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Adaptation of subpopulations of the Norway spruce needle endophyte *Lophodermium piceae* to the temperature regime

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ABSTRACT

Lophodermium piceae represents the most common Norway spruce needle endophyte. The aim of this study was to find out whether subpopulations of *L. piceae* in climatically different environments (in which Norway spruce occurs natively) are adapted to local thermal conditions. *L. piceae*'s ability for thermal adaptation was investigated by determining growth rates of 163 isolates *in vitro* at four different temperatures: 2, 6, 20 and 25 °C. Isolates were obtained between 1995 and 2010 from apparently healthy needles sampled in Finland, Poland, Switzerland, Italy and southeastern Siberia. The sampling sites represent seven climatically distinct locations. Results were evaluated in relation to the age and geographic origin of the isolate, in addition to the highest and lowest average monthly temperature of the sampling location. We found a significant correlation between the growth rate and the age of the isolate at 25 °C. Variation in growth rates between subpopulations was low compared to within subpopulations. Only at 2 °C did statistically significant differences between the average growth rates of subpopulations emerge. These results suggest that *L. piceae* covers the whole distribution area of Norway spruce but that generally the thermal reaction norm of its subpopulations has not changed according to local temperature ranges, despite high contrast in thermal conditions across this vast area. Therefore, it would appear that the thermal environment is not a crucial factor in assessing the fitness of this fungal species within the native range of Norway spruce.

1. Introduction

Temperature is an essential abiotic factor influencing the biological, ecological and evolutionary processes of any organism in its environment. Fungi living in the tree canopies of boreal forests have to cope, as do their hosts, with high diurnal and seasonal temperature variations which can, for instance in Finland, span up to 60 °C (Finnish Meteorological Institute, 2019). Not all native forest pathogens occur throughout the distribution range of their main native host species, but relatively little is known about how the local temperature pattern can restrict the survival of plant-associated fungi. For instance, *Heterobasidion annosum* s.s. and *Diplodia sapinea* are not present in the most northern Scots pine (*Pinus sylvestris* L.) forests in Europe (Müller et al., 2018a, 2018b). Similarly, *Venturia fraxini*, the most frequently found endophytic fungus in leaves of the European ash (*Fraxinus excelsior*) in Switzerland and Northern Italy, seems to be scarce or even absent in Norway (Schlegel et al., 2018; Hietala et al., 2018). As it is unclear whether direct effects of the northern temperature range restrict their propagation northwards, it is difficult to predict if global warming will extend their distribution area in the future. It remains an intriguing, largely unanswered question as to whether fungal parasites and pathogens in general are as adaptable in the face of climatic fluctuations as their main host plant species are. For instance, do environmentally highly tolerant tree species with extremely wide distribution ranges, such as the Norway spruce (*Picea abies* L. Karst.), host the same parasitic fungi throughout their entire native area?

Norway spruce is indigenous to the boreal forests of northern Europe and the mountainous areas of Central Europe. It gradually transforms morphologically along its distribution area towards the

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northeast and its eastern variant (or Siberian subspecies) is called *P. abies* ssp. *obovata* (Ledeb.). This subspecies' presence spans the Kola Peninsula in northern Europe through Siberia to the coast of the Okhotsk Sea of the Pacific Ocean. It can therefore be said that Norway spruce and its Siberian subspecies thrive in widely differing climatic conditions, including mild coastal sites at sea level in Fennoscandia, continental areas in Siberia, as well as, harsh mountainous sites up to 2000 m a.s.l. in the Alps, Altai and Sayan (Schütt et al., 2006).

Lophodermium piceae (Fuckel) Höhn is the most common endophyte endemic to Norway spruce needles in European forests. The infection frequency increases with needle age and often over 50 % of needles are colonized by this fungus (Barklund, 1987; Sieber, 1988; Solheim, 1995; Müller and Hallaksela, 1998). *L. piceae* infects needles via its air-driven ascospores (Osorio and Stephan, 1991), and it only colonizes small spatial domains in the needle tissue, meaning a single intact needle can accommodate over 30 different *L. piceae* individuals (Müller et al., 2001). The number of *L. piceae* individuals inhabiting a single spruce tree is therefore potentially immense, exceeding the number of needles on the tree. In general, the fungus causes no visible harm to its host.

The adaptation of fungal subpopulations to the local temperature range of their environment has been documented in form of altered thermal reaction norms (temperature-dependent pattern of an activity like growth rate, sporulation etc.) in a number of fungal species associated with various plants (Manici et al., 1995; Laine, 2008; Zhan and McDonald, 2011; Mboup et al., 2012; Stefansson et al., 2013; Müller et al., 2015; Robin et al., 2017). In some cases, thermal adaptation (being manifested as increased performance, such as growth rate or reproduction success, of a strain or subpopulation at different thermal conditions than in its initial/native environment) has been shown to proceed rapidly (de Crecy et al., 2009; Lendenmann et al., 2016). Rapid thermal adaptation within a few years or decades is not necessarily based solely on changes in the relative abundance of individual genotypes in a population. In the case of *Cryphonectria parasitica*, adaptation has been known to occur in clonal lineages after their range expansion to a number of areas in France over a couple of decades (Robin et al., 2017). Intraclonal adaptation has also been witnessed in *Phytophthora infestans* (Mariette et al., 2016) in regard to the latent period, lesion growth and spore production which varied between strains according to local temperatures at their origin.

We can hypothesize that especially the fungal species colonizing the upper canopy of trees must demonstrate higher adaptability to variable temperatures than species dwelling in the roots and basal sections of their host where the daily and seasonal temperature fluctuation is less significant. Even in the ground vegetation of forested sites, temperature shifts are likely considerably smaller than in the canopy which is exposed to stronger solar radiation fluctuations. *L. piceae* colonizes intact Norway spruce needles all around the canopy where it may prevail as an endophyte for years before needle senescence (Osorio and Stephan, 1991; Müller and Hallaksela, 2000). At the time of senescence, the fungus becomes active and forms hysterothecia (generally not before the needles fall and join the litter layer on the ground) which liberates ascospores capable of infecting untouched spruce needles, thereby starting a new life cycle. The close association of *L. piceae* with Norway spruce provides an opportunity to investigate fungal adaptation to various thermal conditions since Norway spruce thrives in climatically highly variable conditions.

The aim of this study was to examine whether *L. piceae* is present throughout the distribution area of the Norway spruce across the Eurasian boreal forests, despite its widely diverging weather patterns, and to test the hypothesis that the growth rates of isolates from locations with highly different temperature conditions perform differently at both the high and low ends of their active temperature range, i.e., that their selection is based among others, on the local temperature regime. *L. piceae* was chosen as the model species because it is found abundantly on Norway spruce needles, is easy to isolate and to cultivate on ordinary growth media. Furthermore, *L. piceae* has a simple life cycle compared, for instance, to plant pathogenic rust fungi, so using only one trait, i.e., mycelial growth rate *in vitro*, may be sufficient in measuring the degree of adaptation to site-specific thermal conditions (Pringle and Taylor, 2002).

2. Material and methods

2.1. Needle sampling and fungal isolation

Sampling and isolation of all but the Swiss isolates were performed as follows. Third year twig segments (delimited by terminal bud scars in previous years) were cut from twigs of mature Norway spruces at 1.5–3 m in height and transported in plastic bags (as hand luggage or by mail) to the laboratory where they were subjected to the isolation procedure within three weeks of being cut from their trees. Samples were obtained from four European countries and from southeastern Siberia (Table 1 and Fig. 1S). Fungi of these samples were considered to represent seven subpopulations based on geographical distance and/or distinct differences in climatic conditions of the sampling locality.

The twig segments were surface sterilized with sodium hypochlorite, as described by Müller and Hallaksela (1998). Four slices of approximately 0.5 mm were cut from the base of symptomless green needles after removal of the petiole. These needle slices were incubated on water agar for one month at 20 °C in darkness. Single hyphal tips were removed under the microscope from the plates with a modified Pasteur pipette and transferred to modified orange serum (MOS) agar plates (Müller et al., 1994). After cultivation at 20 °C for three to four weeks, small hyphal samples from colony edges were removed and transferred to fresh MOS agar plates. Agar plugs with hyphal samples were transferred after three to four weeks into 2 ml Cryo-vials (Nalgene, Sigma–Aldrich, Saint Louis, USA) and preserved at 5 °C until experimental use.

Swiss isolates were obtained as follows. Ten healthy ≥ five-year-old needles were collected from ten trees at the Gandberg (46°58′40″N 9°06′00″E) and the Albis (47°17′30″N 8°29′53″E) sites, i.e. 100 needles per site. At the Creux du Van site (46°55′59″N 6°43′25″E), 20 healthy ≥ five-year-old needles were collected from six trees, i.e. 120 needles. The needles were processed within 48 hours of collection as described in Sieber (1989). After surface-sterilization, the needles were cut in half and incubated in 90 mm Petri dishes containing TMEA (20 $g \cdot l^{-1}$ malt extract, 15 $g \cdot l^{-1}$ agar 50 mg $1 \cdot l^{-1}$ oxytetracycline hydrochloride).

The isolates were identified as *L. piceae* based on their ITS-DNA sequences (Müller et al., 2007) using a 98 % similarity threshold. All isolates used in this study were collected and isolated from different tree individuals.

2.2. Cultivation of the isolates at various temperatures

In order to evaluate the suitability of the colony area for growth rate measurement, we determined the effect of temperature on the colony weight-to-area ratio of eight Finnish isolates (Helsinki). The isolates were cultivated in duplicate in Petri dishes (diam. 9 cm) on malt extract (2 %) agar at four different temperatures until the colonies reached an area of ca. 20 cm^2: at 2 °C for 138 d, at 6 °C for 97 d, at 20 and 25 °C for 28 d. The agar was covered with cellophane membranes (Surface Specialities; Wigton, UK). At the end of incubation, the areas of the fungal colonies were evaluated. The

Table 1
Origin of the *Lophodermium piceae* isolates used in this study.

Subpopulation	Locality	Number of isolates	Isolation year	Elevation (meters a.s.l.)	Average monthly air temperature (°C)	
					Lowest	Highest
1. Belarus	Minsk surrounding	23	2006	200–250	−3.0	18.0
2. Italy						
Italy	Trento, Passo di Lavazé	13	2005–2006	1500	−3.5	14.0
Italy	Trento, Pomarolo	11	2005	680	−3.5	14.0
	Sum	24				
3. North-Finland						
North-Finland	Hetta	9	2008	300	−14.8	13.7
North-Finland	Ivalo	1	2007	130	−14.8	13.0
North-Finland	Saariselkä	3	2007	300	−14.8	13.0
North-Finland	Ylläs	4	2005	300–400	−14.8	13.7
	Sum	17				
4. South-Finland						
South-Finland	Helsinki	19	2010	25	−5.7	17.2
South-Finland	Joutsa	3	2005	100	−8.7	16.0
South-Finland	Padasjoki	5	1995	160	−8.7	16.0
South-Finland	Perniö	7	2005–2006	50	−6.5	16.1
South-Finland	Sipoo	2	1995	70	−5.7	16.9
South-Finland	Tuusula	6	1995	60	−5.7	16.9
South-Finland	Ylämaa	4	2002	40	−6.5	16.1
	Sum	46				
5. Russia	Tunkinskii Valley, Buryatia (southeast Siberia)	14	2008	650–750	−25.5	17.0
6. Switzerland I						
Switzerland	Albis	16	2007	830	−1.0	16.7
Switzerland	Gandberg	8	2004	1370	−3.0	9.0
	Sum	24				
7. Switzerland II						
Switzerland	Creux du Van[a] permafrost site	15	2005	1200	−2.0	4.0
Sum/range		163	1995–2010	25–1500	−1.0–−25.5	4.0–18.0

[a] The trees grow in permafrost soil and reach maximum heights of only 20 cm. We therefore used values estimated from data presented by Delaloye et al. (2003) for the inner part of the scree slope, although the air temperatures 2 m above the ground are considerably higher.

colonies were then removed from the cellophane membranes, dried at 105 °C for 17–24 hours and weighed.

The growth rate of the isolates was measured at four temperatures; two low and two high temperatures within their active temperature range. The thermal reaction norm of *L. piceae* was determined by cultivating twelve Finnish isolates from Helsinki at seven different temperatures (2–30 °C). The isolates were cultivated in Petri dishes (diam. 9 cm) on malt extract (2 %) agar and their colony area was determined using a planimeter on upside down plates. The colony area was determined at four time points which differed between temperatures in order to represent roughly the same growth phase at each temperature: at 2 °C on days 47, 64, 99 and 138; at 6 °C on days 26, 48, 72 and 97; at 20 and 25 °C on days 7, 14, 21 and 28. Accordingly, growth rate values were obtained for each isolate at each temperature for four time periods by dividing the increase in colony size between two consecutive measurements with the number of days of incubation. The highest rates were used for both graphs and the statistical analyses in order to exclude a possible time lag at the beginning and a possible downturn in growth rate at the end of the incubation period. Cultivation of all isolates at the four temperatures was started in Mar 2011. At that time, the isolates had been stored at 5 °C for 1–15 y. All cultivation at the given temperatures were performed during one experiment.

2.3. Statistical analyses

Statistical analyses were performed in R (R Core Team, 2018). Differences in the dry weight per area ($mg{\cdot}cm^{-2}$) of the eight Finnish isolates from Helsinki at different temperatures (20 °C vs. 2, 6 and 25 °C) were analysed by means of a linear mixed model (LMM) using the package ‘nlme’ and function ‘lme’ (Pinheiro et al., 2018). "Fungal isolate" was used as a random factor in the model to take into account the fact that repeated measurements of one fungal strain are more similar than the dry weights of randomly collected *L. piceae* isolates.

The effect of differing origins (northern Finland, southern Finland, Siberia, Belarus, Switzerland, Switzerland permafrost, Italy) on the development of *L. piceae* ($cm^2\ d^{-1}$) at different temperatures (2, 6, 20, 25 °C) was investigated using LMMs (Pinheiro et al., 2018, package ‘nlme’, function ‘lme’, $n = 163$). One model per temperature was estimated. Thus, the growth rate of each strain was used as a response variable in the models, and the origin (subpopulation), as a factor as well as the age of the fungal strain (years since isolation) were considered explanatory variables. The age of each fungal isolate was included in each model since it may have an effect on their performance (Müller et al., 2015). As a random factor we used the original locality of each isolate. Differences between the Swiss permafrost and other subpopulations were investigated, as the growth rate in the permafrost population was expected to deviate most from the others. Zhan and McDonald (2011) proposed that temperature sensitivity, measured as the difference between the growth rates at two given temperatures, is a better indicator of thermal adaptation than measurements at individual temperatures. Therefore, similar models were estimated for the differences in growth rate at 6 vs. 2 °C, 20 vs. 6 °C, 20 vs. 2 °C, and 25 vs. 20 °C (the difference being measured as the growth rate at the higher temperature minus the growth rate at the lower temperature divided by the mean growth rate at the two temperatures).

LMMs were used to investigate the effect of the mean air temperature in C° of the coldest and warmest months (of the location from which the isolate was obtained) on the growth rate (cm^2 d^{-1}) of *L. piceae* at different temperatures (2, 6, 20 and 25 °C) by using function 'lme' in R package 'nlme' (Pinheiro et al., 2018). The growth rate was a response variable in the model, and the mean temperature during the coldest month, the mean temperature during the warmest month and the age of the fungal strain were used as explanatory variables. Correlation between the explanatory variables was low ($r < 0.21$). Subpopulation and the locality of origin were input as nested random factors in the model (this takes into account the fact that fungal isolates within a subpopulation and within a specific locality may be more similar than randomly collected fungal strains). Similar models were also estimated for the differences in the growth rate between two temperatures, i.e. temperature sensitivity (6 vs. 2, 20 vs. 6, 20 vs. 2 and 25 vs. 20 °C). The package 'AICcmodavg' was used to obtain predictions for the linear mixed effect model (Mazerolle, 2017).

3. Results

3.1. Methodological examinations with Finnish isolates

The dry weight-to-area ratio did not vary significantly between the various temperatures used (Table 2). The growth rate of the Finnish isolates generally varied between the four cultivation periods, and the highest rate was reached at different time periods depending on the isolate and the applied temperature. At temperatures below 20 °C, most isolates reached the highest growth rate during the first cultivation period but at 20 and 25 °C, the average growth rate remained fairly constant throughout all four cultivation periods (Fig. 1). According to the thermal reaction norm determined, the highest growth rate takes place at 20–25 °C but growth also occurs at 2 °C (Fig. 2). The highest disparity among the Finnish isolates was observed at 20 and 25 °C. At these temperatures some isolates showed highest growth during the first cultivation period, while other isolates were most active during one of the other periods (Fig. 3). The variation between replicates was negligible compared to that of between isolates. At 25 °C, eleven out of twelve Finnish isolates grew but none did so at 28 °C.

3.2. Differences between subpopulations of different countries

The divergence in growth rate between investigated subpopulations was highest at 20 and 25 °C, however only at the lowest temperature of 2 °C were significant differences observed (Fig. 4). The Swiss subpopulation from the permafrost site (Creux du Van) exhibited a significantly higher growth rate at 2 °C compared to the Belarusian ($p = 0.022$) and Italian subpopulation ($p = 0.029$). At 2 °C, the growth rate of a given isolate correlated negatively with the average air temperature of the warmest month in its location of origin (model 1 in Table 3, Fig. 5). The age of the isolates may have influenced the growth rates seen at 25 °C since the oldest isolates showed higher growth rates than the youngest ones (model 4 in Table 3). In addition, the growth rate's sensitivity to the change in temperature from 20 to 25 °C varied significantly with isolate age (model 7 in Table 3). Growth rate responses to temperature changes did not, however, vary significantly with mean air temperatures of the warmest or coldest months (models 5–8 in Table 3).

Table 2
Dry weight of southern Finnish *Lophodermium piceae* colonies per area at various temperatures. Differences were insignificant (LMM, $p > 0.050$).

Temperature	n	Dry weight/area ($mg \cdot cm^{-2}$)	Std. dev.
2 °C	14	8.1	4.9
6 °C	15	8.4	5.1
20 °C	15	6.6	1.0
25 °C	12	7.4	2.4

4. Discussion

The presence of *L. piceae* in the temperate climates of Poland, Italy and southern Finland and the cold climate of northern Finland close to the timberline, in the extremely harsh environments of southeastern Siberia as well as a Swiss permafrost site suggests that the occurrence of *L. piceae* faces no restriction on a thermal level across the entirety of Norway spruce's natural habitat, including its subspecies. This result supports the view that airborne parasitic fungi tend to occur throughout the whole natural distribution range of their main host plants, i.e. where they share a common evolutionary history. Global warming can only be expected to alter the distribution of these parasites if their host plants' distribution changes. The situation differs in the case of parasitic fungi that are vectored by insects, for instance, because they are also reliant on the vector's own dissemination, which may be sensitive to changes in climate. In addition, invasive pathogens may face hindrance during spreading into new regions if they have never experienced climatic conditions similar to that of their new host species' habitat in their own native distribution range. For instance, *Phytophthora cinnamomi*, an invasive species in Europe, does not cause disease in northern Europe, despite the presence of susceptible hosts (Vettraino et al., 2005; Camilo-Alves et al., 2013).

Statistically significant differences between growth rates of subpopulations of *L. piceae* were found at 2 °C. However, differences were only rendered statistically significant with the inclusion of the Swiss subpopulation of the permafrost site (Fig. 4). This result is surprising, since the mean monthly temperatures still varied substantially among sampling sites, even if the Swiss permafrost site was excluded, i.e. by up to 14 °C in the coldest month and up to 9 °C in the warmest month (Table 1). For comparison, in two other investigations even differences in mean monthly summer temperatures of less than 2 °C between sampling locations appear to have resulted in local adaptation of plant pathogenic fungi (Laine, 2008; Robin et al., 2017).

In several previous investigations on other fungal plant parasitic species, significant variations were found *in vitro* at certain temperatures between the activity of subpopulations from various thermally differing locations (Table 4). Growth rate on agar dishes has been the most common activity measured in these previous investigations. None of the previous studies on thermal adaptation among fungal subpopulations included sampling locations with such extreme differences in mean coldest or warmest month temperatures as the present study. Based on our results and those previously reported, it appears that the degree of thermal adaptation varies considerably depending on the fungal species. Investigations on further fungal species incorporating several fitness measures (such as reproduction and infectivity) are required before general conclusions can be drawn.

The variation in growth rates within subpopulations at a given temperature was considerable (Fig. 5). This result is consistent with that obtained in earlier studies on *H. annosum*, a root and butt rot basidiomycete (Müller et al., 2015). Also in some prior studies on growth rates of other plant parasitic fungi, considerably higher disparity within, as opposed to between, subpopulations has been reported (Manici et al., 1995; Stefansson et al., 2013).

The variation of growth rates between isolates was highest at the temperature range in which the isolates were most active; at 20 and 25 °C (SD > 50 % of the mean value, Fig. 2). In this range, the difference between isolates was clearly higher than that observed between those of *Heterobasidion parviporum* (Müller et al., 2015).

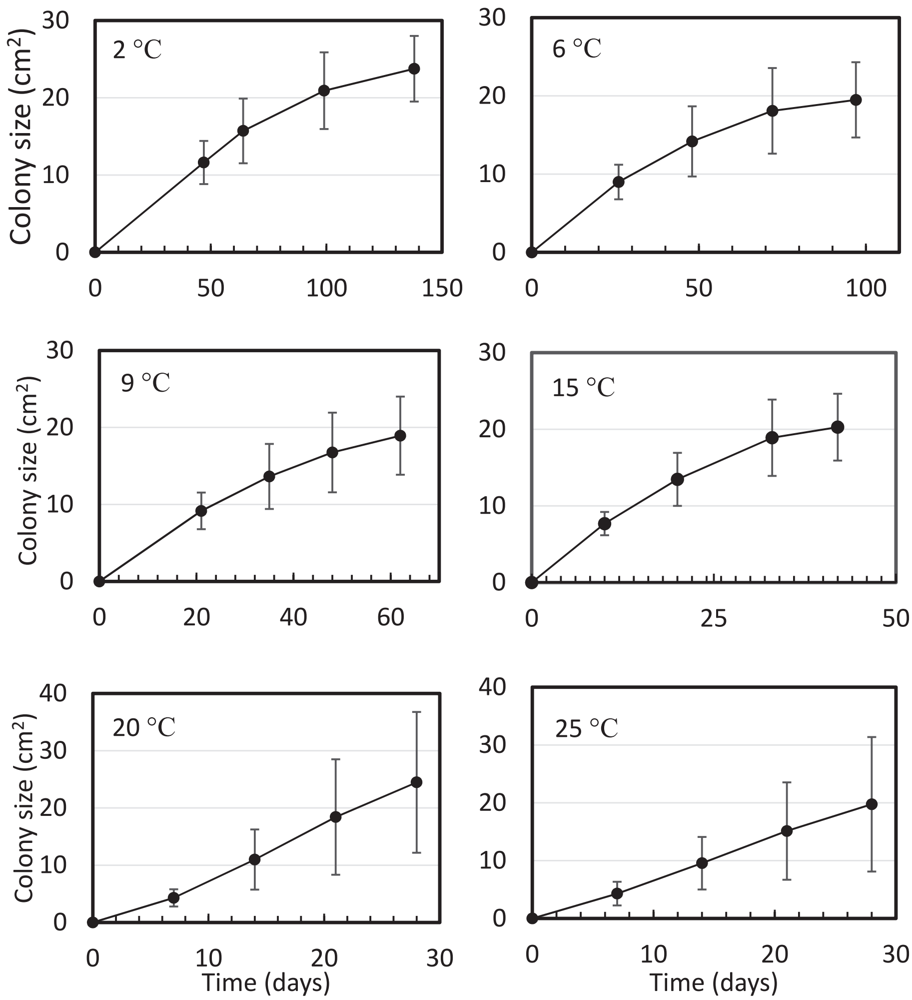

Fig. 1. Growth of *Lophodermium piceae* isolates on malt extract agar at various temperatures (mean of 12 southern Finnish isolates). Vertical bars indicate the standard deviation of the mean values.

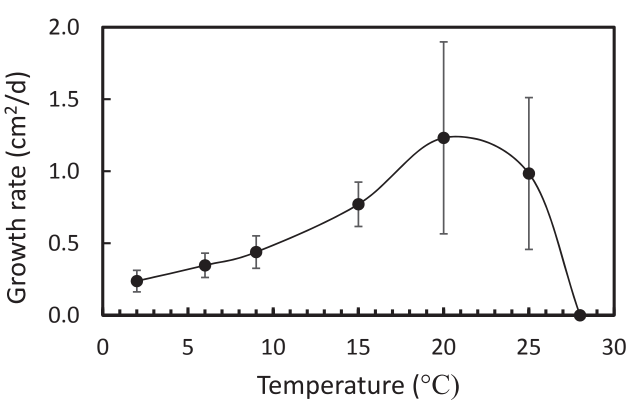

Fig. 2. Growth rate response of *Lophodermium piceae* to temperature (mean of 12 southern Finnish isolates). Vertical bars indicate the standard deviation of the mean values.

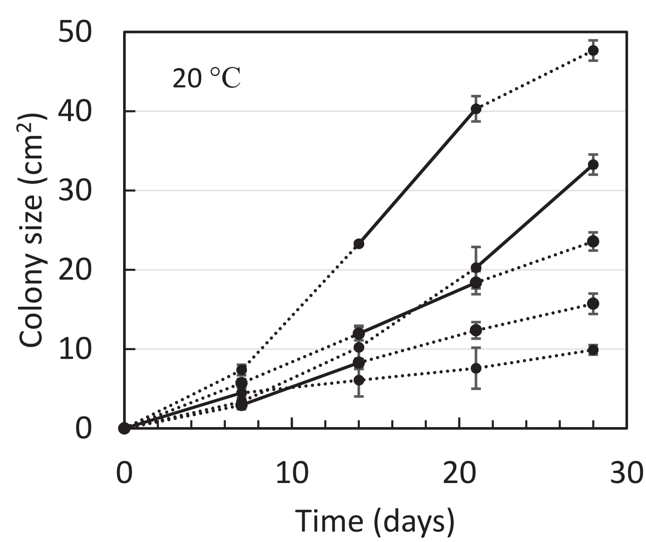

Fig. 3. Colony growth of five Finnish *Lophodermium piceae* isolates at 20 °C showing different growth patterns. Solid line indicates the time span with highest growth rate. Vertical bars indicate the variation between two replicates.

This observation coupled with the low upper temperature limit of <28 °C (Fig. 2) suggest that the growth rate at temperatures exceeding 15 °C is not crucial for the fitness of this species, meaning that the optimal temperature range of 20–25 °C observed for growth of this fungus is not a result of an evolutionary adaptation to

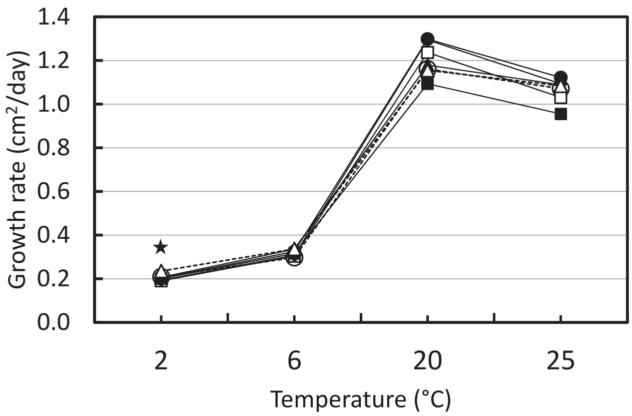

Fig. 4. Growth rates of *Lophodermium piceae* isolates representing seven subpopulations (14–46 isolates/subpopulation) at four temperatures. Symbols: —●— northern Finland, --○-- southern Finland, —□— Belarus, —▲— Switzerland, --△-- Switzerland permafrost site, —♦— Italy, —■— Siberia. Significant differences between subpopulations are indicated with: *$p < 0.05$.

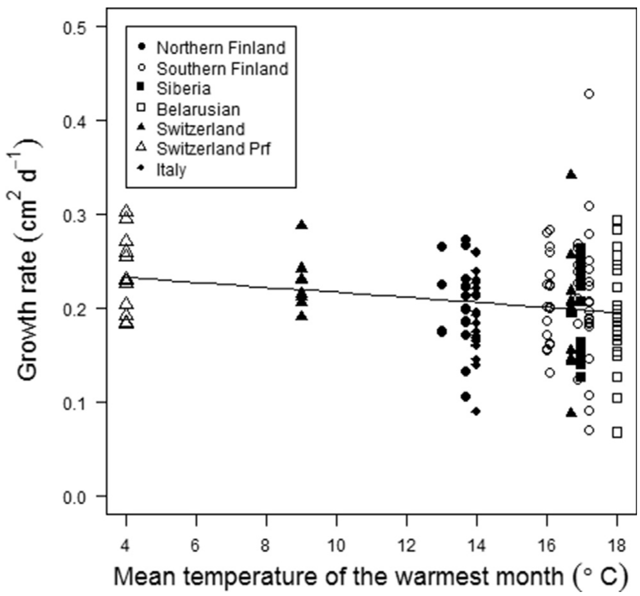

Fig. 5. Growth rate of *Lophodermium piceae* isolates of seven subpopulations at 2 °C as the function of the mean air temperature of the warmest month in the area from where the isolate originates. The regression line is significant at $p = 0.026$ (LMM).

its thermal environment. This conforms to earlier observations that the optimal temperature for microbial communities determined *in vitro* often deviates from temperatures existing in their native environments. For example, the optimum growth temperatures of fungi adapted to life in Arctic and Antarctic sites generally surpass 15 °C, which is in considerable excess of their environment's temperature range (Robinson, 2001). This phenomenon has been explained by 'fundamental trade-offs' in the physiology of fungi (Angilletta et al., 2003; Wallenstein and Hall, 2012).

It is difficult to explain why the growth of *L. piceae* at low temperatures from 2 to 15 °C slowed down over time while the same isolates grew at 20 and 25 °C at an almost constant rate to reach the same final colony size (Fig. 1). High fluctuation in growth patterns, i.e. variable timings of peak growth rate, amongst isolates and its dependence on temperature (Figs. 1 and 3) accounts for the measurements at several time periods and the application of only the highest recorded rate for further evaluations.

The observation that colony dry weight per area ($mg \cdot cm^{-2}$) is rather independent of incubation temperature, is in line with the presumption that colony area increase is a valid measure for estimating a thermal reaction norm for growth of *L. piceae* (Table 2), but we are as yet unable to ascertain whether growth rate *in vitro* is a valid indicator for the temperature dependence of its fitness *in situ*. Some earlier research supports this assumption. Mycelial surface area was a fitting predictor of the spore production by *Aspergillus niger* (de Visser et al., 1997) and the mycelial growth rate of *Agaricus bisporus* proved to be a useful indicator of inbreeding depression (Xu, 1995). On the other hand, distinct fungal activities can follow different thermal reaction norms, as in the case of *Phlebia phlebioides* that decomposed wood most rapidly just above 30 °C, whereas maximum linear extension on agar occurred at ca. 38 °C (Loman, 1962).

Both the growth rate at 25 °C and the difference in growth rates between 20 and 25 °C correlated positively with the age of the isolates (Table 3). In a previous study, *H. parviporum* isolates were preserved at 5 °C from two up to 12 y, after which their respiration activity correlated negatively with age at temperatures beyond 15 °C (Müller et al., 2015) and this was explained by means of phenotypic plasticity (i.e. changes in physiology, behaviour and/or morphology according to the fungus' response to a unique environment). Phenotypic plasticity has also been observed in other past studies, and Zhan and McDonald (2011) estimated that it contributes considerably to the thermal adaptation of *Mycosphaerella graminicola*, a wheat pathogen. However, phenotypic plasticity is inadequate in explaining a positive correlation between

Table 3
The effect of the mean temperature of the coldest and warmest months (in the locations where isolates were collected) and culture age on growth rate, as well as the difference in growth rate of *Lophodermium piceae* at different temperatures (temperature sensitivity) according to LMMs. Statistically significant ($p \le 0.05$) coefficients with standard errors and *p*-values are marked in bold ($n = 163$).

Model	Response variable	Intercept		Explanatory variables					
				Mean temperature of the coldest month (°C)		Mean temperature of the warmest month (°C)		Culture age	
		Coeff. ± SE	*p*	Coeff. ± SE	*p*	Coeff. ± SE	*p*	Coeff. ± SE	*p*
1	Growth rate at 2 °C	**0.242 ± 0.017**	**<0.001**	$-1.037 \times 10^{-4} \pm 0.001$	0.869	**−0.003 ± 0.001**	**0.026**	$1.314 \times 10^{-4} \pm 0.001$	0.909
2	Growth rate at 6 °C	**0.364 ± 0.025**	**<0.001**	$-2.144 \times 10^{-4} \pm 8.854 \times 10^{-4}$	0.813	−0.003 ± 0.002	0.060	$-8.037 \times 10^{-4} \pm 0.002$	0.630
3	Growth rate at 20 °C	**1.260 ± 0.159**	**<0.001**	0.003 ± 0.006	0.581	$1.864 \times 10^{-4} \pm 0.010$	0.985	−0.008 ± 0.010	0.447
4	Growth rate at 25 °C	**1.084 ± 0.109**	**<0.001**	$7.290 \times 10^{-4} \pm 0.004$	0.854	−0.008 ± 0.007	0.276	**0.014 ± 0.007**	**0.042**
5	Rate at 6 − rate at 2 °C	**36.184 ± 7.572**	**<0.001**	0.064 ± 0.292	0.830	0.535 ± 0.475	0.285	−0.029 ± 0.363	0.937
6	Rate at 20 − rate at 6 °C	**106.900 ± 9.058**	**<0.001**	0.071 ± 0.306	0.822	0.429 ± 0.571	0.468	−0.039 ± 0.546	0.943
7	Rate at 20 − rate at 25 °C	−12.774 ± 11.934	0.286	−0.280 ± 0.421	0.519	−0.886 ± 0.714	0.241	**2.209 ± 0.790**	**0.006**
8	Rate at 20 − rate at 2 °C	**130.951 ± 8.334**	**<0.001**	0.064 ± 0.298	0.835	0.539 ± 0.529	0.330	−0.012 ± 0.451	0.979

Table 4
Earlier research results showing adaptation of fungal subpopulations to local thermal conditions; i.e. observations of statistically significant correlations in fitness of isolates at controlled temperatures *in vitro* with thermal conditions at collection sites of the isolates.

Fungus	Disease	Differences of mean air temperatures between locations from where the subpopulations were sampled	Fitness measure	Reference
Macrophomina phaseolina	Charcoal rot on sunflowers	7 °C in winter	Growth rate (cm^2/day) on petri dishes at 15, 25, 30, 35 and 40 °C	Manici et al. (1995)
Podosphaera plantaginis	Powdery mildew on a grass	<1 °C, Jun–Aug	Sporulation time and spore production on detached host leaves *in vitro* at 17, 20 and 23 °C	Laine (2008)
Mycosphaerella graminicola	Leaf blotch disease on wheat	11.2 °C, annual	Growth rate (cm^2/day) on Petri dishes at 15 and 22 °C	Zhan and McDonald (2011)
Puccinia striiformis f.sp. *tritici*	Wheat yellow/ stripe rust	Not reported (the authors supposed that the temperatures in southern France are higher than in northern France)	Infectivity on wheat seedlings *in vitro* and germination rate at 7, 10, 15, 20 and 25 °C	Mboup et al. (2012)
Rhynchosporium commune	Leaf blotch disease on barley	12.7 °C, annual	Growth rate (cm^2/day) on Petri dishes at 12, 18 and 22 °C	Stefansson et al. (2013)
Heterobasidion parviporum	Root and but rot on Norway spruce	8.4 °C during warmest month and 25.4 °C during coldest month	Respiration rate on spruce saw dust at 0, 2, 6, 15, 20, 25, 30 and 33 °C	Müller et al. (2015)
Cryphonectria parasitica	Chestnut blight	1 °C in Jul–Oct and 1.9 °C for annual temperatures	Radial growth rates (mm/day) on agar plates at 12, 15, 28 and 33 °C	Robin et al. (2017)

isolate growth rate and age at a temperature significantly higher than 5 °C, used here for preservation of the cultures.

No significant variation in temperature sensitivity (or RD, the relative difference of activity, e.g. growth rate between two temperatures) was found between subpopulations. This coincides with results of a prior study on *Heterobasidion parviporum*, where considerably more significant contrasts in activity among subpopulations were observed at constant temperatures (2, 6, 15, 20, 30 °C) in comparison to between temperature sensitivity-values ($RD_{0\text{-}6}$ and $RD_{6\text{-}20}$, Müller et al., 2015). These results conflict with previous findings (Zhan and McDonald, 2011), according to which temperature sensitivity would be a better indicator of thermal adaptation for fungi than activity at a constant temperature.

Due to the low variation between the growth rates of subpopulations sampled from various thermal conditions, it can be assumed that the thermal environment of the spruce canopy and the litter layer below it does not act as a crucial factor ruling the fitness of *L. piceae*. The occurrence of *L. piceae* in multiple locations spanning a wide range of climates supports this view.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.09.008.

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