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Planting exotic relatives has increased the threat posed by *Dothistroma septosporum* to the Caledonian pine populations of Scotland

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Running Title: Multiple origins of *Dothistroma septosporum*

Abstract

To manage emerging forest diseases and prevent their occurrence in the future, it is essential to determine the origin(s) of the pathogens involved and identify the management practices that have ultimately caused disease problems. One such practice is the widespread planting of exotic tree species within the range of related native taxa. This can lead to emerging forest disease both by facilitating introduction of exotic pathogens, and by providing susceptible hosts on which epidemics of native pathogens can develop. We used microsatellite markers to determine the origins of the pathogen *Dothistroma septosporum* responsible for the current outbreak of Dothistroma needle blight (DNB) on native Caledonian Scots pine (*Pinus sylvestris*) populations in Scotland, and evaluated the role played by widespread planting of two exotic pine species in the development of the disease outbreak. We distinguished three races of *D. septosporum* in Scotland, one of low genetic diversity associated with introduced lodgepole pine (*Pinus contorta*), one of high diversity probably derived from the DNB epidemic on introduced Corsican pine (*Pinus nigra* subsp. *laricio*) in England, and a third of intermediate diversity apparently endemic on Caledonian Scots pine. These races differed for both growth rate and exudate production in culture. Planting of exotic pine stands in the UK appears to have facilitated the introduction of two exotic races of *D. septosporum* into Scotland which now pose a threat to native Caledonian pines both directly and through potential hybridisation and introgression with the endemic race. Our results indicate that both removal of exotic species from the vicinity of Caledonian pine populations, and restriction of movement of planting material are required to minimise the impact of the current DNB outbreak. They also demonstrate that planting exotic species that are related to native species reduces rather than enhances the resilience of forests to pathogens.

47 **Keywords**: *Dothistroma septosporum*, microsatellite, tree disease, genetic structure, pine,
48 needle blight, emerging disease

Introduction

Over recent decades a dramatic rise in the incidence of tree disease epidemics has occurred on a global scale (Stenlid *et al.* 2011; Wingfield *et al.* 2015). Unregulated global trade in live plants, which facilitates the introduction of exotic pathogens, is largely responsible for this phenomenon (Brasier 2008; Santini *et al.* 2013). However there are a variety of other forestry practices that may be contributing significantly to our current tree disease problems (Ennos 2015). One that deserves particular attention is the practice of planting exotic species in areas occupied by closely related native tree taxa (Burgess & Wingfield 2017). In these situations disease outbreaks can arise in two ways.

The first involves transfer of endemic pathogen species or races from the native to the related exotic tree (Gilbert & Webb 2007; Gilbert *et al.* 2012). The exotic species may prove susceptible to these native pathogens due to lack of previous co-evolution (Ennos, 2015). The natural resistance of the exotic may also be compromised because it is poorly adapted to the novel environment into which it has been planted (Read 1968; Karlman *et al.* 1984). High density planting in monoculture and reduced genetic diversity of the exotic host may further exacerbate disease problems. An epidemic of the native pathogen may therefore build up on the exotic plantation species. The pathogen pressure generated by this epidemic may be severe enough to produce damage on the (previously resistant) native tree species (Ennos 2001).

The second route to epidemic disease involves the inadvertent introduction, along with the exotic tree species, of one of its co-evolved pathogens. The native species may suffer serious

damage because it has no history of co-evolution with the introduced pathogen (Anagnostakis 1987). Disease problems can also arise on the exotic plantation species if the novel environmental conditions that it encounters either favour the introduced pathogen directly (Gibson, 1972), or impose stress on the exotic species and increase its susceptibility to disease (Schoenweiss 1975, 1981). Introduced pathogens may also hybridise with closely related native pathogens to generate genotypes that are more virulent than either parent (Brasier 2000; Brasier *et al.* 2004; Stukenbrock, 2016).

Well documented examples of disease outbreaks associated with planting of exotic relatives of native species include the white pine blister rust *Cronartium ribicola* (Lasch.) Dietr. epidemic on *Pinus strobus* L. in Europe that followed planting of this species within the native range of European five needled pines at the end of the nineteenth century (Hummer 2000), and the epidemic of *Gremmeniella abietina* (Lagerberg) Morelet on *Pinus contorta* Douglas ex Loudon when this species was introduced into Sweden in the 1990's alongside native *Pinus sylvestris* L. (Karlman *et al.* 1994). More recently the ash dieback epidemic in Europe caused by *Hymenoscyphus fraxineus* has been linked with planting of Asian *Fraxinus mandschurica* Rupr. within the native range of European ash *Fraxinus excelsior* L. (Gross *et al.* 2014).

Given the diversity of ways in which planting of exotic relatives can give rise to tree disease epidemics, detailed forensic studies of such situations are needed to establish the origin(s) of the pathogens responsible and devise appropriate control measures. If the pathogen involved is native, removal of the exotic species may be sufficient to eliminate the disease threat. However if the pathogen is introduced, the prospects for the native species may be poor,

involving death of many trees and recovery only after a prolonged period during which there is evolution of enhanced host resistance (Gomulkiewicz & Holt 1995). Where both exotic and native pathogens are responsible the outcome is less predictable and will depend on the extent of genetic interactions between the pathogen sources (Brasier *et al.* 2001, 2004). Here we use microsatellite markers to analyse the origin(s) of the pathogen *Dothistroma septosporum* (Dorog.) Morelet responsible for a recent outbreak of Dothistroma needle blight (DNB) on pine in Scotland (Brown *et al.* 2012). We highlight the role of two exotic pine species in facilitating the DNB outbreak, and assess the threat that *D. septosporum* now poses to native pine populations.

DNB caused by the ascomycete *D. septosporum*, is currently the most important foliar disease of pine worldwide, affecting 82 host pine species across six continents (Drenkhan *et al.* 2016). Needle infection by rain-splashed conidia (Gibson 1972) or wind-borne ascospores (Funk & Parker 1966) leads to defoliation, reduction in growth and, in severe cases, death of trees (Brown & Webber 2008). *D. septosporum* is believed to be endemic on indigenous pine populations in the northern hemisphere (Welsh *et al.* 2009; Drenkhan *et al.* 2013). From here it has spread and caused severe damage to exotic pine plantations in the southern hemisphere (Barnes *et al.* 2014). Most recently DNB has emerged as a problem in plantations in the northern hemisphere; in North America on *P. contorta* (Roach *et al.* 2015); in mainland Europe on *Pinus nigra* (Poir.) Maire (Fabre *et al.* 2012; Tomsovsky *et al.* 2013; Boron *et al.* 2016; Drenkhan *et al.* 2016); and in Britain on *P. sylvestris*, *P. nigra* and *P. contorta* (Brown & Webber 2008).

In Britain the native host for *D. septosporum,* Scots pine *P. sylvestris*, comprises two distinct populations. Caledonian pines represent the remnants of native *P. sylvestris* populations that recolonised Scotland after the last ice age. They are confined to the Scottish Highlands, are highly fragmented, and their distribution has been reduced to less than 1% of its former area (Steven & Carlisle 1959; Forestry Commission Scotland, 1998). Nevertheless they retain high genetic diversity (Kinloch *et al.* 1986; Wakowiak *et al.* 2011) and are of enormous conservation value because they support one of the few intact, semi-natural forest ecosystems remaining in Britain (McVean & Ratcliffe 1962; Mason *et al.* 2004). Outside the Caledonian pinewoods *P. sylvestris* stock derived from Forestry Commission seed orchards is used to establish plantations, and the species is extensively naturalised throughout British woodlands, representing 17% of total conifer area (Forestry Commission 2015).

Two exotic pine species closely related to Scots pine have been introduced to Britain and grown in large scale plantations for the last 60-100 years. Corsican pine *P. nigra* subsp. *laricio* accounts for 13% of conifer stands in England, and has been successfully introduced at a small number of coastal sites in Scotland (Forestry Commission 2015). Lodgepole pine *P. contorta* of two subspecies (*contorta* and *latifolia*) is grown principally in Scotland where it makes up 10% of the conifer area (Lines 1987; Forestry Commission 2015). Plantations of lodgepole pine often occur adjacent to, or even within Caledonian pine stands.

DNB was first found in Britain in the 1950s in southern English nurseries on four exotic species; Corsican pine, lodgepole pine, *Pinus ponderosa* Douglas ex C. Lawson and *Pinus bungeana* Zucc. ex Endl. (Murray & Batko 1962). No infection or damage to Scots pine was reported. Over the next 40 years the presence of *D. septosporum* was recorded sporadically in

143 southern England and southern Wales (Brown & Webber 2008), and in the 1980s on Scots
144 pine in northern Scotland (British Mycological Society 2014) but was not associated with
145 significant damage. However from 2000 onwards serious epidemics of DNB broke out in
146 England on plantations of Corsican pine, with some infection of adjacent Scots pine. The
147 very high level of damage led to a moratorium on plantings of Corsican pine in 2006 (Brown
148 & Webber 2008). Subsequently DNB has been reported in Scotland on Corsican, lodgepole
149 and plantation Scots pine, and on all three species in forest nurseries. Serious conservation
150 concerns were raised in 2011 when *D. septosporum* was discovered in Caledonian pine
151 populations where it had not previously been recorded (Brown *et al.* 2012).

152

153 To clarify the origins of the *D. septosporum* population in Scotland, assess the role of the
154 exotic Corsican and lodgepole pine species in its appearance, and inform management plans
155 for its control, particularly in the Caledonian pinewoods, we initiated a detailed analysis of
156 the genetic structure of the pathogen across its hosts within Scotland. Recent work by Mullet
157 *et al.* (2017), using microsatellite marker analysis of a large sample of *D. septosporum* from
158 across the whole of Britain, has demonstrated that individuals can be assigned to one of three
159 major genetic groups (see Figure 2 in Mullett *et al*., 2017). These comprise: a genetic group
160 with low diversity, present only in Scotland and found predominantly on lodgepole pine
161 (DAPC cluster 1 of Mullett *et al.*, 2017) hereafter referred to as the lodgepole pine race, LPR;
162 a genetic group with a markedly southern distribution found largely on Corsican pine (DAPC
163 clusters 3-7 and 10-12 of Mullett *et al.*, 2017) designated here the southern race, SR; and a
164 Britain wide but predominantly northern genetic grouping loosely associated with Scots pine
165 (DAPC clusters 2, 8 and 9 of Mullett *et al.*, 2017), named here the native pine race, NPR;
166 LPR shows genetic similarities with samples from lodgepole pine in Canada, while SR

clusters genetically with samples from northern France where it is found mainly on Corsican pine (Mullett *et al.*, 2017).

The aim of the present study was firstly to determine the involvement of the three genetic groupings of *D. septosporum* identified by Mullett *et al.* (2017) in the current outbreak of DNB in the native Caledonian pinewoods. We also sought to understand the degree to which the LPR, SR and NPR races are associated with different pine hosts in Scotland, and to determine the geographic distribution of these races. To do this we designed a sampling scheme that explicitly included samples from Caledonian pinewood populations and in which we took population samples from adjacent stands of different hosts so that the effects of host species and geographic location on the frequencies of the races could be determined independently.

Four different categories of host population were recognised in the sampling; Caledonian Scots pine populations; Scots pine plantations; lodgepole pine plantations; and Corsican pine plantations. Replicate sites throughout Scotland containing adjacent stands of these different host population types were identified, and from these sites population samples of *D. septosporum* were isolated. Clustering based on microsatellite data was used to assign individuals to races, assess the distribution of races with respect to host type within each site, and to ascertain the geographic pattern of races among sites. Further samples were obtained from isolated Caledonian pinewood sites and from infected pine nurseries to measure the proportions of *D. septosporum* races present in these situations. Analysis of mating type loci and multilocus microsatellite genotypes was used to infer the reproductive systems of the three races. In addition the races were compared in culture to establish whether they differed

191 significantly for important phenotypic characters. We then developed a scenario, based on
192 our results, to account for the current distribution of *D. septosporum* races in Scotland,
193 highlighting the role played by exotic plantations of Corsican and lodgepole pine, and
194 assessed the likely impact of *D. septosporum* on Caledonian pine populations.

195

Materials and Methods

Sampling

We performed targeted sampling of *Dothistroma septosporum* (Dorog.) Morelet outbreaks identified in disease surveys in naturally regenerated and planted forest stands (Table 1, Figure 2). Classes of sample site and associated sampling strategies were:

i. Mixed plantations of Scots (*P. sylvestris*) and Corsican pine (*P. nigra* subsp. *laricio*). Three sites were sampled in 2015 (Culbin Forest (n=39), Torrs Warren (n=33), and Tentsmuir (n=30)). At each site roughly equal numbers of isolations were made from the two host species.

ii. Caledonian Scots pine sites with adjacent lodgepole pine (*P. contorta*) stands. Three sites were sampled in 2014 (Glen Einig (n=29), Glen Garry (n=35), Inshriach Forest (n=32)) and two in both 2014 and 2015 (Glen Affric (n=21), Dundreggan (n=39)). At each site we made isolations from roughly equal numbers of the two hosts. In addition we collected two lodgepole pine isolates from Strathpeffer in 2015 (n=2).

iii. Caledonian Scots pine sites isolated from exotic pine plantations.
Two sites were sampled in 2015, Glen Tanar (n=15), and Beinn Eighe (n=23).

iv. Forest nursery sites with reported disease outbreaks.
Samples were isolated by Forest Research (Alice Holt) during annual forest nursery DNB surveys between 2011 and 2015. Nursery samples were classified into three categories; southern Scotland (n=24), northern Scotland (n=13) and northern England (n=3). All other information relating to the samples and their location remains confidential.

At each site, we sampled needles bearing conidiomata from 15-40 individuals of the relevant tree species (current year or second year growth needles). At Caledonian pine sites infected needles originated mostly from naturally regenerated saplings, though in some cases mature trees were sampled. A single genotype of *D. septosporum* was obtained from each tree.

Single spore cultures were isolated following the procedure described by Mullet *et al.* (2015), with modifications described in Piotrowska *et al.* (2016). Cultures were stored in three ways as described by Mullett & Barnes (2012); as agar cubes at 4 °C, water storage at 4 °C and 15 % glycerol stocks at -80 °C. In addition to our Scottish collection, Forest Research at Alice Holt provided a single isolate of *D. septosporum* from each of three North American populations of lodgepole pine (Nass Valley, Brown Bear (1 and 7) and Kispiox, Buckley Canyon)) (Table 1).

DNA extraction and genotyping

i. DNA extraction

Fungal mycelium was collected from cultures on agar plates into 2 ml cryovial tubes, freeze-dried overnight (Alpha 1-4 LDplus, Christ, Osterode am Harz, Germany) and tissue-lysed (Tissue Lyser LT; Qiagen, Hilden, Germany) prior to DNA extraction (~ 20 mg of lyophilized tissue). DNA extraction was performed using DNeasy Plant Mini Kit (Qiagen), following the manufacturer's guidelines. DNA for genotyping was re-suspended in sterile distilled water (SDW) and stored at -20 °C for further use.

ii. Mating type assay

Mating type variants for *D. septosporum* were determined using species-specific primer combinations developed by Groenewald *et al.* (2007). The amplification reactions were

carried out using GoTaq Green Master Mix (Promega, Madison, USA). Each reaction comprised 1x Promega Master Mix, 200 nM of each forward (F) and reverse (R) primers, 12.5 ng of DNA and SDW up to 25 µl. The thermocycler (GeneAmp PCR System 9700 thermocycler, Applied Biosystems, Foster City, CA) conditions included initial denaturation at 94 °C for 5 minutes, followed by 36 cycles of denaturation at 95 °C for 20 seconds, annealing at 60 °C for 30 seconds and extension at 72 °C for 40 seconds, and a final extension at 72 °C for 5 minutes. To determine mating type variants, samples were run on 1.2 % agarose gels, and band sizes corresponding to mt-1 and mt-2 were scored manually against the Quick-Load Purple 100 bp DNA Ladder (New England BioLabs, Ipswich, USA). Both positive and negative controls for each mating type were run on every PCR plate.

*iii. Microsatellite scorin*g

To investigate the population structure of *D. septosporum*, we scored 11 microsatellite loci, using primers developed by Barnes *et al.* (2008). Economic fluorescence labelling (Schuelke 2000) was used in all genotyping assays; F primers were tailed at the 5' end with M13 universal primer and M13 primer was labelled with 6-Carboxyfluorescin (6FAM) dye at the 5' end. We grouped microsatellite primers into three multiplex combinations: (i) MixI: Doth_E, Doth_F, Doth_I, Doth_K, M13_FAM; (ii) MixII: Doth_J, Doth_M, Doth_DS1, Doth_DS2, M13_FAM; (iii) MixIII: Doth_G, Doth_L, Doth_O, M13_FAM. The amplification reactions were performed using Multiplex PCR Kit (Qiagen) with the following thermocycler conditions (GeneAmp PCR System 9700 thermocycler): initial denaturation at 95 °C for 15 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 90 seconds, extension at 72 °C for 60 seconds, and final extension at 60 °C for 30 minutes. For MixI and MixII PCR components comprised 1x Master Mix, 0.2 µM of each R and M13 primer, 0.05 µM of each F primer, 12.5 ng of DNA template and

RNase free water (Qiagen) up to a final volume of 25 µl. For MixIII the following modifications of primers' concentrations were used: M13 at 0.2 µM, primer Doth_L at 0.2 µM of R and 0.05 µM of F, primers Doth_G and Doth_O at 0.1 µM of R and 0.025 µM of F. Genotyping reactions were run on the ABI 3730 sequencer (Applied Biosystems) at Edinburgh Genomics (UK) using size standards GS500LIZ (Life Technologies, Thermo Fisher Scientific, Wilmington, USA). Allele sizes were scored in Peak Scanner Software (v 2.0, Applied Biosystems) and binned manually for population genetic analysis.

Population genetic analysis of genotype data

i. Genotypic clustering

In order to infer the number of genetic clusters within Scottish populations of *D. septosporum*, we performed analysis in R studio (v 1.0.136) using the adegenet package (v 2.0.1, Jombart 2008; Jombart & Ahmed 2011). The isolates were assigned to genetic groups using the multivariate Discriminant Analysis of Principal Components method (DAPC, Jombart *et al.*, 2010). This method of clustering was chosen because it makes no assumptions about the mating system of the organisms concerned. The optimal number of clusters was inferred using the *find.cluster* function by computing both BIC (Bayesian Information Criterion) and WSS (within sum of squares) statistics for increasing number of clusters.

ii. Genetic diversity and divergence among and within genetic clusters

All the input files for genetic analysis were prepared in CREATE software (v 1.37, Coombs *et al.* 2008). Percentage of polymorphic loci, number of alleles, number of unique alleles possessed by each cluster, genetic diversity over all loci (H_t), as well as genetic divergence

(θ_{st}) between *D. septosporum* clusters and among populations within these clusters, were calculated using the FSTAT programme (v 2.9.3.2, Goudet 1995, 2002). H_t was calculated according to Nei's (1987) unweighted estimator. Overall genetic differentiation between the clusters, among populations within the clusters, as well as pairwise differentiation between populations was measured using Weir & Cockerham's (1984) estimator of θ_{st}. Significance of θ_{st} was tested with multiple bootstrapping over loci.

iii. Multilocus structure of races

For each cluster the number of multilocus genotypes was found using the program MLGsim (Stenberg *et al*. 2003). The program was also used to estimate which multilocus genotypes represented multiple times have a low probability (P<0.05) of being the product of sexual reproduction. These genotypes were then treated as clonal replicates to generate a clone-corrected data set. Allele frequencies used in the MLGsim simulation were those estimated in the complete dataset (prior to removal of clonal genotypes).

Analysis of multilocus structuring of the races was conducted in three ways. In the first analysis, the program Multilocus 1.3 Beta (Agapow & Burt 2001) was used to estimate the index of association among loci (I_A) and mean correlation among loci (r_D), with significance estimated using 1000 randomisations. In the second analysis, the proportion of locus pairs showing significant association (Weir 1996) was determined in FSTAT with allele permutations. In the third analysis the extent of genetic differentiation between the two populations of opposite mating type within a race (θ_{mt}) was computed using FSTAT. If sexual reproduction is prevalent, there will be no significant genetic differentiation between

populations of opposite mating type (Ennos & Hu *in prep.*). Analyses were conducted both on the original and on the clone-corrected data sets. Additionally, the extent of genetic differentiation (θ_{mt}) between the clone-corrected mt-1 population in the SR race and the clone-corrected mt-1 population in the NPR race was calculated in FSTAT.

Analysis of growth rate and exudate production

To investigate possible genetically determined phenotypic differences between *D. septosporum* clusters identified with genetic markers and between populations within these clusters, we examined the rate of fungal colony growth and exudate production *in vitro*. We randomly selected five individuals from three populations within each of the three *D. septosporum* races identified above. Initial cultures were grown on Dothistroma sporulating medium (DSM), (Bradshaw *et al*. 2000) at 20 °C at 12 h dark/12 h light mode (Gallenkamp, INF 780C, Weiss Technik Konigswinter, Germany). Mycelial plugs of 8 mm diameter were excised from the colony and sub-cultured onto fresh DSM media. Samples were incubated for a further 8 weeks, in 24 hour darkness. In total we used 6 growth cabinets (3- MIR-254 incubator; Sanyo, Osaka, Japan and 3- Gallenkamp, INF 780C), 3 temperatures (10 °C, 17.5 °C, 22.5 °C) and 2 technical replicates for each temperature, resulting in a total number of n=270 observations. Growth of isolates was measured as increase in the colony radius (mm) from week 0 to week 8.

In culture *D. septosporum* produces the exudate dothistromin which is known to be a virulence factor in DNB (Kabir *et al.*, 2015). Exudate production of isolates was scored at week 8 according to the degree to which it discoloured the growth medium using a four point

scale: no exudate=0, low=1, medium=2, high=3 (Figure S1). Statistical analysis of growth rate and exudate production was performed in Minitab v. 17 (Minitab Inc., State College, PA). Data were analysed in a split plot ANOVA framework with individual incubators as plots. The analysis was used to infer the significance of the following factors: race (fixed), temperature (fixed) and their interaction; population (nested within race, random effect) and isolate (nested within isolates and populations).

Results

Microsatellite genotyping and genotypic clustering

A total of n=338 isolates were successfully scored for both mating type and the 11 SSR loci (Table 1). All of the SSR loci were polymorphic, but total number of alleles per locus varied from 3 at locus O to 37 at the hypervariable M locus (Table S1). Clustering of the multilocus genotypes using DAPC analysis in the adegenet package inferred three discrete genetic clusters within the total *D. septosporum* population in Scotland (Figure 1).

Host and geographic distributions of genetic clusters

The highly genetically divergent cluster of *D. septosporum* isolates revealed by the DAPC analysis was isolated only from lodgepole pine (Table 1, Figure 2). This was true even at sites where adjacent stands of Caledonian Scots pine had been sampled and in nursery collections where isolates had also been made from both Scots and Corsican pine. This cluster will hereafter be referred to as the lodgepole pine race of *D. septosporum* (LPR). We found LPR in all sites where isolations were made from lodgepole pine with the exception of the northern Caledonian pinewood site at Glen Einig. In some locations, such as Glen Affric and Glen Garry, LPR was the predominant race on lodgepole pine, whereas in others, such as Dundreggan, it comprised a small proportion of lodgepole pine infections (Table 1, Figure 2).

In contrast to LPR, the remaining two genetic clusters distinguished by the DAPC analysis were found on all three host pine species. However they were very distinct in their geographic distributions. The first of these clusters (n= 92) predominated in samples from more southern locations, and its frequency declined towards the north of Scotland (Table 1,

Figure 2). In recognition of its distribution, this genetic cluster will be referred to as the southern race (SR) of *D. septosporum*. In the combined nursery samples from southern Scotland and northern England, 26 of the 27 isolates were from SR, while only 3 of 13 isolates from northern Scottish nurseries belonged to this race. In the mixed Scots and Corsican pine plantations the frequency of SR declined from south to north; from 88% in Torrs Warren through 47% in Tentsmuir to 26% in Culbin Forest. SR was not isolated from Caledonian Scots pine trees except in the most easterly population at Glen Tanar where it was present at a frequency of 33%. In more westerly and northerly Caledonian pine sites, SR was either absent or present at a very low frequency (total 4 isolates) on lodgepole rather than Scots pine. Where it occurred at intermediate frequency in mixed plantations of Scots and Corsican pine e.g. at Tentsmuir, SR was significantly associated with Corsican pine (χ^2=6.47, d.f. =1, P<0.05).

The third genetic cluster recognised by the DAPC analysis (n=209) was strongly associated with Caledonian Scots pine (Table 1, Figure 2). Of the 130 isolations made from Caledonian pine trees, 125 belonged to this cluster, which will henceforth be referred to as the native pine race (NPR). As well as being present on Caledonian Scots pine, NPR was also isolated from adjacent stands of lodgepole pine and was the main race present in northern Scottish nursery samples. In mixed stands of Scots and Corsican pine NPR predominated in the north at Culbin Forest, but its frequency declined to the south (Figure 2). Where present at intermediate frequency at Tentsmuir it was preferentially found on Scots pine (χ^2=6.47, d.f. =1, P<0.05).

Genetic diversity and divergence among and within D. septosporum races

LPR was the least diverse of the three *D. septosporum* races (Table 2). It was characterised by very low allelic richness *A*=1.55 +/- 0.16, low gene diversity H_t=0.041 and relatively low percentage of polymorphic loci, 55%. It had the smallest total number of alleles n=17, although 7 of these were private alleles distributed over 6 loci. Three of these alleles (E-243, G-195, I-319) were shared with the three individuals sampled from North American populations (Table 1).

In contrast, SR was characterised by the highest level of genetic variation (Table 2). All of its loci were polymorphic, with 68 alleles in total, 21 of which were unique and present across 7 loci. SR displayed both high allelic richness *A*=5.50 +/- 1.25 and high gene diversity H_t=0.550. Allelic richness of SR was significantly higher in the population occupying plantations (*A*=3.75) than on Caledonian pine (*A*=2.64) (*P*=0.015).

The NPR race was intermediate in genetic diversity between LPR and SR (Table 2). 91% of loci were polymorphic and total number of alleles (n=68) and allelic richness *A*=4.23 +/- 1.60 were high. However gene diversity H_t=0.182 was much lower than in SR because allelic diversity was present predominantly at a single hypervariable locus (M). NPR showed the highest number of unique alleles (n=22) with 16 of these found at the hypervariable locus M (Table S1). Although mean allelic richness was greater on Caledonian pine (*A*=4.45) than on plantation pines (*A*=3.91) this difference was not significant (*P*=0.348).

The three *D. septosporum* races were strongly genetically differentiated from each other with overall θ_{st}=0.558, *P*<0.01. LPR diverged the most from the other two races, showing pairwise

411 θ_{st}=0.8037 (P<0.05) with NPR and θ_{st}=0.5119 (P<0.05) with SR. The other two races, NPR
412 and SR, were genetically closer, with a moderate but still significant level of divergence
413 θ_{st}=0.3997 (P<0.05).
414
415 Within the LPR race, there was no significant genetic differentiation (θ_{st}=0.075, P>0.05)
416 among the major populations scored (Inshriach Forest, Glen Garry, Dundreggan, Glen
417 Affric). For SR there was low but significant genetic differentiation (θ_{st}=0.082, P<0.01)
418 among the plantation and nursery populations (Torrs Warren, Tentsmuir, Culbin Forest,
419 Southern Scotland nursery) (Table S2). SR also showed significant genetic differentiation
420 between Caledonian pine and plantation populations (θ_{st}=0.101, P<0.01). In NPR there was
421 low but significant genetic divergence among Caledonian pine populations (θ_{st}=0.071,
422 P<0.01) but much higher genetic differentiation among plantation populations θ_{st}=0.318
423 (P<0.01) (Table S3). Mean pairwise genetic differentiation between Caledonian pine
424 populations and the northernmost Culbin Forest plantation population was low (θ_{st}=0.040),
425 while mean pairwise differentiation between Caledonian pine populations and the two
426 plantation populations located further south was much higher (Tentsmuir θ_{st}=0.147, Torrs
427 Warren θ_{st}=0.656) (Table S3).
428
429 *Multilocus structure and mating type variation of races*
430 LPR comprised only 5 multilocus genotypes (MLGs), among which one was a clonal MLG
431 (Table 3). All of the individuals within this race were of one mating type variant, mt-2. There
432 was high and significant linkage disequilibrium among loci, in both the original (I_A=0.842,
433 r_D=0.176, P=0.003) and clone-corrected datasets (I_A=0.576, r_D=0.124, P=0.015), as expected
434 if LPR reproduces asexually.

435

436 SR was characterised by the highest number of MLGs n=58, 11 of which were clonal MLGs
437 (Table 3). Mating type variants, mt-1 and mt-2, were present in a 1:1 ratio (χ^2=0.043, d.f.=1,
438 P=0.835), which suggests the possibility of sexual reproduction within SR populations. There
439 was however significant linkage disequilibrium among loci present in the original (I_A=0.663,
440 r_D=0.067, P<0.001) and clone-corrected datasets (I_A=0.322, r_D=0.033, P<0.001). We also
441 found a significant amount of genetic divergence between the two mating type populations
442 (original θ_{mt}=0.107, P<0.01; clone-corrected θ_{mt}=0.022, P<0.05), rejecting the hypothesis of
443 random mating within SR populations. The results are compatible with SR being
444 predominantly asexual with low levels of sexual reproduction.

445

446 A moderate number of MLGs n=51 were present in the NPR population with a relatively
447 small number of clonal MLGs (n=5) (Table 3). Within NPR multilocus genotypes were often
448 distinguished by allelic differences at a single hypervariable locus. Linkage disequilibrium
449 was high and significant in both the original (I_A=2.037, r_D=0.245, P<0.001) and clone-
450 corrected datasets (I_A=2.203, r_D=0.261, P<0.001). Although both mating type variants (mt-1
451 and mt-2) were present in NPR populations, the frequency of individuals bearing the mt-1
452 allele was limited to only 3% of the population. Genetic differentiation was high and
453 significant between the two mating type populations within NPR (original θ_{mt}=0.378, P<0.01;
454 clone-corrected θ_{mt}=0.396, P<0.01). The mating system of NPR is therefore likely to be
455 predominantly asexual. Genotypes possessing the mt-1 variant were noticeably more
456 genetically variable than those possessing mt-2 (mt-1 Ht=0.507, mt-2 Ht=0.153, P=0.002).

457

458 *Growth rate and exudate production of races at different temperatures*

We found significant differences in mycelial growth rate among the races ($F_{2,6}$ = 20.40, P=0.002), and significant interaction between race and temperature for this character ($F_{4,12}$ = 13.31, P<0.001) (Table S4) (Figure 3**a**). This interaction remained significant even when the LPR isolates were removed from the analysis ($F_{2,56}$ = 5.93, P=0.005). The three *D. septosporum* races showed different temperature optima for growth. LPR exhibited the slowest growth at all three temperatures tested, NPR was the fastest growing race at 10 °C and 22.5 °C, while SR exhibited the fastest growth at 17.5 °C.

There were also significant differences in exudate production among races ($F_{2,6}$=11.94, P=0.008) and a significant race x temperature interaction ($F_{4,12}$ = 5.61, P=0.009) (Table S4). LPR produced the least exudate at all three temperatures, NPR produced the most exudate at 10 °C, and SR at the remaining two temperature points, 17.5 °C and 22.5 °C (Figure 3**b**).

Discussion

Our molecular analysis of the *D. septosporum* population in Scotland was able to assign individuals to the three major genetic groups previously recognised by Mullett *et al.* (2017) in their Britain wide study. In agreement with Mullett *et al.* (2017) we found that the first of these groups, LPR, possesses very low genetic variability and is completely asexually reproducing. In addition our analysis of mixed sites provided no evidence of LPR on trees other than lodgepole pine, implying a high degree of host adaptation. In contrast we found the second race SR to be highly genetically variable with the potential for sexual reproduction, again in line with the results of Mullett *et al.* (2017). Our results further show that within Scotland SR is mainly southern in distribution, occurs on all three pine species, but is preferentially found on Corsican rather than Scots pine where adjacent stands of these two hosts occur, implying a modest degree of host specialisation. Finally we have shown that NPR, the third major genetic grouping distinguished by Mullett *et al*. (2017), is the predominant race on Caledonian pine, and preferentially infects Scots pine when present in mixture with Corsican pine. In our purely Scottish sample, NPR possesses intermediate levels of variability and reproduces largely asexually.

Besides confirming and documenting the host and geographic distributions within Scotland of the genetic groups recognised by Mullett *et al.* (2017), we have also shown that LPR, SR and NPR are highly significantly different both in their growth rate response to temperature and their level of production of the exudate dothistromin, implicated as a virulence factor in DNB (Kabir *et al.*, 2015). This provides strong evidence that these groupings represent important biological, ecological and evolutionary units within *D. septosporum*, and that it is therefore appropriate to regard them as distinct races within the species.

The very low genetic and clonal variability of LPR strongly suggests that it derives from recent introduction of a limited number of isolates. However despite low variability, LPR harbours 7 alleles that are absent from SR and NPR, three of which were detected in the limited sample of North American isolates that were genotyped. These observations support the hypothesis of Mullett *et al.* (2017) that LPR has been introduced into Scotland on needle debris accompanying lodgepole pine seed imports from North America. Although seed importation is generally considered a low biosecurity risk, there are at least two other examples where it has led to transfer of important pine pathogens between North America and Europe (*Lecanosticta acicola* (Janousek *et al.* 2015); *Gremmeniella abietina* (Hamelin *et al.* 1998)).

SR is the most genetically variable of the three races implying a large effective population size as expected in an epidemic population. Equality of mating type frequencies and low (though significant) correlation among markers together with limited differentiation across mating types suggests that SR may practice a low frequency of sexual reproduction. Therefore some long distance dispersal by ascospores could occur in this race. Given the preference of SR for Corsican pine, a predominantly southern distribution, and near absence from Caledonian pine, SR is likely to have originated via recent dispersal from the epidemic of DNB on Corsican pine in England. Transfer of SR to northern plantations in Scotland via infected nursery stock is suggested firstly by the prevalence of SR on nursery material from south Scotland. In addition the genetic composition of the SR population found on southern nursery samples is very similar to that at Tentsmuir and Culbin Forest (low genetic

519 differentiation), a result that would be expected if infected nursery stock from southern
520 nurseries was the source of SR at these northern sites.

521

522 The third and most enigmatic of the *D. septosporum* races, NPR, is strongly associated with
523 Caledonian pine in our study (125 out of 130 isolates derived from Caledonian pine). NPR
524 was also isolated from lodgepole pine adjacent to Caledonian pine stands and was
525 predominant in northern Scottish nurseries. In plantations the frequency of NPR declined
526 from north to south and data from Mullett *et al.* (2017) indicates that the equivalent 'northern
527 Scottish group' is infrequent south of the Scottish border. A plausible hypothesis to account
528 for this host and geographic distribution is that NPR is a northern race of *D. septosporum*
529 endemic on Caledonian pine, that has recently spread to adjacent lodgepole pine stands,
530 plantations of Corsican pine in northern Scotland, and pine nurseries in the same geographic
531 area.

532

533 Genetic support for the hypothesis that NPR represents an endemic race is equivocal.
534 Although allelic richness is relatively high (due to hypervariability at a single locus) overall
535 gene diversity is low. The ratio of mating types in NPR is very highly skewed and there is
536 strong allelic correlation among markers and genetic differentiation between mating types, all
537 suggesting that NPR reproduces asexually. Neither low genetic diversity nor asexual
538 reproduction are generally considered to be attributes of endemic populations. On the other
539 hand NPR possesses 22 alleles that are not found in the most closely related race SR. Such
540 race specific alleles can only have accumulated by mutation if the races have been isolated
541 for a considerable number of generations.

542

543 To reconcile these results we hypothesise that *D. septosporum* arrived in Scotland with pine
544 populations colonising from continental Europe after the last glaciation (Sinclair *et al.*, 1998).
545 Following isolation of the Caledonian pine populations from their continental counterparts
546 about 8Kybp, we propose that the *D. septosporum* population went through a population
547 bottleneck, leading to loss of genetic variation. We further propose that the population was
548 selected for reproductive assurance under conditions at the range edge that were unfavourable
549 for sexual reproduction, leading to evolution of a predominantly asexual breeding system.
550 Where environmental factors hinder outcrossing sexual reproduction, mating system
551 transitions to uniparental reproduction are known to have taken place in a wide range of
552 plants and animals (Avise 2015; Holsinger 2000) and are equally likely to have occurred in
553 fungi (Taylor *et al.* 2015).

554

555 If *D. septosporum* has been endemic in Caledonian pine populations it appears curious that its
556 presence was not noted before 2011 (Brown *et al.*, 2012). One explanation may be that the
557 proposed endemic race NPR, which apparently causes little damage to needles, has been
558 overlooked, with attention being focussed on more damaging needle pathogens such as
559 *Lophodermium seditiosum* (Minter & Millar, 1980). Another explanation may be that
560 populations have recently increased in size due to more favourable environmental conditions
561 for *D. septosporum*. This would be consistent with a rise in annual temperature of 0.75^{o}C,
562 and an increase of 23% in annual rainfall in Scotland since 1970 (Met Office, 2017).
563 Circumstantial evidence that *D. septosporum* has had a long term endemic presence in the
564 Caledonian pine populations comes from studies of geographic variation in their DNB
565 susceptibility. Pine populations from areas of high rainfall, where conditions are most

566 favourable to *D. septosporum*, show significantly lower susceptibility than those in low
567 rainfall areas, the pattern expected under long term co-evolution (Perry *et al.* 2016b).

568

569 Drawing together the information outlined above and that from Mullett *et al.* (2017), we can
570 put forward a tentative scenario to account for the current situation in Scotland. Prior to
571 planting of exotic conifers in Britain, we suggest that NPR was present both as a co-evolved
572 endemic pathogen causing minimal damage in Caledonian pinewoods and on other Scots pine
573 populations in Scotland. From the 1930s Corsican pine was widely planted in England and
574 more locally in Scotland. In the 1950s the SR race was introduced into southern England
575 probably from France (Mullett *et al.* 2017), possibly aided by transfer of diseased nursery
576 stock. In the 1990s SR spread to genetically susceptible high density Corsican pine stands in
577 East Anglia and expanded massively to create a DNB epidemic made particularly serious by
578 a succession of unusually wet and warm summers (Brown & Webber 2008). High disease
579 pressure may have facilitated limited adaptation to adjacent Scots pine, which was previously
580 immune to attack (Murray & Batko 1962). From these footholds SR spread northwards
581 through England and into Scotland in the 2000s via the stepping stones of vulnerable
582 Corsican pine and to a lesser extent Scots pine plantations. SR has now established an outlier
583 population in the most easterly Caledonian pine site (Glen Tanar) via natural dispersal, and in
584 more northerly Caledonian pine sites by movement of infected lodgepole pine. The derived
585 nature of SR in Caledonian pine sites is supported by its lower genetic diversity compared to
586 that found on plantation pines.

587

588 Concurrent with northward movement of SR, the major Corsican pine plantation in northern
589 Scotland (Culbin Forest) appears to have become infected by the endemic NPR race of *D.*

590 *septosporum* dispersed from Caledonian pine populations present in the region, with
591 subsequent spread to plantations further south. This direction of transfer is supported by the
592 low differentiation between the NPR population on Caledonian pine and that at Culbin
593 Forest, and increasing differentiation between Caledonian pine populations and plantation
594 populations to the south. Transfer via asexual spores may have been augmented by planting
595 of stock infected with NPR from northern Scottish nurseries. Meanwhile lodgepole pine
596 plantations established in the vicinity of Caledonian pinewoods have become infected by
597 NPR, but this has done little damage. However inadvertent introduction of the lodgepole pine
598 adapted LPR race from North America has led to devastating DNB outbreaks mainly on the
599 *latifolia* subspecies of lodgepole pine (Brown & Webber 2008).

600

601 Our scenario implies that the planting of related exotic species has significantly altered the
602 biodiversity threat posed by *D. septosporum* to Caledonian pine. The presence of susceptible
603 Corsican pine throughout Britain has facilitated the introduction of race SR from continental
604 Europe, while planted lodgepole pine has brought with it a second exotic race of *D.*
605 *septosporum* LPR from North America. The two exotic races are now sympatric with race
606 NPR which prior to their arrival was not causing any significant damage. What are the risks
607 posed by these novel introductions?

608

609 Analysis of the outcome of natural inoculations by the SR race present at Torrs Warren
610 showed high genetic variation for susceptibility both within and among populations of
611 Caledonian pine (Perry *et al.* 2016b). Thus although SR is likely to cause some damage to
612 Caledonian pine populations, this could be mitigated if populations are able to naturally
613 regenerate, allowing evolution of greater resistance to SR by natural selection (Cavers &

614 Cottrell 2015). Our isolation results provide no evidence that LPR has established on
615 Caledonian pine. Therefore if lodgepole pine is removed from the vicinity of native
616 Caledonian pine, the impact of LPR may be minimal. However Mullet *et al.* (2017) did
617 record rare presence of the 'lodgepole population' on Scots pine, so the possibility of host
618 switching onto Caledonian pine cannot be discounted.

619

620 Our conclusion that introduction of SR and LPR into Caledonian pine populations will have
621 limited impact are conditional on a lack of genetic interaction among the three races of *D.*
622 *septosporum* now present. In this respect we note that introduction of SR brings with it a high
623 frequency of isolates carrying the mt-1 allele which is absent or at very low frequency in the
624 other two races. If the races are sexually compatible, this opens up the possibility for
625 hybridisation between them and rapid evolution of either a new hybrid race (Brasier *et al.*
626 2001, 2004), or introgression of important genetic attributes among the races (Paoletti *et al.*
627 2006). In either circumstance, the virulence of the races may be increased, causing more
628 serious damage to the Caledonian pine populations (Stukenbrock 2016).

629

630 Tentative evidence for hybridisation between NPR and SR comes from an analysis of the
631 small number of NPR isolates from Caledonian pine that carry the mt-1 allele. Genetic
632 differentiation of these isolates from mt-2 isolates of the NPR mating type is much larger (θ_{st}
633 = 0.369, P<0.01) than from mt-1 isolates in the SR race (θ_{st} = 0.048, n.s.). A possible
634 explanation is that the mt-1 mating types within NPR are actually hybrids between the SR
635 and NPR races. Analysis of the genomic constitution of these isolates, now underway, will
636 provide further evidence for or against this hypothesis (Stukenbrock 2016).

637

638 Possible steps to reduce spread of SR into Caledonian pine include a moratorium on
639 movement of nursery stock of either Scots or lodgepole pine into Caledonian pinewood sites,
640 and removal of plantations of either Scots, lodgepole or Corsican pine from areas within the
641 range of sexual spore dispersal. If the SR race nevertheless becomes established within
642 Caledonian pine populations, control of DNB would be best achieved by promoting
643 management of the pinewoods for natural regeneration (Cavers & Cottrell 2015) to maximise
644 the opportunity for greater resistance to evolve (Perry *et al.* 2016a, b).

645

646 Overall our results demonstrate the power of adopting a forensic forest pathology approach in
647 which population genetic analysis of molecular markers is used to unravel the origins and
648 subsequent evolution of emerging forest pathogens. The approach has already provided
649 previously inaccessible information on the introduction pathways and subsequent behaviour
650 of forest pathogens in genera such as *Heterobasidion* (Garbelotto *et al.* 2013), *Cryphonectria*
651 (Dulech *et al.* 2010) and *Hymenoschyphus* (Gross *et al.* 2014). Crucially this approach can
652 identify previously cryptic but evolutionarily important units within recognised
653 morphological taxa which may possess very different ecological attributes (Perez *et al.* 2012).
654 Understanding these ecological differences may be key to explaining the epidemiology of the
655 associated disease.

656

657 From a wider forest policy viewpoint our analysis of DNB in Scotland provides a clear
658 illustration of the dangers of establishing plantations of exotic tree taxa that are related to and
659 share pathogens with native tree species (Gilbert & Webb 2007; Gilbert *et al.* 2012; Burgess

& Wingfield 2017). Establishment of exotic Corsican and lodgepole pine has led to two separate and economically damaging epidemics of DNB on exotic plantations, caused by two new races of DNB that accompanied their exotic hosts. In addition the presence of these exotic pathogen races has increased the biosecurity threat to the iconic Caledonian pine populations. Our results are highly relevant to recent debate over the merits of introducing exotic species to increase the diversity and resilience of ecosystems (Schlaepfer *et al.* 2011; Vitule *et al.* 2012). Our overall conclusion, based on the outcome of a large scale though unplanned historical experiment, is that planting exotic trees related to native species is likely to decrease rather than increase the resilience of forest ecosystems to disease.

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Author Contributions

MJP, CR, PNH and RAE were all involved in the design and performance of the research together with data collection, analysis and interpretation. MJP and RAE wrote the manuscript with significant input from CR and PNH.

Data Accessibility

Data for this study are available at: (to be completed after acceptance of the manuscript)

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878

879 Table 1. Sampling sites, their locations and number of trees from which *Dothistroma septosporum* isolations
880 were made. Number of isolates of each genetic group identified by Mullett *et al.* (2017) and designated
881 lodgepole pine race (LPR), southern race (SR) and native pine race (NPR) are shown in the format
882 (LPR:SR:NPR).

(i) Mixed plantations of Scots pines and Corsican pines

Site	Year of collection	Total no of isolates	No of Scots pines	No of Corsican pines	Lat/Long
Culbin Forest	2015	39	20 (0:3:17)	19 (0:7:12)	57.632986 -3.6792442
Torrs Warren	2015	33	18 (0:17:1)	15 (0:12:3)	54.865287 -4.8919655
Tentsmuir	2015	30	16 (0:4:12)	14 (0:10:4)	56.413737 -2.8109709

(ii) Caledonian Scots pine sites with adjacent lodgepole pine stands

Site	Year of collection	Total no of isolates	No of Scots pines	No of lodgepole pines	Lat/Long
Glen Einig	2014	29	23 (0:0:23)	6 (0:0:6)	57.957361 -4.7397091
Glen Garry	2014	35	20 (0:0:20)	15 (11:0:4)	57.051089 -4.9790647
Inshriach Forest	2014	32	13 (0:0:13)	19 (10:2:7)	57.097124 -3.9313490
Glen Affric	2014 2015	21	7 (0:0:7)	14 (13:0:1)	57.292428 -4.8939597
Dundreggan	2014 2015	39	29 (0:0:29)	10 (2:2:6)	57.196282 -4.7359641
Strathpeffer	2015	2	0	2 (0:1:1)	57.621310 -4.5325429

(iii) Isolated Caledonian Scots pine forests

Site	Year of collection	No of Scots pines	Lat/Long
Glen Tanar	2015	15 (0:5:10)	57.043444 -2.8552516
Beinn Eighe	2015	23 (0:0:23)	57.629890 -5.3512581

(iv) Forest nursery sites

Site	Year of collection	Total no of isolates
Southern Scotland	2011-2015	24 (0:23:1)
Northern Scotland	2011-2015	13 (1:3:9)
Northern England	2011-2015	3 (0:3:0)

(v) Northern American samples

Site	No of lodgepole pines
Nass Valley, Brown Bear, British Columbia, Canada	2

Kispiox, Buckley Canyon, British Columbia, Canada	1	

Table 2. Genetic diversity measured at 11 microsatellite loci within three *Dothistroma septosporum* races in Scotland.

Race	LPR (n=37)	SR (n=92)	NPR (n=209)
Gene diversity H_t	0.041	0.550	0.182
% Polymorphic loci	55%	100%	91%
Total number of alleles	17	68	68
Allelic richness *A*	1.55 +/- 0.16	5.50 +/- 1.25	4.23 +/- 1.60
Number of unique alleles	7 (over 6 loci)	21 (over 7 loci)	22 (over 4 loci)

888 Table 3. Multilocus structure of *D. septosporum* races in Scotland.

Race	LPR	LPRcc[a]	SR	SRcc[a]	NPR	NPRcc[a]
mt-1:mt-2	0:37	0:36	45:47	26:33	7:202	7:195
Total no MLGs[b]		5		58		51
No clonal MLGs[b]		1		11		5
I_A[c]	0.842**[d]	0.575*	0.663***	0.323***	2.037***	2.203***
r_D[e]	0.176**	0.124*	0.067***	0.033***	0.245***	0.261***
% pairwise l.d.[f]	0	0	46	9	38	27
Θ_{mt}[g]	-	-	0.107**	0.022*	0.378***	0.396**

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890 [a] clone-corrected data set

891 [b] MLG-multilocus genotype

892 [c] I_A- index of association among loci

893 [d] Significance of deviations from expectations under purely sexual reproduction are indicated

894 (*P<0.05, **P<0.01, ***P<0.001)

895 [e] r_D- mean correlation among loci

896 [f] l.d.- linkage disequilibrium

897 [g] Θ_{mt}- genetic differentiation between mating types within races

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908 Figure 1. Plot of scores on first two axes from DAPC analysis (Jombart *et al.*, 2010), based
909 on variation at 11 microsatellite loci, for *Dothistroma septosporum* isolates from Scotland.
910 Races LPR (red), SR (orange) and NPR (blue) are indicated.

911

912 Figure 2. Distribution of *Dothistroma septosporum* races (LPR (red), SR (orange) and NPR
913 (blue)) **a**). on Scots (S) and lodgepole (L) pine at Caledonian pine sites, illustrating data from
914 Table 1, sections (ii) and (iii); **b**). on Scots (S) and Corsican (C) pine in mixed plantations
915 and on pines in northern and southern Scottish nurseries (N), illustrating data from Table1,
916 sections (i) and (iv).

917

918 Figure 3. Variation in **a**). growth rate and **b**). exudate production at three temperatures in
919 three races (LPR, SR and NPR) of *Dothistroma septosporum* from Scotland.

Supplementary Materials for Piotrowska *et al.*

Table S1. The range of allele sizes, number of alleles and allelic richness at 11 microsatellite loci (Barnes *et al.*, 2008) in Scottish populations of *Dothistroma septosporum.*

Locus	Allele range (bp)[a]	Number of alleles across all three races	Allelic richness in LPR[b]	Allelic richness in SR[b]	Allelic richness in NPR[b]
E	231-278	6	2.000	4.98	1.907
F	192-208	4	1.000	2.000	2.686
G	195-205	4	1.000	3.000	2.230
I	319-328	4	2.000	2.957	2.680
J	203-223	7	2.000	5.518	2.773
K	355-438	8	1.000	7.283	2.762
L	339-459	14	2.000	8.215	5.401
M	233-579	37	2.000	16.38	19.85
O	215-224	3	1.000	1.998	1.000
DS1	165-175	5	2.000	2.985	3.731
DS2	388-418	6	1.000	5.120	1.543

[a] all allele sizes include M13 tail length

[b] based on min. sample size of 37 individuals.

925 Table S2. Pairwise genetic differentiation (θ_{st}) between SR populations of *Dothistroma*
926 *septosporum*.

	Torrs Warren	Tentsmuir	Culbin Forest	Southern Scottish Nursery
Caledonian pinewoods	0.1466	0.1134	0.0954	0.1293
Torrs Warren		0.0613	0.0722	0.1091
Tentsmuir			-0.0055	-0.0085
Culbin Forest				0.0559

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930 Table S3. Pairwise genetic differentiation (θ_{st}) between NPR populations of *Dothistroma*

931 *septosporum*.

	Inshriach Forest	Glen Garry	Dundreggan	Glen Affric	Beinn Eighe	Glen Tanar	Torrs Warren	Tentsmuir	Culbin Forest	Northern Scottish Nursery
Glen Einig	0.023	0.074	0.053	0.017	0.079	0.035	0.708	0.139	0.018	0.011
Inshriach Forest		0.087	0.067	0.038	0.060	-0.017	0.623	0.115	0.028	0.009
Glen Garry			0.126	0.056	0.129	0.070	0.585	0.141	0.084	0.051
Dundreggan				0.161	0.127	0.112	0.801	0.218	0.025	0.035
Glen Affric					0.064	0.020	0.638	0.127	0.025	0.027
Beinn Eighe						0.108	0.681	0.191	0.055	0.055
Glen Tanar							0.554	0.099	0.044	0.005
Torrs Warren								0.598	0.709	0.603
Tentsmuir									0.158	0.077
Culbin Forest										-0.015

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Table S4. Analysis of variance of growth rate and exudate production for three races of *Dothistroma septosporum* grown at three temperatures. *P<0.05, **P<0.01, ***P<0.001.

Source of Variation	df	MS Growth rate	MS Exudate production
Temperature	2	487.83*	15.03**
Incubator (Temp)	3	30.83***	0.48
Race	2	475.07**	9.73**
Population (Race)	6	23.30	0.82
Isolate (Race Pop)	36	20.13***	1.32***
Temp x Race	4	111.09***	2.73**
Temp x Pop (Race)	12	8.35*	0.49
Temp x Iso (Race Pop)	72	4.37**	0.46*
Error	127	2.52	0.30

948 Figure S1. Exudate production scoring scale for *Dothistroma septosporum*

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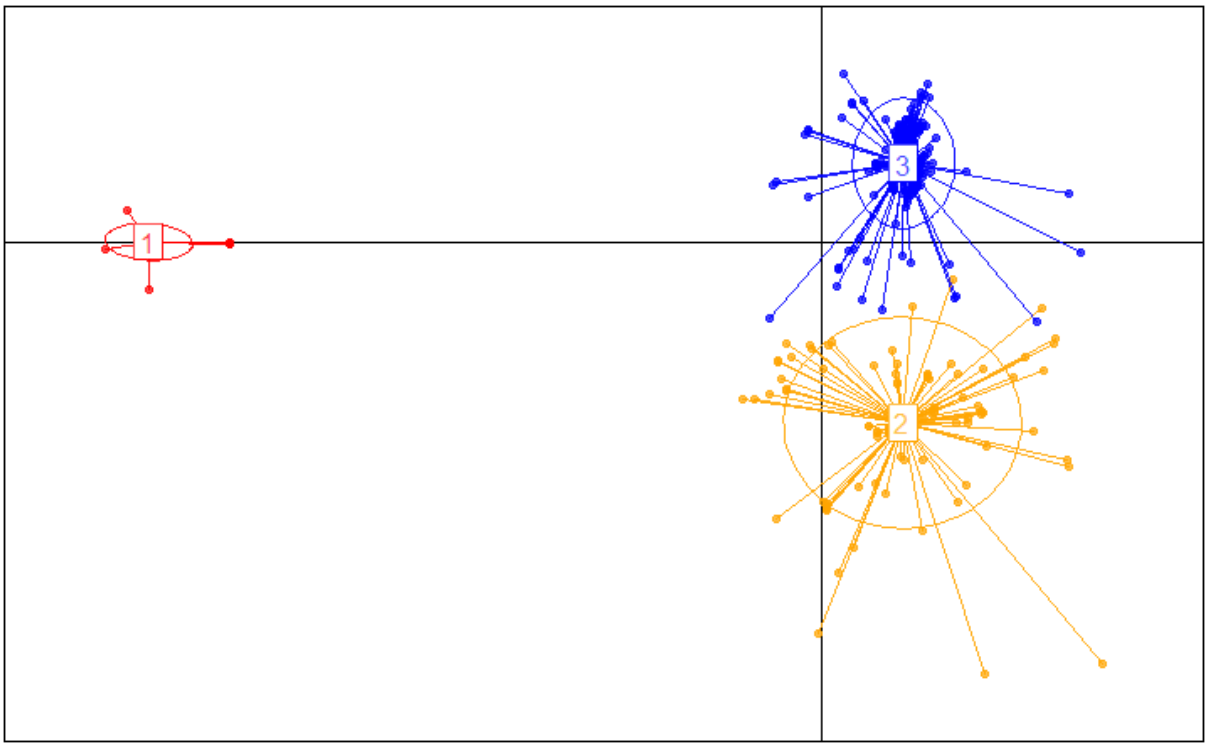

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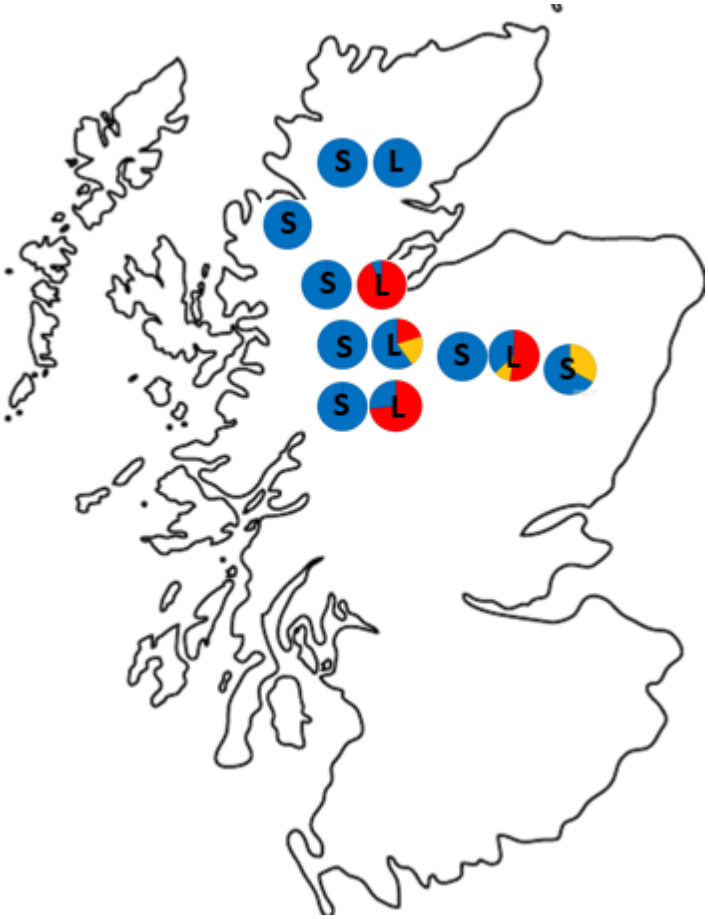

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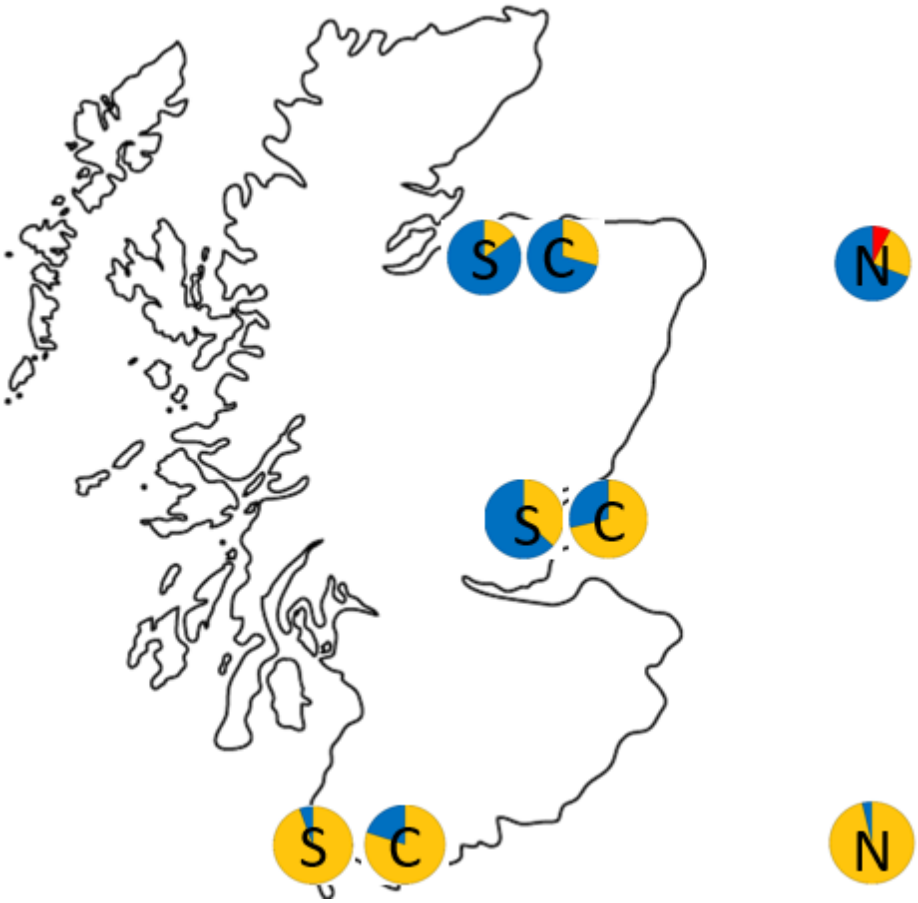

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956 Figure 2b

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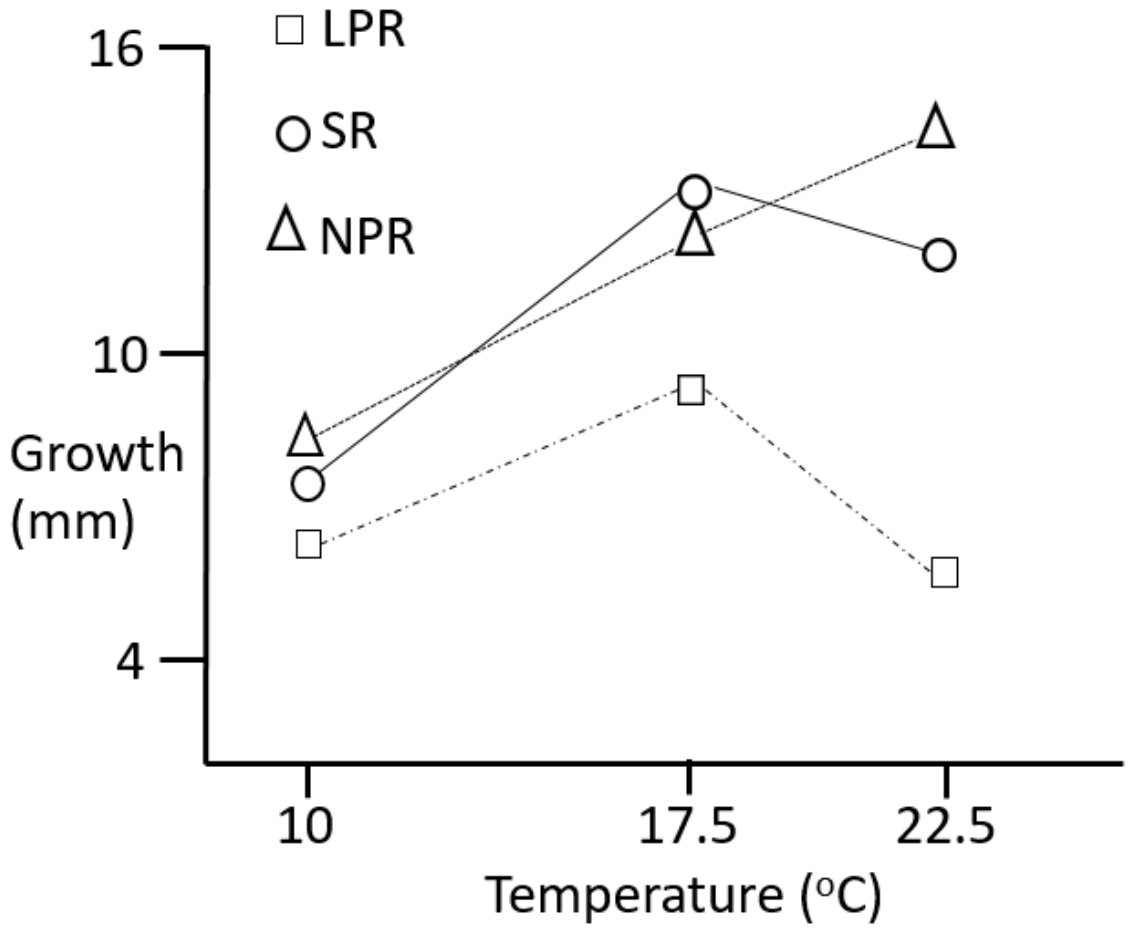

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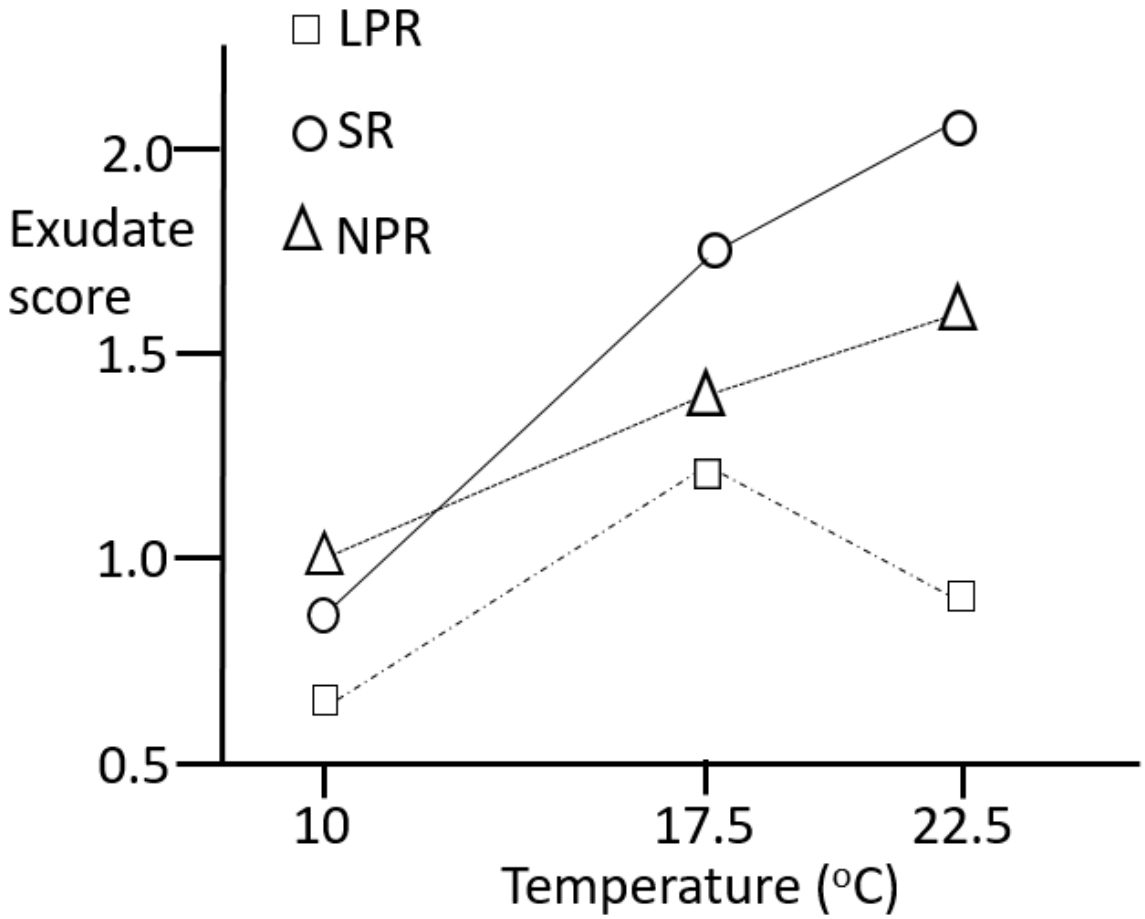
959 Figure 3a



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961 Figure 3b

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