slovakia, Czech and Austria clustered together. Isolates from Poland displayed differences in allele frequencies when compared with those from the rest of Europe and were partitioned into their own cluster (Figure 4). Baysian estimates also indicated a slight potential for K=2 populations (Figure 2). When the partitioning of K=2 was further investigated (Figure 2, Figure 3), the separation was mainly between the populations in the Northern Hemisphere versus those in the Southern Hemisphere, except for Bhutan which grouped together with the Southern Hemisphere (Figure 3).

Mating type distribution

Two products were obtained using the Doth Mat primers including a 823 bp product of the MAT1-1 idiomorph and a 480 bp product pertaining to the MAT1-2 idiomorph. Both mating types were present in all the Northern Hemisphere populations. Only Poland deviated from the null hypothesis of a 1:1 ratio of random mating favouring the MAT1-2 idiomorph (Table 4). Isolates from Kenya and RSA in Africa in the Southern Hemisphere, contained both mating types while Ecuador, Chile, Australia and New Zealand isolates had only the MAT1-2

idiomorph (Table 4). The hypothesis of random mating was rejected in five out of the nine populations that were analysed using the I_A statistic at $P<0.01$ (Table 4). Those that appeared to have random mating were populations from the Czech Republic, Hungary, Bhutan and Kenya. In Austria, when the analyses were refined to 27 isolates from *P. nigra* in one plantation, results indicated random mating with $I_A = 0.27$ and $P = 0.037$.

DISCUSSION

The epidemics caused by *Dothistroma septosporum* in many parts of the world have been exacerbated by planting of susceptible hosts, particularly in monocultures and under environmental conditions conducive to disease development (Gibson 1972, Ivory 1987, Brown *et al.* 2003, Woods *et al.* 2005). Clustering methods, based on multilocus microsatellite markers applied to a worldwide collection of isolates of *D. septosporum,* in this study, partitioned the isolates roughly corresponding to geographic origin. This clustering made it possible to show how the patterns of genetic diversity, population structure and distribution of mating types in the fungus reflect the anthropogenic nature of its worldwide distribution in the Southern Hemisphere. They also provide an interesting insight into the establishment, reproduction and range expansion of *D. septosporum* on non-native trees in the Northern Hemisphere, particularly in Europe.

Other than those for Poland, the European populations of *D. septosporum* were characterised by high levels of genetic diversity, high levels of geneflow, low population differentiation, and the presence of two mating types. Essentially these populations from Austria, Hungary, the Czech Republic and Romania constitute one panmictic population, where most of the genetic variation (91 %) distributed in populations is reflected by the large numbers of unique alleles present in these individual populations. These populations, mainly on exotic *P. nigra,* were genetically very similar, despite their occurrence in different countries. This is explained by the fact that these countries border on each other and thus have no barriers to geneflow.

The absence of physical barriers to geneflow suggests that migration of *D. septosporum* individuals, which was particularly evident between Austria and the Czech Republic, would occur between the European countries. Migration or range expansion of *D. septosporum* during the last 50 years in Europe, due to effective dispersal of conidia and extensive movement of pine material, has evidently resulted in the homogenisation of allele frequencies

in isolates from this region. This has, in turn, resulted in an even distribution of genetic structure of the pathogen in these countries. The range expansion of an apparently native pathogen is not unusual and is well known for many other plant pathogens (Banke & McDonald 2005, Stukenbrock *et al.* 2006, Gladieux *et al.* 2008)

The equal proportions of mating types found in the populations of *D. septosporum* from Europe and the high genetic diversity observed in these isolates, suggests that recombination between individuals is occurring. This is most likely due to sexual reproduction as is common for outcrossing populations of fungi (Milgroom 1996). This interpretation is supported by the fact that in most cases, the populations consisted of unique haplotypes and by the fact that the sexual state of the fungus has been recorded from many European countries (Bradshaw 2004) including those considered in his study. Apparently, regular sexual recombination and population growth over time has gradually dissipated most of the evidence of introduced populations in Europe, which would be revealed by genetic bottlenecks and multilocus linkage disequilibrium (Dlugosch & Parker 2008).

Isolates from Poland represented a clear example of an introduction into an area where a genetic bottleneck (Dlugosch & Parker 2008) has apparently shifted the allele frequency of a population. As a result, the Poland population was partitioned in its own cluster, which would account for the majority of the 9 % among-population variation observed in Europe. Dothistroma needle blight in Poland was first observed in a strip of trees near the edge of an old forest nursery in 1990 (Kowalski & Jankowiak 1998) which could be the source of the introduction into that country, probably via infected nursery stock. Seven years later, *D. septosporum* was found over 500 km away, seriously infecting a 12-year-old seed plantation (Kowalski & Jankowiak 1998), which was also the collection site for the samples in this study. The Poland population is also the only one from Europe analysed in this study that was not in Hardy-Weinberg equilibrium, with the MAT 2 phenotype predominating (73 %) in the samples collected. This however, has not affected the diversity of the pathogen in the region, which is still comparatively high compared to those from the rest of Europe. The Poland population also included many unique alleles that might have resulted from multiple introductions into the country. These introductions would most likely have originated from a source different to other populations considered in this study.

The isolates from Bhutan and USA included in this study were very small in number but they were included in order to determine whether any of the genotypes in those countries might be

present in samples from other countries. This would have made it possible to determine migration patterns for the pathogens. The USA isolates were very distinct from those originating elsewhere in the world and had a large number of unique alleles, despite the small sample size. These populations were genetically isolated with high levels of population differentiation. In contrast, while the Bhutan isolates did not cluster with those from Europe, they shared many of the same alleles. This is particularly interesting because the Himalayas have been suggested as a possible area of origin for *D. septosporum* (Ivory 1990, Ivory 1994). In addition, the isolates were from trees that did not show signs of disease (Barnes *et al.* 2008a), which is a common feature for pathogens present in their native ranges and under selection pressure (Parker & Gilbert 2004, Burdon *et al.* 2006). Human-mediated movement of the pathogen out of an area such as the Himalayas, followed by large natural range expansion could have aided the efficient spread of *D. septosporum* throughout Europe. This would have been strongly supported by planting of susceptible, often non-native hosts for wood production and the spread would have most likely continued gradually from east to west. Recent reports of *D. septosporum* from Finland and Sweden (Hantula, pers. communication) provide an example of such local range expansion of the pathogen.

Patterns of genetic diversity and the clustering of isolates of *D. septosporum* from countries of the Southern Hemisphere sampled, reflect the anthropogenic movement of pines into these regions. In the African cluster, the high levels of genetic variability in the Kenyan and South African populations, and the presence of both mating types, indicate that multiple introduction events of *D. septosporum* have occurred. This is consistent with the long history of pine being moved into these countries (Poynton 1977, Richardson *et al.* 2007) that would have provided many pathways and opportunities for introduction of the pathogen (Lundquist 1987, Diekmann *et al.* 2002). A similar situation has emerged for pine pathogen *Diplodia pinea* (Desm.) J. Kickx. Fil. on *P. radiata* in the Southern Hemisphere (Burgess *et al.* 2001), *Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni populations in Europe (Gobbin *et al.* 2006) and the European race of *Gremmeniella albietina* (Lagerberg) Morelet var. *abietina* in North America (Hamelin *et al.* 1998).

The Kenyan population of *D. septosporum* was the most diverse sample in the Southern Hemisphere as reflected by gene and genotypic diversity as well as the number of unique alleles. It is also the country considered in this study which has had the longest history of commercial *P. radiata* plantations and *D. septosporum* infections (Gibson 1972, Lavery & Mead 1998). The presence of both mating types in a 1:1 ratio in the African countries and the

lack of linkage disequilibrium for the Kenyan isolates suggest that sexual reproduction could be important in the life cycle of the pathogen in Africa. This could account for the high genotypic diversity observed in the populations due to recombination and re-assortment of alleles during sexual reproduction (Milgroom 1996). It is unusual, therefore, that the sexual state of *D. septosporum* has not been reported from Africa (Groenewald *et al.* 2007), although this might simply be due to time and intensity of sampling.

Founder effects were evident in *D. septosporum* populations from Ecuador, Chile, New Zealand and Australia. The low levels of diversity and clonal structure that were observed for isolates from these countries suggests that these populations have lost alleles in association with their movement, arrival, and establishment outside their native range. This is common for introduced pathogens. For example, *Fusarium circinatum* Nirenberg & O'Donnell, which causes pitch canker of pine, has reduced genetic variation in its areas of introduction in comparison to those in its native geographic range (Wikler & Gordon 2000). Linkage disequilibrium and shifts in the reproductive modes of populations are additional indicators of pathogens that have been moved out of their natural range (Taylor *et al.* 1999). Both these indicators are apparent in the populations of *D. septosporum* from South America and Australasia in this study where only the MAT 2 phenotype was observed. These studies using a larger sample size, confirmed the same results obtained by Groenewald *et al.* (2007).

Low genetic diversity and the presence of a small number of clonal lineages in the South American and Australasian cluster observed in this study has clearly not been a limiting factor for the successful establishment and spread of *D. septosporum*. Populations that have gone through a bottleneck resulting in lower levels of genetic diversity, are thought to be at a disadvantage due to the possibility of alleles conferring adaptive abilities to survive in their new environment being deleted (Sakai *et al.* 2001, Allendorf & Lundquist 2003). *D. septosporum* disease epidemics have, however, been most devastating in the Southern Hemisphere. In this case, a small number of virulent genotypes have likely adapted to the environmental conditions and susceptible host tissue and these would have spread, expanding their range via asexual reproduction. This would be similar to the situation for many other introduced plant pathogens including *Phytophthora infestans* (Mont.) de Bary (Goodwin *et al.* 1994), *Diplodia pinea* (Desm.) J. Kickx. Fil. (Burgess *et al.* 2001) and *Ceratocystis fimbriata* (Ellis & Halsted) f. *platani* Walter (Engelbrecht *et al.* 2004).

The results of this study suggest that there has been direct movement of *D. septosporum* between Ecuador and Chile. This was evident from the fact that both countries shared common genotypes, all the alleles were identical and there was a lack of population structure (1 % among population variation) between these countries. Pine plantation forestry based on *P. radiata* has been practiced in Chile since the 1940's (Toro & Gessel 1999) and to a lesser degree in Ecuador since 1925 (Miller 1974). Dothistroma needle blight has been known in Chile since 1957 (Gibson 1972) but was only reported from Ecuador in 1983 (Evans & Oleas 1983). Given the genetic similarity of the *D. septosporum* populations in these countries, it is likely that the pathogen was accidentally moved from Chile that has shared *P. radiata* germ plasm with Ecuador (F. Montenegro, pers. communication).

It has been speculated that *D. septosporum* moved from New Zealand to Australia naturally across the Tasman Sea (Edwards & Walker 1978, Matheson 1985). Results of this study showing a single haplotype present in New Zealand and at two locations sampled in Australia supports this view. However, the Australian samples included additional genotypes and unique alleles not found in New Zealand. This suggests that, despite rigorous quarantine regulations, other sources of introductions have occurred into Australia. These would most likely have been human-mediated due to the geographically isolated position of the continent and they would most probably have occurred through the importation of pine germplasm such as seed.

Results of this study clearly show that the *D. septosporum* populations in New Zealand are clonal. This is consistent with previous studies (Hirst 1997) where only one haplotype was found in samples covering a relatively large area. In that study, collections made in the 1960's were identical to those made 30 years later. It is evident that the same clone continues to persist almost 50 years later. New Zealand is well known to have maintained very strict quarantine regulations for many years (www.maf.govt.nz/quarantine) and evidently, new introductions of *D. septosporum* have not occurred. There was, however, a single isolate in the New Zealand collection that had a mutation at one locus in a microsatellite region resulting in a unique genotype. The observed mutation in a single isolate suggests that *D. septosporum* has the potential to evolve, and thus to overcome the extensive resistance barrier that has been bred into *P. radiata* in that country.

The number of isolates available for this study from Kenya was limited. However, there were no shared genotypes between isolates from New Zealand and Kenya and only a small

proportion (22 %) of the alleles were common to the fungus in these areas. It was thus not possible to validate the hypothesis of Hirst (1997) that *D. septosporum* in New Zealand originated in Kenya. Yet the data suggests that this is probably not the case.

The worldwide distribution of *D. septosporum* has provided an outstanding example of human intervention, albeit unintentional, facilitating both the movement and the establishment of this pathogen in many areas of the world where pines are planted as non-natives. Although available cultures from countries considered in this study varied in number, intriguing patterns of local range expansion and global spread emerged. There remains substantial opportunity to expand knowledge gained in this study with larger numbers of isolates from areas that could not be optimally sampled. Larger numbers of isolates from North America, Central America and the Himalayas are particularly likely to provide evidence for the centre of origin of *D. septosporum*.

In the future, optimal climatic conditions, influenced dramatically by climate change (Woods *et al.* 2005), and the planting of susceptible trees in monocultures are likely to increase the potential for *D. septosporum* to infect, establish, and expand its range. This is apparently already occurring in areas such as British Columbia and parts of Europe. *D. septosporum* provides an outstanding model to study biological invasions due to the fact that it has become established both in the Northern and Southern Hemisphere where it can be viewed alternatively as an alien invasive or a native pathogen, influenced by planting practices, other human activities and climate change. Whereas populations of the pathogen in the Northern Hemisphere are genetically homogenous and sexually recombining, those in the Southern Hemisphere, with the exception of Africa, have low levels of diversity and a single mating type gene. It is thus important to monitor and prevent the further spread of different, possibly more virulent genotypes and opposite mating type genes, into these predominantly monoculture plantations of susceptible hosts.

REFERENCES

ÁESZ Hungary (2002) Magyarország erdőállományai 2001. [Hungarian forests 2001]. Állami erdészeti Szolgálat, Budapest.

Agapow PM, Burt A (2001) Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* **1:** 101-102.

Allendorf FW, Lundquist LL (2003) Introduction: Population Biology, Evolution, and Control of Invasive Species. *Conservative Biology* **17:** 24-30.

Anagnostakis SL (1987) The effect of multiple importations of pests and pathogens on a native tree. *Biological Invasions* **3:** 245-254.

Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution* **19:** 535-544.

Aumonier T (2002) La maladie des bandes rouges toujours en augmentation en [Dothistroma needle blight (*Dothistroma septospora*) still on the increase in 2001]. Les Cahiers du DSF, 1-2002 (La Santé des Forêts [France] en 2000 et 2001) pp 58-60. *Min. Agri. Alim. Pêche Aff. rur. (DERF)*, Paris France.

Banke S, McDonald A (2005) Migration patterns among global populations of the pathogenic fungus *Mycosphaerella graminicola. Molecular Ecology* **14:** 1881-1896.

Barnes RD (1970) The prospects for re-establishing *Pinus radiata* as a commercially important species in Rhodesia. *South African Forestry Journal* **72:** 17-19.

Barnes I, Cortinas MN, Wingfield MJ, Wingfield BD (2008b) Microsatellite markers for the red band needle blight pathogen, *Dothistroma septosporum*. *Molecular Ecology Resources* **8:** 1026-1029.

Barnes I, Crous PW, Wingfield MJ, Wingfield BD (2004) Multigene phylogenies reveal that red band needle blight of *Pinus* is caused by two distinct species of *Dothistroma, D. septosporum* and *D. pini. Studies in Mycology* **50:** 551-565.

Barnes I, Kirisits T, Akulov A, Chhetri DB, Wingfield BD, Bulgakov TS, Wingfield MJ (2008a). New host and country records of the Dothistroma needle blight pathogens from Europe and Asia. *Forest Pathology* **38:** 178-195.

Bednářová M, Palovčíková D, Jankovský L (2006) The host spectrum of Dothistroma needle blight *Mycosphaerella pini* E. Rostrup – new hosts of Dothistroma needle blight observed in the Czech Republic. *Journal of Forest Science* **52:** 30-36.

Bradshaw RE (2004) Dothistroma (red-band) needle blight of pines and the dothistromin toxin: a review. *Forest Pathology* **34:** 163-185.

Brasier CM (2001) Rapid evolution of introduced plant pathogens via interspecific hybridization. *BioScience* **51:** 123-133.

Brasier CM, Kirk SA, Pipe ND, Buck KW (1998) Rare interspecific hybrids in natural populations of the Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi*. *Mycological Research* **102:** 45-57.

Brown A, Rose D, Webber J (2003) Red band needle blight of pine. *Forestry Commission Information Note 49.*

Brown JKM, Hovmøller MS (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297:** 537-541

Burdon JJ, Thrall PH, Ericson L (2006) The current and future dynamics of disease in plant communities. *Annual Review of Phytopathology* **44:** 19-39.

Burgess T, Wingfield BD, Wingfield MJ (2001) Comparison of genotypic diversity in native and introduced populations of *Sphaeropsis sapinea* isolated from *Pinus radiata*. *Mycological Research* **105:** 1331-1339.

Clark L (1988) Novel non-templated nucleotide addition reactions catalyzed by prokaryotic and eukaryotic DNA polymerases. *Nucleic Acid Research* **16:** 9677-9686.

Cockerham CC, Weir BS (1993) Estimation of gene-flow from F-statistics. *Evolution* **47:** 855-863.

Desprez-Loustau ML, Robin C, Buée M, Courtecuisse R, Garbaye J, Suffert F, Sache I, Rizzo DM (2007) The fungal dimension of biological invasions. *Trends in Ecology and Evolution* **1:** 472-480.

Diekmann M, Sutherland JR, Nowell DC, Morales FJ, Allard G, editors. (2002) *FAO/IPGRI Technical guidelines for the safe movement of germplasm.* No. 21. *Pinus* spp. Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* **17 :** 431-449.

Doroguine M (1911) Une maladie cryptogamique du Pin. *Bulletin Trimestriel de la Société Mycologique de France* **27:** 105-106

Edwards DW, Walker J (1978) *Dothistroma* needle blight in Australia. *Australian Forest Research* **8:** 125-137.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [Argania spinosa (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics* **92:** 832-839.

Elton CS (1958) The ecology of invasions by animals and plants. *London: Methuen, 181pp.*

Engelbrecht CJB, Harrington TC, Steimel J, Capretti P (2004) Genetic variation in eastern North American and putatively introduced populations of *Ceratocystis fimbriata* f.*platani. Molecular Ecology* **13:** 2995-3005.

Evanno G, Regnault S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure. A simulation study. *Molecular Ecology* **14:** 2611-2620.

Evans HC (1984) The genus *Mycosphaerella* and its anamorphs *Cercoseptoria*, *Dothistroma* and *Lecanosticta* on pines, CMI Mycological Paper no. 153. Surrey U.K.: Commonwealth Agricultural Bureaux.

Evans HC, Oleas AR (1983) Pathology of *Pinus radiata* in Ecuador with special reference to Dothistroma. *Tropical Pest Management* **29:** 316-320.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* **164:** 1567-1587.

Ganley RJ, Bradshaw RE (2001) Rapid identification of polymorphic microsatellite loci in a forest pathogen, *Dothistroma pini*, using anchored PCR. *Mycological Research* **105:** 1075-1078.

Gibson IAS (1972) Dothistroma blight of *Pinus radiata. Annual Review of Phytopathology* **10:** 51-72.

Gibson IAS, Christiansen P, Munga F (1964) First observations in Kenya on a foliage disease of pines caused by *Dothistroma pini* Hulbary. *Commonwealth Forest Review* **45:** 67-76.

Gilmour JW (1967) Distribution and significance of the needle blight of pines caused by *Dothistroma pini* in New Zealand. *Plant Disease Reporter* **51:** 727-730.

Gladieux P, Zhang X-G, Afoufa-Bastien D, Valdebenito Sanhueza R-M, Sbaghi M, Le Cam B (2008) On the origin and spread of the scab disease of apple: out of Central Asia. *PLoS ONE*. **3**, e1445.doi:10.1371/journal.pone.0001455

Gobbin D, Rumbou A, Linde CC, Gessler C (2006) Population genetic structure of *Plasmopara viticola* after 125 years of colonization in European vineyards. *Molecular Plant Pathology* **7:** 519-531.

Goodwin SB, Cohen BA, Fry WE (1994) Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the national academy of sciences of the United States of America* **91:** 11591-11595.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Groenewald M, Barnes I, Bradshaw RE, Brown A, Dale A, Groenewald JZ, Lewis KJ, Wingfield BD, Wingfield MJ, Crous PW (2007) Characterization and worldwide distribution of the mating type genes in the Dothistroma needle blight pathogens. *Phytopathology* **97:** 825-834.

Hamelin RC, Lecours N, Laflamme G (1998) Molecular evidence of distinct introductions of the European race of *Gremmeniella abietina* into North America. *Phytopathology* **88:** 582-588.

Hirst P (1997) Genetic diversity of *Dothistroma pini* in New Zealand. Masterate Thesis. Massey University: New Zealand.

Ivory MH (1987) Diseases and disorders of pines in the tropics. *Overseas Development Administration,* Oxford Forestry Institute.

Ivory MH (1990) Needle diseases of pines in Nepal. *Banko Janakari* **2:** 209-212.

Ivory MH (1994) Records of foliage pathogens of *Pinus* species in tropical countries. *Plant Pathology* **43:** 511-518.

Jankovský L, Bednářová M, Palovčíková D (2004) Dothistroma needle blight *Mycosphaerella pini* E. Rostrup, a new quarantine pathogen of pines in the CR. *Journal of Forest Science* **50:** 319-326.

Karadzić D (1988) *Dothistroma septospora* (Dorog.) Morelet: rasprostranjenje, biljke domacini i znacaj. [*Dothistroma septospora*: distribution, hosts and importance]. Glasnik Sumarskog Fakulteta, *Univerzitet u Beogradu* **70:** 133-144.

Karadzić D (1989) *Scirrhia pini* Funk et Parker. Life cycle of the fungus in plantations of *Pinus nigra* Arn. in Serbia. *European Journal of Forest Pathology* **19:** 231-236.

Kowalski T, Jankowiak R (1998) First record of *Dothistroma septospora* (Dorog.) Morelet in Poland: A contribution to the symptomology and epidemiology. *Phytopatologia Polonica* **16:** 15-29.

Krstić M (1958) Nezabeležene fitopatološke pojave u rasadnicima i šumama Srbije. *Zaštita bilja* **45:** 75-79.

Lavery PB, Mead DJ (1998) *Pinus radiata*: a narrow endemic from North America takes on the world. In: Ecology and biogeography of *Pinus* (Richardson DM, ed) pp 432-449. Cambridge University Press, Cambridge, United Kingdom.

Lundquist JE (1987) Fungi associated with *Pinus* in South Africa. Part II. The Cape. *South African Forestry Journal* **140:** 4-15.

Magnuson VL, Ally DS, Nylund SJ, Karanjawala ZE, Rayman JB, Knapp JI, Lowe AL, Ghosh S, Collins FS (1996) Substrate nucleotide-determined non-templated addition of adenine by taq DNA polymerase: Implications for PCR-based Genotyping and cloning. *Biotechniques* **21:** 700-709.

Marincović P, Strajković B (1969) Ogledi suzbijanja *Dothistroma pini* Hulbary u kulturama crnog bora na Deliblatskom Pesku. *Deliblatski Pesak Zbornik radova I.* 247-252.

Matheson AC (1985) Quarantine and Dothistroma needle blight of pines. Standing Committee on Forestry, Division of Forest Research CSIRO. pp 6.

Maynard Smith J, Smith NH, O'Rourke M, Spratt BG (1993) How clonal are bacteria? *Proceedings of the National Academy of Sciences USA* **90:** 4384-4388.

Milgroom MG (1996) Recombination and the multilocus structure of fungal populations. *Annual Review of Phytopathology* **34:** 457-477.

Miller AD (1974) *Pinus radiata* in Ecuador, its ecology and growth. *Unasylva* **26:** 30-34.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the USA* **70:** 3321-3323.

Parker AK, Collis DG (1966) Dothistroma needle blight of pines in British Columbia. *Forestry Chronicle* **42:** 160-161.

Parker IM, Gilbert GS (2004) The evolutionary ecology of novel plant-pathogen interactions. *Annual Review of Ecology Evolution and Systematics* **35:** 675-700.

Peakall ROD, Smouse PE (2006) Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6:** 288-295.

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. *Bioscience* **50:** 53-65.

Poynton RJ (1977) Tree planting in Southern Africa. Vol. 1 The Pines. *South African Forestry Research Institute*.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155:** 945-959.

Richard NM, Lonsdale (2001) Humans as global plant dispersers: Getting more than we bargained for. *BioScience* **51:** 95-102.

Richardson DM, Rundel PW, Jackson ST, Teskey RO, Aronson J, Bytnerowicz A, Wingfield MJ, Procheş Ş (2007) Human impacts in pine forests: Past, Present, and Future. *Annual Review of Ecology, Evolution, and Systematics* **38:** 275-297.

Rogers DL (2002) *In situ* genetic conservation of Monterey pine (*Pinus radiata* D. Don): Information and recommendations. Report No. 26. University of California Division of Agriculture and Natural Resources, Genetic Resources Conservation Program, Davis CA USA.

Rossman AY (2001) A special issue on global movements of invasive plants and fungi. *BioScience* **51:** 93-94.

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller

SG (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics* **32:** 305-332.

Stukenbrock EH, Banke S, McDonald BA (2006) Global migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum. Molecular Ecology* **15:** 2895-2904.

Szabó I (1997) A *Dothistroma septosporum* (Dorog.) Morlet fellépése feketefenyő-ültetvényeken. [Occurrence of *Dothistroma septospora* in Austrian pine plantations]. *Erdészeti Lapok* **132:** 44-45.

Taylor JW, Jacobson D, Fisher M (1999) The evolution and asexual fungi: Reproduction, Speciation and Classification. *Annual Review of Phytopathology* **37:** 197-246.

Toro J, Gessel SP (1999) Radiata pine plantations in Chile. *New Forests* **18:** 33-44.

Weir BS (1996) *Genetic Data Analysis II*. Sinauer Associates, Sunderland, MA.

Wikler K, Gordon TR (2000) An initial assessment of genetic relationships among populations of *Fusarium circinatum* in different parts of the world. *Canadian Journal of Botany* **78:** 709-717.

Wingfield MJ (2003) Increasing threat of diseases to exotic plantation forests in the Southern Hemisphere: lessons from Cryphonectria canker. *Australasian Plant Pathology* **32:** 133-139.

Wingfield MJ, Slippers B, Roux J, Wingfield BD (2001) Worldwide movement of exotic forest fungi, especially in the tropics and the Southern Hemisphere. *BioScience* **51:** 134-140.

Woods A, Coates KD, Hamann A (2005) Is an unprecedented Dothistroma needle blight epidemic related to climate change? *BioScience* **55:** 761-769.

Woods AJ (2003) Species diversity and forest health in northwest British Columbia. *The Forestry Chronicle* **79:** 892-897.

Table 1: Isolates of *Dothistroma septosporum* from different countries and hosts used in this study

Country	Locality	Host	Number of isolates	Date collected	Collectors
SOUTH AFRICA	Hogsback, Eastern Cape	*P. radiata*	73	Aug 2001	J. Roux
	Haenertsburg area, Tzaneen, Limpopo	*P. radiata*	27	Sep 2002	I. Barnes
KENYA	Napkoi	*P. radiata*	11	Jan 2001	J. Roux
ECUADOR	Lasso Highlands, Cotopaxi	*P. muricata*	17	2001	M.J. Wingfield
CHILE	Canteras plantation, Bio Bio, VIII Region	*P. radiata*	27	2001	M.J. Wingfield
	Canteras plantation, Bio Bio, VIII Region	*P. radiata*	1	2007	R. Ahumada
	Dollinco, Valdivia, X Region	*P. radiata*	23	2001	M.J. Wingfield
	Naguilan, Valdivia, X Region	*P. radiata*	8	2001	M.J. Wingfield
NEW ZEALAND	Karioi	*P. ponderosa*	1	Aug 2001	M. Dick
	Karioi	*P. contorta*	1	Aug 2001	M. Dick
	FRI Nursery, Rotorua	*P. radiata*	3	Aug 2001	M. Dick
	Hokonui Forest	*P. ponderosa*	1	Aug 2001	M.J. Wingfield
	Lake Okareka, Rotorua	*P. radiata*	1	Aug 2001	M.J. Wingfield
	Kaingora Forest	*P. radiata*	15	Feb 2003	M.J. Wingfield
AUSTRALIA	Canberra (A.C.T)	*P. radiata*	6	2000	K. Old
	State Forests of New South Wales in Tumut	*P. radiata*	35	June 2003	A.J. Carnegie
BHUTAN	Yusipang, Thimphu dzongkhag	*P. radiata*	3	July 2005	T. Kirisits, M. J. Wingfield
	Ura, Bumthang dzongkhag	*P. wallichiana*	1	May 2005	H. Konrad
	Tangsibi, Bumthang dzongkhag	*P. wallichiana*	6	July 2005	T. Kirisits, M. J. Wingfield
	Lamey Goemba, Bumthang dzongkhag	*P. wallichiana*	2	July 2005	T. Kirisits, N. Gyeltshen
HUNGARY	Botanical garden of the University of West Hungary, Sopron	*P. mugo*	5	August 2005	T. Kirisits
	Near Diszel	*P. nigra*	45	May 2007	I. Barnes
AUSTRIA	Hollenstein/Ybbs, Lower Austria	*P. sylvestris*	5	July 2004	T. Kirisits
		P. mugo	5	July 2004	T. Kirisits
		P. nigra	2	July 2004	T. Kirisits
		P. cembra	1	July 2004	T. Kirisits
	Forest experimental garden, “Knödelhütte”, BOKU, Vienna	*P. peuce*	4	April 2004	T. Kirisits
	Thenneberg, province Lower Austria	*P. nigra*	27	June 2004	T. Kirisits
	Near Wr. Neustadt, Lower Austria	*P. sylvestris*	1	June 2006	T. Kirisits
	Raumberg, Styria	*P. mugo*	2	June 2006	T. Kirisits
	National park “Gesäuse”, Styria	*P. sylvestris*	8	June 2006	T. Kirisits
		P. mugo	2	June 2006	T. Kirisits
POLAND	Miechów Forest District, Goszcza Forest Unit, Near Cracow	*P. nigra*	34	June 2003	T. Kowalski
CZECH REPUBLIC	Tisnov, Riegrova road	*P. nigra*	1	July 2006	L. Jankovsky
	South Moravia, Lanzhot, Forest district Lanzhot, Loc:	*P. nigra*	12	July 2006	L. Jankovsky
SLOVAKIA	Strazovske vrchy, valley between Zliechov and Kosecke Podhradie	*P. nigra*	24	July 2006	L. Jankovsky
ROMANIA	Valea Putnei (near fishing pond), Suceava	*P. nigra*	4	July 2007	G. Hoch
U.S.A.	Lochsa Historical Ranger Station, Idaho	*P. ponderosa*	1	2004	L.M. Carris
	Missoula Lola National Forest, Montana	*P. contorta v. latifolia*	9	May 2006	D. Six

Table 2: Allele frequencies of *D. septosporum* isolates from different countries based on 12 microsatellite markers

		EUROPE						ASIA	NORTH AMERICA	AFRICA		SOUTH AMERICA		AUSTRAL-ASIA	
Locus	Allele	Austria	Czech	Hungary	Poland	Romania	Slovakia	Bhutan	USA	Kenya	RSA	Chile	Ecuador	Australia	New Zealand
DS1	Allele A	0.02	-	-	-	-	0.04	0.08	-	-	0.74	-	-	-	-
	Allele B	0.02	-	-	-	-	-	0.92	-	-	-	1	1	1	1
	Allele C	**0.02**[1]	**0.23**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele D	0.30	0.15	0.25	0.07	0.67	0.35	-	0.86	0.22	0.03	-	-	-	-
	Allele E	0.11	-	0.11	0.33	-	0.17	-	-	0.78	-	-	-	-	-
	Allele F	0.20	0.08	0.25	0.22	-	-	-	-	-	0.23	-	-	-	-
	Allele G	0.18	0.23	0.25	-	0.33	0.35	-	0.14	-	-	-	-	-	-
	Allele H	**0.05**	**0.08**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele I	**0.07**	**0.15**	**0.06**	**0.37**	**-**	**0.09**	-	-	-	-	-	-	-	-
	Allele J	**0.02**	**0.08**	**0.08**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
DCB2	Allele A	0.56	0.38	0.58	1	0.67	0.83	-	1	0.67	1	0.25	0.5	0.5	1
	Allele B	0.44	0.62	0.42	-	0.33	0.17	1	-	0.33	-	0.75	0.5	0.5	-
F	Allele A	**0.02**	**-**	**0.03**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele B	0.64	0.62	0.33	0.74	-	0.70	1	-	0.78	0.23	1	1	1	1
	Allele C	0.33	0.38	0.64	0.26	1	0.30	-	-	0.22	0.77	-	-	-	-
	Allele D	-	-	-	-	-	-	-	**0.57**	-	-	-	-	-	-
	Allele E	-	-	-	-	-	-	-	**0.43**	-	-	-	-	-	-
E	Allele A	**-**	**-**	**0.03**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele B	**0.02**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele C	0.13	0.38	0.19	0.22	-	0.39	-	-	-	0.03	-	-	-	-
	Allele D	0.73	0.62	0.69	0.78	1	0.43	0.83	0.14	0.44	0.57	-	0.08	-	-
	Allele E	0.09	-	0.08	-	-	0.09	-	-	-	-	-	0.08	-	-
	Allele F	0.02	-	-	-	-	0.09	-	-	0.56	0.40	-	-	-	-
	Allele G	-	-	-	-	-	-	0.08	0.14	-	-	-	-	-	-
	Allele H	-	-	-	-	-	-	-	**0.71**	-	-	-	-	-	-
	Allele I	-	-	-	-	-	-	0.08	-	-	-	0.08	0.08	-	-
	Allele J	-	-	-	-	-	-	-	-	-	-	-	0.08	1	0.50
	Allele K	-	-	-	-	-	-	-	-	-	-	0.33	0.25	-	0.50
	Allele L	-	-	-	-	-	-	-	-	-	-	**0.50**	**0.33**	-	-
	Allele M	-	-	-	-	-	-	-	-	-	-	**0.08**	**0.08**	-	-
G	Allele A	-	-	-	-	-	-	-	**0.71**	-	-	-	-	-	-
	Allele B	**0.02**	-	-	-	-	-	-	-	-	-	-	-	-	-
	Allele C	0.09	0.08	0.08	0.04	-	0.04	1	0.14	0.44	0.60	-	-	-	-
	Allele D	0.78	0.92	0.92	0.96	1	0.96	-	0.14	0.56	0.40	0.75	0.75	-	-
	Allele E	0.09	-	-	-	-	-	-	-	-	-	0.25	0.25	1	1
	Allele F	**0.02**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
J	Allele A	0.02	-	0.06	-	-	-	0.17	0.14	-	-	-	-	-	-
	Allele B	-	-	-	-	-	-	-	**0.86**	-	-	-	-	-	-
	Allele C	0.13	-	0.03	0.19	0.33	-	0.08	-	-	0.40	1	1	-	-
	Allele D	0.09	0.23	0.19	0.19	-	0.04	0.17	-	1	0.54	-	-	-	-
	Allele E	0.44	0.54	0.53	0.48	-	0.78	0.33	-	-	0.06	-	-	-	-
	Allele F	0.27	-	0.17	0.07	0.67	0.04	0.08	-	-	-	-	-	-	-
	Allele G	0.02	0.15	-	0.07	-	**-**	0.08	-	-	-	-	-	0.75	1
	Allele H	-	0.08	0.03	-	-	**-**	-	-	-	-	-	-	0.25	-
	Allele I	**-**	**-**	**-**	**-**	**-**	**0.13**	-	-	-	-	-	-	-	-
	Allele J	-	-	-	-	-	-	**0.08**	-	-	-	-	-	-	-
	Allele K	**0.02**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
O	Allele A	0.11	-	0.06	0.19	0.33	-	-	-	0.33	0.31	-	-	-	-
	Allele B	0.87	1	0.94	0.81	0.67	1	1	-	0.67	0.69	1	1	1	1
	Allele C	0.02	-	-	-	-	-	-	1	-	-	-	-	-	-
M	Allele A	**0.02**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele B	0.07	0.08	0.06	0.37	-	0.09	0.08	-	-	0.60	-	-	-	-
	Allele C	-	-	0.06	-	-	**-**	-	-	-	-	-	-	1	1
	Allele D	0.36	0.46	0.56	0.33	0.33	0.48	0.08	0.86	0.33	0.40	-	-	-	-
	Allele E	-	-	0.08	-	-	**-**	-	0.14	-	-	-	-	-	-
	Allele F	0.11	0.15	0.06	-	0.33	**-**	0.25	-	-	-	-	-	-	-
	Allele G	0.09	-	-	-	-	**-**	0.08	-	-	-	1	1	-	-
	Allele H	**0.18**	**-**	**0.06**	**0.07**	**-**	**0.04**	-	-	-	-	-	-	-	-
	Allele I	0.09	-	-	0.11	0.33	0.09	0.25	-	-	-	-	-	-	-
	Allele J	**0.02**	**0.23**	**0.03**	**0.04**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele K	-	0.08	-	-	-	0.04	0.17	-	-	-	-	-	-	-
	Allele L	0.02	-	0.08	-	-	**-**	0.08	-	-	-	-	-	-	-
	Allele M	**0.02**	**-**	**-**	**-**	**-**	**0.22**	-	-	-	-	-	-	-	-
	Allele N	-	-	-	-	-	0.04	-	-	0.11	-	-	-	-	-
	Allele O	**0.02**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele P	**-**	**-**	**0.03**	**0.07**	**-**	**-**	-	-	-	-	-	-	-	-

Table 2: Continued…

		EUROPE						ASIA	NORTH AMERICA	AFRICA		SOUTH AMERICA		AUSTRAL-ASIA	
Locus	**Allele**	**Austria**	**Czech**	**Hungary**	**Poland**	**Romania**	**Slovakia**	**Bhutan**	**USA**	**Kenya**	**RSA**	**Chile**	**Ecuador**	**Australia**	**New Zealand**
I	Allele A	0.02	-	-	-	-	0.30	-	0.86	-	-	-	-	-	-
	Allele B	0.58	0.54	0.97	0.59	0.67	0.57	0.75	-	0.22	0.03	0.25	0.25	1	1
	Allele C	0.13	-	-	-	-	**-**	0.17	-	0.78	0.86	0.58	0.75	-	-
	Allele D	0.02	-	-	0.30	-	0.04	-	0.14	-	0.11	0.17	-	-	-
	Allele E	0.18	0.38	0.03	0.11	0.33	0.09	0.08	-	-	-	-	-	-	-
	Allele F	**0.07**	**0.08**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
L	Allele A	0.04	0.08	0.06	-	-	-	-	0.14	-	-	-	-	-	-
	Allele B	**0.04**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele C	0.56	0.62	0.50	0.19	0.33	0.61	0.17	-	-	-	-	-	-	-
	Allele D	0.04	0.08	0.06	-	-	0.09	0.58	0.14	-	-	-	-	-	-
	Allele E	**-**	**-**	**-**	**0.78**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele F	**-**	**-**	**0.03**	**-**	**-**	**0.04**	-	-	-	-	-	-	-	-
	Allele G	**-**	0.08	-	-	-	**-**	0.17	-	-	-	-	-	-	-
	Allele H	0.04	-	0.11	-	-	0.04	-	-	1	-	-	-	-	-
	Allele I	**0.02**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele J	**0.02**	**-**	**0.19**	**-**	**-**	**0.04**	-	-	-	-	-	-	-	-
	Allele K	**-**	**-**	**-**	**0.04**	**-**	**0.04**	-	-	-	-	-	-	-	-
	Allele L	**-**	**-**	**-**	**-**	**0.33**	**-**	-	-	-	-	-	-	-	-
	Allele M	**-**	**0.08**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele N	**0.11**	**0.08**	**0.06**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele O	0.04	-	-	-	-	**-**	-	-	-	-	-	0.25	0.75	1
	Allele P	**-**	-	-	-	-	**-**	-	-	-	-	-	-	**0.25**	**-**
	Allele Q	0.02	-	-	-	-	**-**	-	0.14	-	-	-	-	-	-
	Allele R	0.02	-	-	-	-	0.04	0.08	-	-	0.80	-	-	-	-
	Allele S	**-**	**-**	**-**	**-**	**0.33**	**-**	-	-	-	-	-	-	-	-
	Allele T	**-**	-	-	-	-	**-**	-	-	-	-	**1**	**0.75**	-	-
	Allele U	**-**	-	-	-	-	0.09	-	-	-	0.20	-	-	-	-
	Allele V	**-**	-	-	-	-	-	-	**0.43**	-	-	-	-	-	-
	Allele W	**0.02**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele X	-	-	-	-	-	-	-	**0.14**	-	-	-	-	-	-
K	Allele A	**0.47**	**0.54**	**0.61**	**0.30**	**0.33**	**0.04**	-	-	-	-	-	-	-	-
	Allele B	-	-	-	-	-	**-**	**0.17**	-	-	-	-	-	-	-
	Allele C	**-**	**0.08**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele D	-	-	-	-	-	0.04	-	-	-	-	0.17	-	-	-
	Allele E	0.18	0.08	0.08	0.04	0.33	0.48	0.58	0.14	1	0.40	0.42	0.25	1	1
	Allele F	0.13	0.08	0.06	0.37	-	0.04	0.08	0.57	-	0.29	0.08	-	-	-
	Allele G	0.07	0.15	0.08	0.30	-	0.30	-	0.14	-	0.14	-	0.08	-	-
	Allele H	0.04	-	-	-	-	**-**	0.17	-	-	0.17	-	-	-	-
	Allele I	**0.07**	**-**	**0.03**	**-**	**0.33**	**0.09**	-	-	-	-	-	-	-	-
	Allele J	**0.02**	**0.08**	**0.14**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele K	0.02	-	-	-	-	-	-	0.14	-	-	-	-	-	-
	Allele L	-	-	-	-	-	-	-	-	-	-	**0.33**	**0.67**	-	-
DS2	Allele A	**0.07**	**-**	**0.03**	**0.04**	**-**	**0.04**	-	-	-	-	-	-	-	-
	Allele B	**0.02**	**-**	**0.03**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele C	**0.09**	**0.15**	**0.09**	**0.04**	**0.33**	**0.09**	-	-	-	-	-	-	-	-
	Allele D	**-**	**-**	**0.06**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele E	0.40	0.31	0.23	0.33	-	0.09	-	-	0.11	-	-	-	-	-
	Allele F	0.09	0.08	0.20	-	0.33	0.39	-	-	-	-	1	1	1	1
	Allele G	0.07	-	0.09	-	-	**-**	-	-	-	0.34	-	-	-	-
	Allele H	0.07	-	0.03	0.44	-	**-**	-	1	-	-	-	-	-	-
	Allele I	**-**	**0.15**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele J	**0.02**	**0.15**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele K	-	-	0.03	-	-	**-**	-	-	0.89	0.40	-	-	-	-
	Allele L	0.02	-	-	-	-	**-**	0.17	-	-	-	-	-	-	-
	Allele M	**-**	**-**	**-**	**-**	**-**	**0.22**	-	-	-	-	-	-	-	-
	Allele N	-	-	-	-	-	**-**	0.83	-	-	0.26	-	-	-	-
	Allele O	**-**	**-**	**0.03**	**-**	**-**	**0.04**	-	-	-	-	-	-	-	-
	Allele P	**0.04**	**0.08**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele Q	**-**	**-**	**-**	**0.15**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele R	**0.04**	**-**	**0.14**	**-**	**-**	**0.04**	-	-	-	-	-	-	-	-
	Allele S	**0.04**	**-**	**-**	**-**	**-**	**-**	-	-	-	-	-	-	-	-
	Allele T	**0.02**	**0.08**	**0.06**	**-**	**0.33**	**0.09**	-	-	-	-	-	-	-	-

[1]Allele frequencies in bold represent alleles that are unique to the continent

Table 3: Summary diversity statistics of *Dothistroma septosporum* isolates within populations and regions

	CONTINENT	COUNTRY	N[1]	No. of haplotypes[2]	Total no. of alleles	Unique alleles	% alleles unique	% loci polymorphic	H[3]	Allelic richness	D[4]
Northern Hemisphere		AUSTRIA	61	45	85	10	11.76	100	0.60	4.32	0.979
		CZECH	13	13	47	3	6.38	92	0.53	3.55	1
		HUNGARY	50	36	61	2	3.28	100	0.50	3.63	0.977
		POLAND	34	27	39	2	5.13	92	0.46	2.81	0.980
		ROMANIA	4	3	25	2	8	75	0.41	N/A	0.833
		SLOVAKIA	24	23	53	2	3.77	92	0.49	3.42	0.996
	EUROPE	**Total**	**186**	**147**	**109**	**46**	**42.20**	**100**	**0.58**	**4.00**	**0.995**
	ASIA (Bhutan)	**Total**	**12**	**12**	**36**	**2**	**5.55**	**67**	**0.33**	**2.68**	**1**
	NORTH AMERICA (USA)	**Total**	**10**	**7**	**28**	**7**	**25**	**75**	**0.31**	**2.32**	**0.911**
Southern Hemisphere		KENYA	11	9	23	2	8.70	75.00	0.31	2.28	0.964
		RSA	100	35	30	0	0	92	0.43	1.91	0.857
	AFRICA	**Total**	**111**	**44**	**37**	**2**	**5.41**	**100**	**0.49**	**2.57**	**0.884**
		CHILE	59	12	22	0	0	42	0.22	1.79	0.835
		ECUADOR	17	12	24	0	0	50	0.24	1.92	0.934
	SOUTH AMERICA	**Total**	**76**	**20**	**27**	**4**	**14.81**	**50**	**0.24**	**1.90**	**0.873**
		AUSTRALIA	41	4	15	1	6.67	25	0.10	N/A	0.618
		NEW ZEALAND	22	2	13	0	0	8	0.04	N/A	0.091
	AUSTRALASIA	**Total**	**63**	**5**	**16**	**1**	**6.25**	**33**	**0.12**	**1.33**	**0.528**

[1] N = Total number of isolates

[2] Equivalent to samples that have been clone corrected

[3] H = Nei's (1973) gene diversity

[4] Genotypic diversity

Table 4: Linkage disequilibrium tests and the distribution and frequency of mating types within world-wide populations of *Dothistroma septosporum*

Countries	Clone-corrected sample size	Clonal fraction	Index of association (I_A)	*P*-vlaue[1] of I_A	Frequencies		χ^2 values
					MAT1-1	MAT1-2	
AUSTRIA	45 (27)[2]	0.26	0.406 (0.27)	< 0.001* (0.037)	0.44	0.56	0.56
CZECH	13	0.00	0.222	0.093	0.46	0.54	0.08
HUNGARY	35	0.30	0.11	0.146	0.56	0.44	0.50
POLAND	27	0.21	0.704	< 0.001*	0.27	0.73	5.54 [3]
ROMANIA	3	0.25	-	-	1	0	-
SLOVAKIA	23	0.04	0.433	0.002*	0.45	0.55	0.18
BHUTAN	12	0.00	-0.155	0.718	0.42	0.58	0.33
USA	7	0.30	2.850	< 0.001*	0.86	0.14	3.57
KENYA	9	0.18	0.730	0.011	0.67	0.33	1.00
SOUTH AFRICA	35	0.65	0.702	< 0.001*	0.46	0.54	0.26
CHILE	12	0.80	-	-	0	1	-
ECUADOR	12	0.29	-	-	0	1	-
AUSTRALIA	4	0.90	-	-	0	1	-
NEW ZEALAND	2	0.91	-	-	0	1	-

[1] *The null hypothesis of random mating is rejected at P<0.01

[2] Values in parenthesis indicate the results obtained from the refined analyses of I_A for 27 isolates from *P. nigra* in one plantation in Austria.

[3] Mating type frequencies depart from the null hypothesis of a 1:1 ratio at $P < 0.05$ with 1 degree of freedom.

Table 5: Analyses of molecular variance within populations, among populations and between regions of *Dothistroma septosporum*

Dataset	Number of populations	Number of regions	% Variation						ϕPT-statistics	*P*- value[1]
			Among Regions	df	Among Pops	df	Within Pops	df		
Africa	2	1	-	-	33%	1	67%	42	0.333	0.001*
South America	2	1	-	-	1%	1	99%	22	0.006	0.319
Australasia	2	1	-	-	4%	1	96%	4	0.040	0.537
Europe	6	1	-	-	9%	5	91%	141	0.088	0.001*
North America	1	-	-	-	-	-	-	-	-	-
Asia	1	-	-	-	-	-	-	-	-	-
All Regions	14	6	32%	5	8%	8	61%	226	0.395	0.001*
Northern vs Southern Hemisphere	14	2	11%	1	25%	12	64%	226	0.358	0.001*

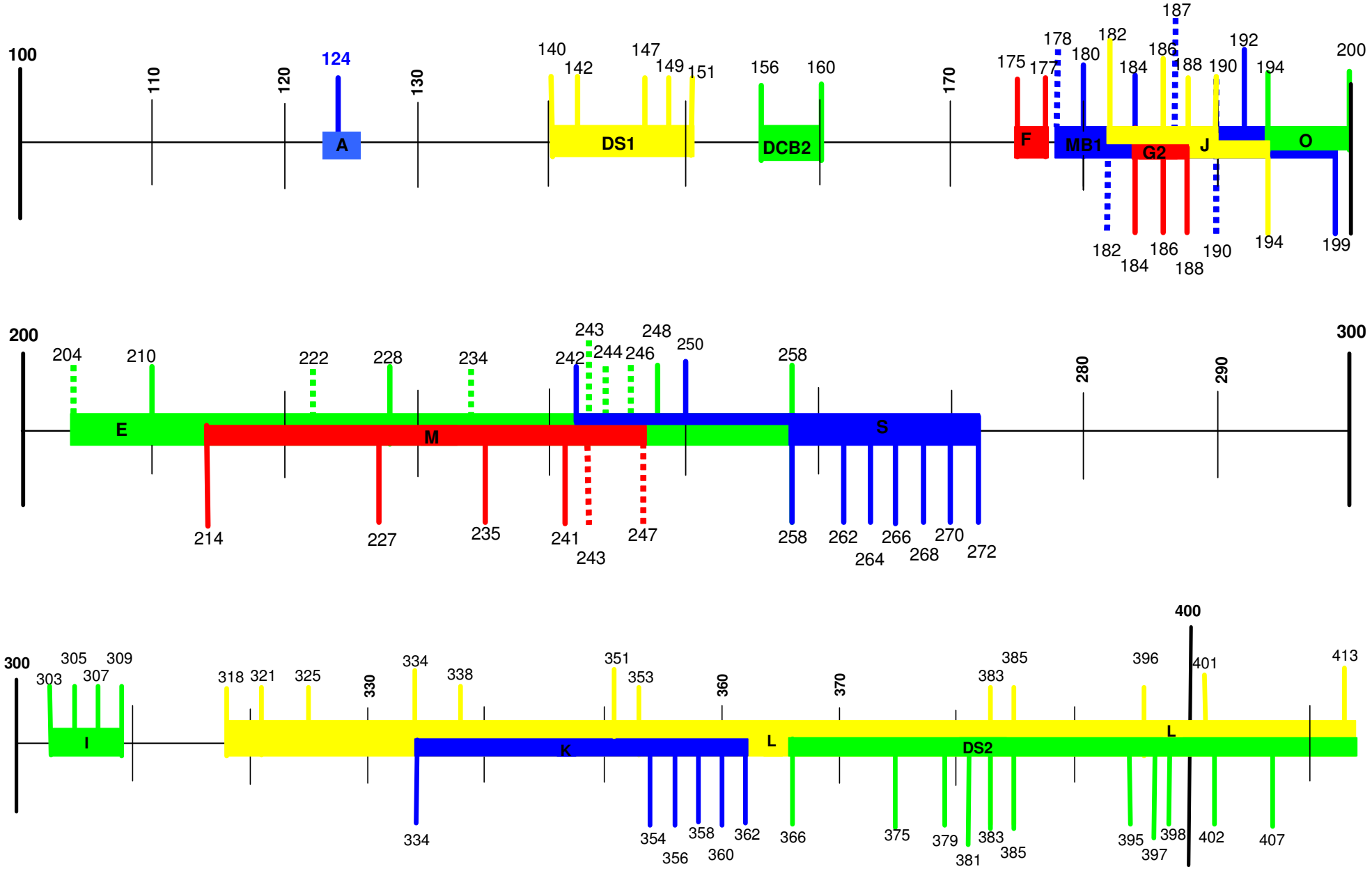
[1]P-value based on 1 000 randomizations.

*The null hypothesis of no population structure is rejected at P<0.01.

Table 6: Pairwise population differentiation (above the diagonal) as measured by θ and number of migrants measured as M=[(1/θ) - 1]/2 (below the diagonal). Theta estimates in bold were significantly greater than zero (P<0.05) based on permutation tests

	RSA	KENYA	ECUADOR	CHILE	N. ZEALAND	AUSTRALIA	POLAND	AUSTRIA	CZECH	SLOVAKIA	HUNGARY	ROMANIA	BHUTAN	USA
RSA	-	**0.333**	**0.502**	**0.530**	**0.515**	**0.557**	**0.343**	**0.291**	**0.358**	**0.351**	**0.347**	**0.326**	**0.453**	**0.507**
KENYA	1.00	-	**0.580**	**0.604**	**0.579**	**0.616**	**0.388**	**0.283**	**0.357**	**0.348**	**0.362**	**0.419**	**0.487**	**0.592**
ECUADOR	0.50	0.36	-	0.007	0.557	0.580	0.467	0.338	0.431	0.417	0.437	0.536	0.526	0.680
CHILE	0.44	0.33	76.42	-	0.614	0.618	0.492	0.356	0.447	0.440	0.453	0.570	0.530	0.702
NEW ZEALAND	0.47	0.36	0.40	0.31	-	0.040	0.437	0.310	0.377	0.357	0.398	0.461	0.515	0.661
AUSTRALIA	0.40	0.31	0.36	0.31	12	-	0.494	0.356	0.425	0.418	0.435	0.573	0.515	0.704
POLAND	0.96	0.79	0.57	0.52	0.64	0.51	-	0.114	0.156	0.166	0.165	0.210	0.424	0.457
AUSTRIA	1.22	1.27	0.98	0.90	1.11	0.90	3.89	-	0.004	0.071	0.029	0.014	0.264	0.387
CZECH	0.90	0.90	0.66	0.62	0.83	0.68	2.71	113.14	-	0.077	0.032	0.070	0.316	0.455
SLOVAKIA	0.92	0.94	0.70	0.64	0.90	0.70	2.51	6.54	5.99	-	0.099	0.130	0.372	0.439
HUNGARY	0.94	0.88	0.65	0.60	0.76	0.65	2.53	16.74	15.13	4.55	-	0.039	0.360	0.457
ROMANIA	1.03	0.69	0.43	0.38	0.58	0.37	1.88	35.21	6.64	3.35	12.32	-	0.437	0.474
BHUTAN	0.60	0.53	0.45	0.44	0.47	0.47	0.68	1.39	1.08	0.84	0.89	0.64	-	0.622
USA	0.53	0.45	0.44	0.47	0.47	0.68	1.39	1.08	0.84	0.89	0.64	1.08	0.84	-

Figure 1. Multiplexed allele sizes and range of loci generated with 15 fluorescently labelled microsatellite markers designed by Ganley *et al.* (2001) and Barnes *et al.* (2008b). Combined allele sizes, in base pairs, were obtained by screening the microsatellite markers on a few isolates from Chapter 4 and from this chapter.


100
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186
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188
190
192
194
200
A
DS1
DCB2
F
MB1
G2
J
O
182
184
186
188
190
194
199
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204
210
214
222
227
228
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E
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303
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318
321
325
330
334
338
351
353
360
370
383
385
396
400
401
413
I
K
L
L
DS2
334
354
356
358
360
362
366
375
379
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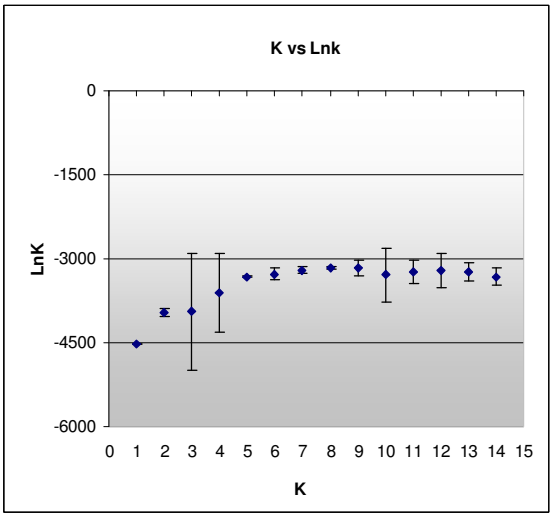
397
398
402
407

Figure 2: Optimal number of populations (K=5) as determined by the LnK and DeltaK values obtained from STRUCTURE analyses using the admixture model with correlated allele frequencies.


K vs Lnk
LnK
0
-1500
-3000
-4500
-6000
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
K

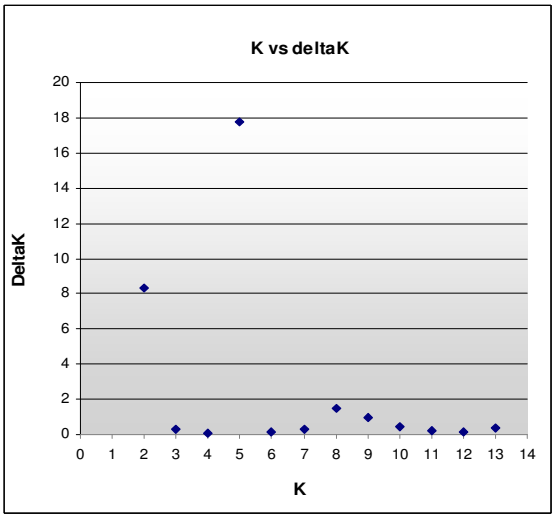
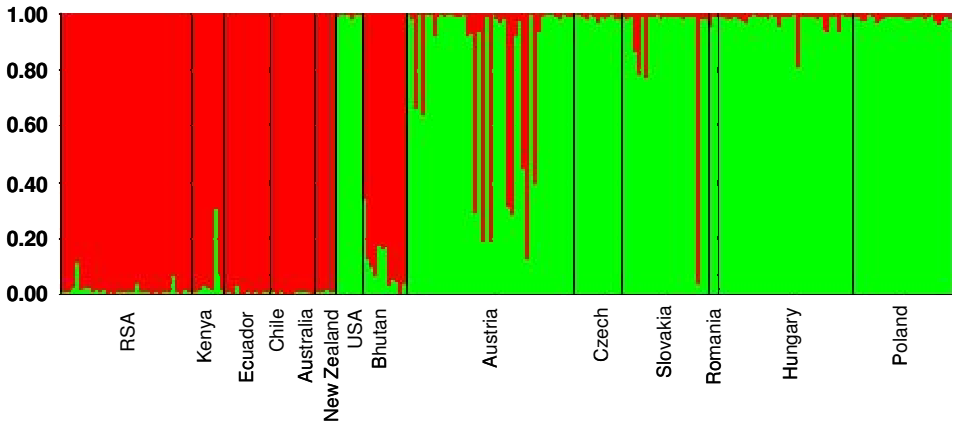
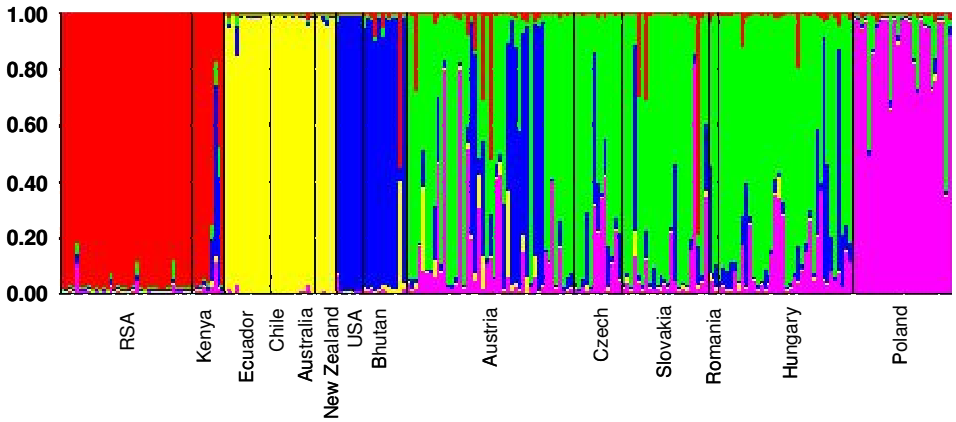

K vs deltaK
DeltaK
20
18
16
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
K

Figure 3: Assignment of individuals into K=2 populations (above), that separate most of the Northern and Southern Hemisphere isolates, and the optimal population size of K= 5 (below), using the program STRUCTURE.

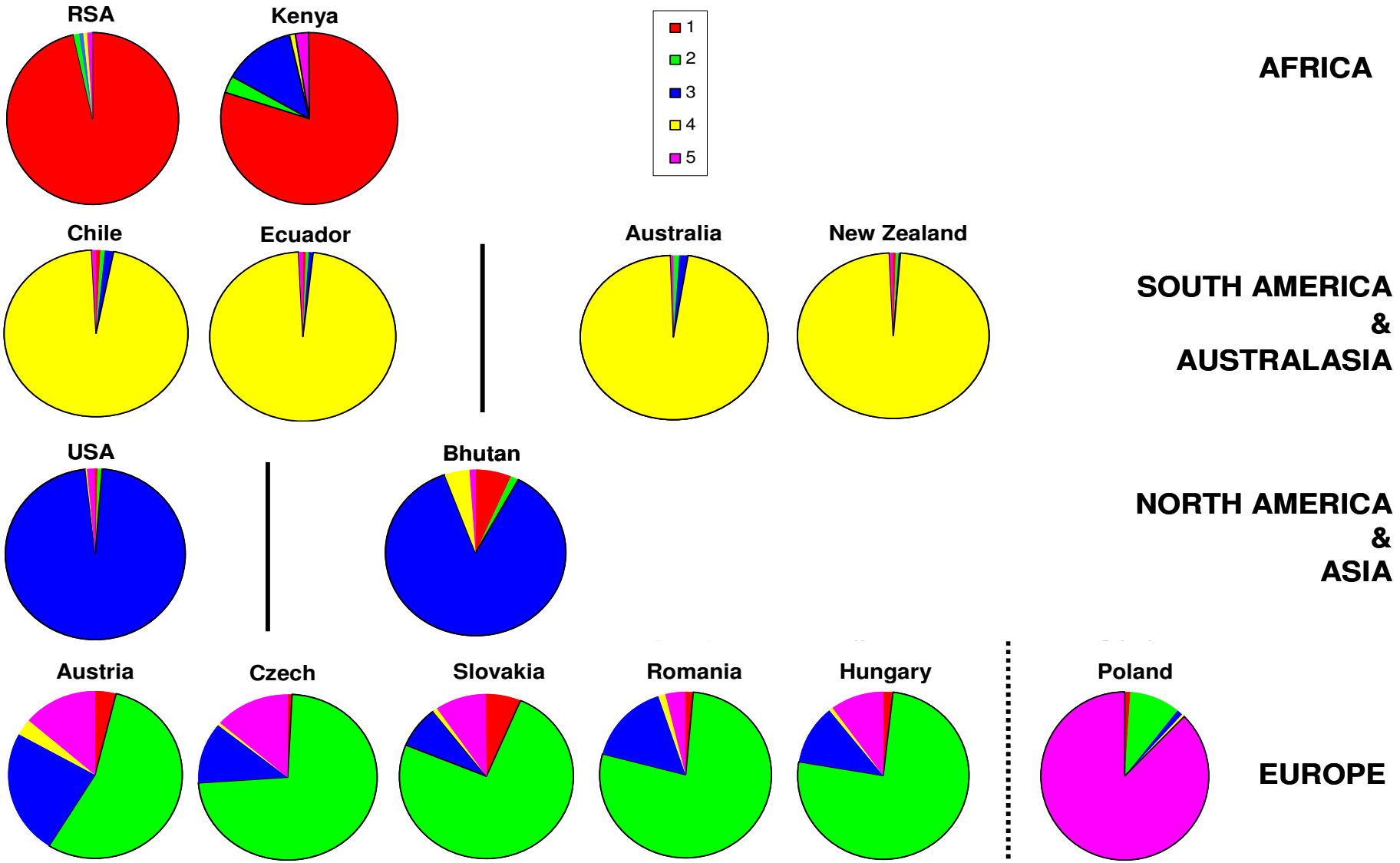


K = 2



K = 5
(Optimal)

Figure 4: Proportion of individuals within countries assigned to one of five clusters as identified by STRUCTURE analyses.


RSA
Kenya
1
2
3
4
5
AFRICA
Chile
Ecuador
Australia
New Zealand
SOUTH AMERICA
&
AUSTRALASIA
USA
Bhutan
NORTH AMERICA
&
ASIA
Austria
Czech
Slovakia
Romania
Hungary
Poland
EUROPE