Fungal and cyanobacterial gene expression in a lichen symbiosis: Effect of temperature and location

Sophie S. Steinhäuser [a], Ólafur S. Andrésson [a], Arnar Pálsson [a], Silke Werth [a,b,c]

[a] Life and Environmental Sciences, University of Iceland, Sturlugata 7, 101 Reykjavik, Iceland

[b] Institute for Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria

[c] Corresponding author's details: silke.werth@uni-graz.at, phone: +43 316 380 5645, fax: +43 316 380 9880

Abstract

The capacity of species to cope with variation in the physical environment, e.g. in temperatures and temperature fluctuations, can limit their spatial distribution. Organisms have evolved cellular mechanisms to deal with damaging effects of increased temperature and other aggravation, primarily through complex molecular mechanisms including protein refolding and DNA repair. It is of interest to see whether these responses vary with geographic location, with high vs. low elevation and on the coast vs. inland, indicating long-term acclimatization or genetic adaptation. As mutualistic symbioses, lichens offer the possibility of analyzing molecular stress responses in a particularly tight interspecific relationship. For this study, we have chosen the widespread cyanolichen *Peltigera membranacea,* a key player in carbon and nitrogen cycling in terrestrial ecosystems at northern latitudes. We ask whether increasing temperature is reflected in mRNA levels of selected damage control genes, and do the response patterns show geographical associations? Using real-time PCR quantification of 38 transcripts, differential expression was demonstrated for nine cyanobacterial and nine fungal stress response genes (plus the fungal symbiosis-related *lec2* gene) when the temperature was increased from 5°C to15°C and 25°C. Principle component analysis (PCA) revealed two gene groups with different response patterns. Whereas a set of cyanobacterial DNA repair genes and the fungal *lec2* (PC1 group) showed an expression drop at 15°C vs. 5°C, most fungal candidates (PC2 group) showed increased expression at 25°C vs. 5°C. PC1 responses also correlated with elevation. The correlated downregulation of *lec2* and cyanobacterial DNA repair genes suggests a possible interplay between the symbionts warranting further studies.

Keywords: lichens; gene expression; candidate genes; quantitative real-time PCR (qPCR); heat stress; symbiosis; environmental stress response mechanisms

1. Introduction

Climate change and global warming call for studies of how changing environmental conditions affect species survival. Changes in species composition and reduced biodiversity can negatively affect ecosystems and therefore also services they provide for human society (Burkle et al. 2013; Schröter 2005). In the past decades, there have been numerous reports on rapid climatic changes on global scales, impacting both terrestrial and marine ecosystems (Diffenbaugh and Field 2013; Penuelas et al. 2013; Raven et al. 2005). Increasing temperatures cause major ecosystem changes affecting species on many levels, from metabolism and growth to general species interactions and ecosystem productivity (Penuelas et al. 2013). For a variety of terrestrial organisms (e.g. butterflies, birds, higher plants), increased temperatures have been shown to affect breeding, flowering, or the length of the growth season (Parmesan 2006). This can result in asynchronies in the population dynamics of species, uncoupling predator-prey or insect-host interactions and thus even leading to extinctions of local populations (Parmesan 2006). However, climate change is not expected to have the same effect on populations of a species occurring in distinct habitats. For example, a study showed that epiphytic floras are altered more rapidly in open rural areas than in forested areas (Aptroot and van Herk 2007). Therefore, the effect of global warming on a species can be rather complex.

Tolerance of stress associated with changing environmental conditions is an important property allowing organisms to persist in changing environments. Environmental change may e.g. lead to fluctuations, as well as extremes, in temperature, hydrological regime or light conditions, leading to physiological strain for most organisms. There are several strategies enabling the survival of species under pressure due to environmental changes, like migration to favorable habitats or local adaptation (which has a genetic base and acts over long time scales) (Aitken et al. 2008). A third survival strategy is acclimatization, gradual long-term physiological adjustment to the changed local environment (Sork et al. 2010). This is in contrast to short-term physiological adjustment, e.g. to laboratory conditions, which is referred to as ‘acclimation’. As one aspect of phenotypic plasticity, acclimatization is based on the capacity of a single genotype to develop variable phenotypes in different environments, manifested in altered biochemistry, physiology, morphology, behavior or life history (Whitman and Agrawal 2009). Acclimatization is an ongoing process, reversible and not passed on to the next generation. Differences in the capacity of species for acclimatization has important implications for their persistence in changing environments and may allow *in-*

situ tolerance of changed environmental conditions, thus playing an important role for species to successfully cope with environmental variability (Aitken et al. 2008; Davis 2005; Manel et al. 2010). Thus, migration, genetic adaptation and *in-situ* tolerance of new, stressful conditions (achieved by changes in acclimatization) are key mechanisms for coping with a changing climate.

One of the most important mechanisms behind *in-situ* tolerance is the environmentally induced alteration of gene expression, resulting in changes in physiology enabling species to tolerate altered abiotic conditions. At the molecular level, both prokaryotes and eukaryotes have evolved multiple methods to sense and respond to changes in their abiotic environment and develop in-situ tolerance, most notably environmental stress-response (ESR) mechanisms that include pathways commonly activated under environmental stress such as drought or heat (Apte et al. 1998; Che et al. 2013; di Pasqua et al. 2013; Enjalbert et al. 2006; Gasch 2007; Plesofsky-Vig and Brambl 1998; Sato et al. 2008; Young 2001; Zahrl et al. 2007). Stress-responsive regulatory and damage repair pathways are important for enhancing survival (Wang et al. 2015). Common stress-response regulation pathways such as the Hog1 mitogen-activated protein kinase pathway and the TATA-binding protein mediated pathway induce the transcriptional upregulation of ESR related genes such as heat shock protein (Hsp) or DNA repair genes (Wang et al. 2015). In addition to protein refolding and denaturation systems, DNA damage repair pathways such as the global SOS response system play an important role in repairing stress-related DNA damages (Cheng et al. 2012; Nagashima et al. 2006; Odsbu and Skarstad 2014; Sargentini and Smith 1986; Voloshin et al. 2003).

To determine how species react to environmental changes, it is interesting to include the level of species interactions since species generally do not live in isolation, but with other species. One of the most remarkable interactions between different species is mutualism. In terrestrial ecosystems, mutualistic plant- or fungal-bacterial symbioses are widely distributed (Albright et al. 2010; Antonyuk and Evseeva 2006; del Campo et al. 2013; McCowen et al. 1986; Timmusk and Wagner 1999; Yahr et al. 2004). Symbiosis partners can communicate and strongly influence each other at the molecular level as shown for the plant-bacterial symbiosis of wheat and the rhizobacterium *Azospirillum brasilense* and for *Arabidopsis thaliana* and its rhizobacterial symbiont *Paenibacillus polymyxa* (Antonyuk and Evseeva 2006; Timmusk and Wagner 1999). Lichens are an important form of mutualistic interaction, dominating in over 12% of all terrestrial ecosystems, (Honegger 2012). Lichens are characterized by the symbiotic association of a filamentous fungus (mycobiont) with one or

several photoautotrophic organisms (photobionts), which can be green algae or cyanobacteria (Honegger 2012; Lutzoni and Miadlikowska 2009). Lichens can be very sensitive to changes in their environment such as air pollution (Hawksworth 1970; Nimis 2002). Using the bioclimatic envelope approach, studies have predicted major future range shifts for lichen-forming fungi in response to climate change (Ellis et al. 2007a; Ellis et al. 2007b). However, the molecular background of how lichen populations might react to large-scale climatic changes such as global warming remains to be investigated in more detail. One strategy through which lichens might be able to tolerate climatic changes is the modulation of gene expression, allowing individuals to cope with environmental extremes and stress. In non-lichenized fungi, there have been several studies on gene expression under environmental stress (Gasch 2007), but only few recent studies have investigated gene expression in lichen-forming fungi and their photobionts (Athukorala and Piercey-Normore 2015; Joneson et al. 2011). In the desert lichen *Endocarpon pusillum*, expression of fungal genes involved in osmoregulation, metabolism and protein repair has been investigated in response to drought (Wang et al. 2015). However, no studies have yet been performed in order to identify and characterize expression of ESR genes in both the lichen-forming fungus and its cyanobacterial photobionts with increased temperature.

The membranaceous dog lichen, *Peltigera membranacea*, is a key player in carbon and nitrogen cycling in terrestrial ecosystems at northern latitudes due to its high abundance and its symbiosis with nitrogen-fixing *Nostoc* cyanobacteria (Miadlikowska and Lutzoni 2004). In this study, we investigated the mRNA levels of selected ESR genes of both mycobiont and photobiont of *P. membranacea* to answer the question whether temperature increases of 10°C or 20°C cause stress responses, reflected in altered mRNA levels in both symbionts (question 1). The term 'gene expression' is used for the quantity of mRNA relative to a reference gene, measured by quantitative PCR. In addition to examining the gene expression of ESR candidates, expression of two fungal lectin genes (*lec1* and *lec2*) was investigated as lectin genes are thought to play a role in the symbiotic interaction (Manoharan et al. 2012; Miao et al. 2012). The impact of fungal lectins on chemotropism of green-algal photobionts has already been described for several other lichens (Diaz et al. 2011; Legaz et al. 2004; Molina and Vicente 2000; Wang et al. 2014). For wheat, it has been suggested that differential expression of wheat lectin can affect nitrogen fixation and plant growth-stimulating processes in its bacterial symbiont (Antonyuk and Evseeva 2006). Therefore, the expression analysis of *lec1* and *lec2* in the *P. membranacea* mycobiont and in its photobiont *Nostoc* might provide information about the interplay between the symbionts under thermal stress. Moreover, we

asked the question whether lichen populations from climatically more variable inland habitats are affected differently by increased temperatures than lichens from more stable coastal habitats (question 2). More specifically, we asked if expression of the candidate genes at elevated temperatures shows a coastal-inland clinal pattern even after lichen acclimation to laboratory. Site-specific differences in gene expression at different temperatures could imply that either physiological acclimatization has been retained after laboratory acclimation, or that gene expression might vary depending on the genomic background of the studied individuals.

2. Material and Methods

2.1 Sampling

Sampling was performed in South-West Iceland in June 2014, once the highland sites were accessible. Four sea-exposed sites and four inland sites were chosen for sampling (see Table 1, Fig. 1), all characterized by sandy or rocky ground and open vegetation (bryophytes, graminoids) without trees. In addition to the categorical variable "habitat" (sea-exposed vs. inland), the continuous variables "sea-distance" and "elevation" were taken into account to assess site-related differences (Table 1). Temperatures at five sampling sites (RY7, HF2, LA1, HV3 and LL3) were recorded in the years prior to sampling using iButton data loggers (Maxim Integrated, San José, California, USA) and showed greater temperature variation in the inland (LA1, HV3 and LL3) than at the coast (RY7 and HF2) (see Fig. S1). From each of the eight sampling sites, thalli of five *Peltigera membranacea* individuals with a size of about 7 x 7 cm were randomly collected keeping a minimum distance of 2 m between individuals and were cleaned from attached bryophytes and leaf litter. The total number of individuals included in our study was 40.

------ Table 1 ------

------ Figure 1 ------

2.2 Acclimation phase and temperature experiment

When gene expression is quantified in samples obtained under field conditions, it is difficult to impossible to assure that recent environmental conditions have been the same for all samples. Hence, we decided to quantify gene expression in samples subjected to controlled temperatures, after performing an initial acclimation to cold conditions in the laboratory.

All samples were placed in closed petri dishes on filter paper and acclimated to 5°C under ~30 lux illumination (Sylvania GRO-LUX F18W, Germany) in the laboratory during 3 weeks under identical conditions (light, temperature, etc.). Samples were placed randomly and rotated regularly to ensure similar light exposure, were sprayed with distilled water regularly, and were left to dry at least once a week. Then, marginal tissue (ca. 25 mm^2) from one lobe of each fully hydrated lichen was sampled into RNAlater (Life Technologies). Subsequently, all 40 lichen thalli were transferred into a growth chamber (Controlled Environment Incubator Shaker, New Brunswick Scientific Co.) with the same light conditions as previously and sequentially exposed to two higher temperatures, 15°C and 25°C, for 3 hours each. After both treatments, tissue was sampled (from different lobes of the same individual) resulting in three tissue samples (5°C, 15°C and 25°C) per individual for gene expression quantification. The temperatures were chosen based on the recorded temperatures at the sampling sites in June. Temperatures of 25°C or even higher do occur in the natural habitat in sunny conditions. Under these circumstances, the lichens dry out and become metabolically inactive. In rainy conditions, lichens do usually not face such high temperatures in Iceland.

2.3 RNA extraction, DNase digestion and cDNA synthesis

RNA extraction was performed according to the manufacturer's protocol using the RNeasy Plant Mini Kit (Qiagen, Germantown MD, USA). After elution of extracted RNA in RNase-free H_2O, RNA concentration and quality were checked using a NanoDrop® ND-1000 UV/Vis-Spectrophotometer (Thermo Scientific, Carlsbad CA, USA). The concentrations varied between 50 and 200 ng/µl. In order to eliminate any remaining genomic DNA, DNase 1 digestion (New England Biolabs, Ipswich MA, USA) was performed with all RNA samples according to the manufacturer´s protocol. All digested RNA samples were adjusted to the same RNA concentration (50 ng/µl) and cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit according to the manufacturer's protocol (Thermo Fisher Scientific, Carlsbad CA, USA). All cDNA samples were diluted to a final concentration of 10 ng/µl. Gene expression was quantified using Real-Time qPCR (RT-qPCR). The final cDNA input per RT-qPCR reaction was 10 ng.

2.4 Reference and candidate genes

The effect of increasing temperature on the expression of *P. membranacea* genes was surveyed in a RNAseq-based transcriptome study (Werth & Andrésson, unpublished). Fungal candidate genes showing expression differences at different temperatures were chosen for our

study. *Nostoc* candidate genes were chosen based on the literature, using genes which we suspected to be affected by temperature stress (e.g. heat shock genes, DNA repair genes, chaperones; Table 2). Initially, expression of a broad set of candidate genes (21 fungal and 18 *Nostoc* genes) was tested in two lichen thalli from one sea-exposed and one inland site to identify genes showing expression differences between the temperature extremes (5°C and 25°C). After further analyses with individuals from another two sea-exposed and two inland sites, ten fungal and nine cyanobacterial candidate genes were chosen and tested in all remaining individuals, sites and for all temperature treatments. The excluded genes did not amplify well (one mycobiont gene, three *Nostoc* genes) or did not exhibit expression differences between temperatures or collecting sites (10 mycobiont genes, six *Nostoc* genes). The final nineteen candidates varied in function from heat-shock and DNA repair to transcriptional regulation and symbiosis-related functions (Table 2).

---- Table 2 ----

The reference genes for the mycobiont (*gpd1* and *tub2*) were chosen based on the literature (Manoharan et al. 2012, Miao et al. 2012) and on the previous RNAseq-based transcriptome study on *P. membranacea* (Werth & Andrésson, unpublished). For both genes, expression stability (as fraction of total cDNA) was confirmed throughout the experiment (standard deviations of Ct values <10%). ANOVA revealed no significant expression differences between temperatures and sites (see Table S1). The *Nostoc* reference genes (*rnpB* and *secA*) were validated in a previous study on *Nostoc* sp. (Pinto et al. 2012). Expression stability was confirmed (standard deviation of Ct values: *rnpB* = 14%; *secA* = 9%) and expression was not significantly different between temperatures or sites.

Peltigera membranacea RT-qPCR primers were designed based on genomic and transcript sequences from the RNAseq study (GenBank accessions KU904448-KU904455; Electronic Supplement, Table S8) as well as sequences from GenBank (Electronic Supplement, Table S9). RNA sequences were aligned against the appropriate DNA sequences in order to locate exons and introns. Regions showing the least nucleotide differences between individual samples were chosen for primer design and primers were designed to cross the boundary of one or two exons in order to be unable to amplify intron-containing genomic DNA. The *Nostoc* RT-qPCR primers were designed based on DNA sequences of the *Nostoc* strains N6 (BioProject Accession PRJNA279350), N232, N210a (Electronic Supplement, Table S9) and a *Nostoc* strain sequenced from *Lobaria pulmonaria* (A. Gagunashvili, unpublished). *Nostoc* primer sequences are reported in the Electronic Supplement, Table S8.

Primers were designed in regions conserved between the different *Nostoc* strains. All primer sequences were checked for self-complementarity, melting temperature (~60°C), GC-content and 3' complementarity to self and the reverse primer according to the MIQE guidelines (Bustin et al. 2009). The amplicon lengths for all candidate genes varied between 90 and 220 base pairs. Primers were ordered from Microsynth (Balgach, Switzerland) (HPLC purified and desalted). All primers were diluted to 5 µM for an input of 0.5 µl in each qPCR reaction (250nM). Amplification efficiency of each primer pair was calculated by linear regression analysis of the raw amplification data (amplification cycle showing exponential expression rise out of cycles 1-40) using LinRegPCR version 11.0. Amplification efficiencies were close to 80% (Electronic Supplement, Table S8).

2.5 RT-qPCR procedure

RT-qPCR was performed using PowerSYBRgreen® PCR Master Mix as recommended by the manufacturer (Thermo Fisher Scientific, Carlsbad, CA, USA). The RT-qPCR was conducted in 10µl total reaction volume on 96-well optical PCR plates (4titude, UK) on an ABI 7500 Real-Time PCR System (Thermo Fisher Scientific, Carlsbad, CA, USA). In addition to technical duplicates of each sample, non-template controls (NTC) were included in each run and for each gene. The RT-qPCR was started with two minutes hold at 50°C followed by a 10 min hot start at 95°C to heat-activate the hot start DNA polymerase. Subsequently, amplification was performed with 40 cycles of 15 s denaturation at 95°C and one minute annealing/extension at 60°C (Ahi et al. 2013). For each gene, a dissociation step was performed (temperature increase from 60°C to 95°C) after the first RT-qPCR run to verify melting temperature and primer specificity.

2.6 Data analysis and statistics

After threshold and baseline adjustment of the RT-qPCR raw data, analysis of relative expression (RQ) was performed according to the MIQE guidelines (Bustin et al. 2009). Based on the Ct-values of each candidate gene (mean of the two technical replicates) and the geometric mean of the Ct-values of both reference genes of *P. membranacea* or *Nostoc*, ΔCt-values were calculated. The number of biological replicates was five, corresponding to the five lichen thalli utilized per site (total sample size: 40). For illustrations, relative expression (RQ) was calculated using the ΔΔCt-method by setting the sample with the lowest expression as reference sample (RQ = 1). Statistical analyses were conducted in R 3.0.2 (R Development Core team, 2013). Multivariate analysis of variance (MANOVA) was used to determine the effect of the factors of interest (temperature, site, habitat, sea distance and elevation) on gene

expression (ΔCt). For further determination of expression differences associated with these factors, principle component analysis (PCA) was performed (R package "stats") followed by linear mixed effects (lme) models and ANOVA. To analyze and visualize expression correlations between all genes we used the R package "corrgram" (Wright 2013). To test and determine the relationship between gene expression in the fungus and the cyanobacteria we analyzed the data with canonical correlation analysis (R package "candisc"), evaluating with the null hypothesis that the canonical correlations in each row and all that follow were zero based on Wilk's lambda. To disentangle the effects of variables, we performed single-gene, multivariate ANOVA using a linear mixed effects model with temperature and site as fixed factors and lichen individual as a random factor. If the ANOVA revealed significant results for the factor temperature, Tukey's honest significant difference test (Tukey 1949) was performed to determine which temperature levels (5°C vs. 15°C, 5°C vs. 25°C, 15°C vs. 25°C) were different from one another with respect to expression of the candidate genes. To investigate habitat differences, mixed effects models with temperature and habitat (sea vs. inland) as fixed factors and individual and site as random factors were used in ANOVA. Additionally, sea distance and elevation were analyzed with linear models to determine if there was a response to continuous environmental variables.

3. Results

Multivariate analysis of variance (MANOVA) indicated a significant effect of the factors of interest (temperature, site, habitat, sea distance and elevation) on expression levels and further suggested a temperature response difference associated with elevation (see Electronic Supplement, Table S2). These correlations were investigated using principle component analysis, general correlation analysis and multifactorial ANOVA for single genes.

3.1 Principle component analysis (PCA)

To further investigate the expression dynamics associated with the various factors, principle component analysis was performed. Since the first three principle components explained ~80% of the total variation (see Electronic Supplement, Table S3), further analysis focused on PC1 (32.4%), PC2 (28.2%) and PC3 (13.6%). The loadings indicated differential contribution of cyanobacterial and fungal genes onto PC1-3 (Fig. 2).

---- Figure 2 ----

PC1 primarily reflected variation in the fungal symbiosis-related gene *lec2* and to a lesser extent *lec1* and the cyanobacterial DNA repair genes *radA*, *recF*, *recN* and *recO*. However most fungal candidate genes contributed mainly to PC2, along with *lec1* (but not *lec2*) and furthermore PC3 had a strong contribution from *lec1* and less from several other genes. The PCA revealed significant differences between temperature treatments for PC1 and PC2 but not for PC3 (Fig. 3, Table 3).

---- Figure 3 ----

---- Table 3 ----

PC1 mainly represented mRNA variation for the fungal *lec2* gene and cyanobacterial DNA repair genes showing a significant decrease at 15°C (and further at 25°C) compared to 5°C (Table 4). In contrast, PC2, with contributions from most other fungal candidate genes, was characterized by a significant increase in mRNA level at 25°C compared to 5°C and 15°C (Table 4). In PC3 (mainly characterized by fungal *lec1*), there was no significant difference between the three temperatures (Table 4).

---- Table 4 ----

For PC1, the expression differed significantly between sites, with the two inland sites UX4 and LL3 differing the most (Fig. 3). Similarly, PC2 and PC3 also showed significant expression differences between sites. For PC2, gene expression in the inland site LL3 differed the most from the other sites, whereas for PC3 the inland site UX4 differed the most (see Electronic Supplement, Fig. S2). The PCA also revealed a significant difference in temperature response between sites for PC2-related genes (Fig. 3, Table 3). Focusing on PC1, the analysis further revealed a difference between sea-exposed and inland sites. However, there was a difference in PC1 with sea distance and, most strongly, with elevation (Table 3) in accord with the MANOVA results. For PC2 and PC3, there was no difference between habitats, but for PC3 a significant correlation with elevation was also found (Table 3). Note, the three environmental variables, elevation, distance to sea and location (inland vs. coast) are associated. A larger follow up study is needed to disentangle the impact of these variables, and their potential dependence on temperature, on the dynamics of gene expression in lichen symbiosis.

3.2 Expression correlations

To investigate the PCA results further, we calculated correlations of the mRNA levels between fungal and cyanobacterial candidate genes. Note, significant correlation coefficients

reflect associations of variables, but do not imply causation (direction of effects, up or down regulation). Expression of the fungal lectin gene *lec2* and the cyanobacterial DNA repair genes *radA*, *recO*, *recF* and *recN* showed a strong positive correlation (Fig. 4).

-----Figure 4 -----

Furthermore, the majority of the other fungal candidate genes involved in stress response regulation, protein folding and DNA repair showed strong positive correlations of expression levels. Expression of the cyanobacterial DNA repair genes mentioned before, and especially the *Nostoc* heat shock gene *groEL*, was negatively correlated with that of cyanobacterial heat shock genes and most of the fungal candidate genes (Table S10). In contrast, expression of the *Nostoc* stress-induced transcriptional repressor *hrcA* was positively correlated with fungal and cyanobacterial heat shock genes (*hsp90*, *hsp88*) and the fungal stress-induced transcriptional activator *msn2*. Generally, the cyanobacterial heat shock genes (*dnaJ*, *hsp90*) showed expression patterns positively correlated with those of the fungal heat shock genes (*hsp88*, *hsp98*) and fungal *msn2*. Expression of the fungal lectin gene *lec1* correlated with *lec2* expression but otherwise generally showed weak correlations.

Canonical correlation analysis revealed significant correlations between the fungal and the cyanobacterial gene expression data set in six cases out of nine (Electronic Supplement, Table S11). The first six canonical correlations were deemed significant, suggesting substantial correlation of gene expression in the two organisms.

3.3 Single-gene analysis (ANOVA)

For a more detailed picture, multifactorial ANOVA was performed to assess correlation of expression with temperature, site, habitat, sea distance and elevation for each candidate gene separately. The single-gene analysis again revealed a significant upregulation of the fungal candidate genes *msn2*, *mot1*, *hhk2* (transcriptional stress response regulation), *swi10* and *UCRNP2_806* (DNA repair), *hsp88*, *hsp98*, *spp* (heat shock/protein repair) at 25°C relative to the reference genes *gpd1* and *tub2* (Electronic Supplement, Tables S4 and S5). Relative expression differences for one representative gene from each functional group are shown in Fig. 5.

-----Figure 5 -----

In contrast, *lec2* expression was significantly lower already at 15°C compared to 5°C (Electronic Supplement, Table S5). Expression of *lec1* was not affected significantly by increased temperatures. The results confirmed the PCA and correlation analysis findings

regarding groups of genes related in temperature response patterns. For all fungal candidates, expression differed significantly between sites and for *mot1*, *hhk2*, *hsp88*, *hsp98* and *UCRNP2_806* the temperature response also differed between sites (Electronic Supplement, Table S4) confirming the PCA results. The ANOVA further confirmed that these differences associated more strongly with sea distance and elevation than with habitat categories (sea vs. inland). Based on the F-values in the ANOVA test statistic, the effect of temperature on gene expression was far greater than the effect of sampling sites.

In the *Nostoc* photobiont, expression of the DNA repair genes *radA*, *recF*, *recN* and *recO* was significantly lower at 15°C compared to 5°C (relative to the *rnpB* and *secA* reference genes) (representatively shown for *radA* in Fig. 6; Tables S6 and S7), and correlated with decreased fungal *lec2* expression. Those data are in accord with the PCA results. While it is impossible to infer causality from those data, a tentative hypothesis would be that *lec2* influences expression of those and other photobiont genes.

-----Figure 6 -----

In contrast, expression of another gene putatively involved in DNA repair, *Npun_F4482,* was upregulated at increased temperatures (Fig. 6, Electronic Supplement, Table S6). Expression of the chaperone gene *groEL* was significantly decreased at 25°C whereas its transcription repressor *hrcA* was significantly upregulated at 15°C vs. 5°C (Fig. 6, Electronic Supplement, Table S7). However, two other cyanobacterial heat-shock genes, *dnaJ* and *hsp90* (representatively shown in Fig. 6), were significantly upregulated at increased temperatures, similar to the fungal heat shock genes *hsp88* and *hsp98* (Figs. 5 and 6), thus confirming the correlation analysis results (Fig. 4). For the DNA repair genes *recF*, *recN* and *radA*, expression differed significantly between sites and *recF* and *recO* also showed different temperature responses between sites (Electronic Supplement, Table S6). While there was no significant difference between sea and inland habitats, the expression of all four DNA repair genes showed a significant correlation with increasing elevation, thus confirming the PCA results. Expression of *groEL, hrcA*, *dnaJ, hsp90* and *Npun_F4482* differed considerably between sampling sites. For *Npun_F4482* and *hsp90,* there was also a difference in expression between habitat categories (sea vs. inland). In general, elevation above sea level and sea distance showed stronger correlations with expression differences for many more genes than the difference between sea-exposed vs. inland habitats (Electronic Supplement, Table S6).

4. Discussion

For our first question whether increased temperatures cause a stress response reflected in altered mRNA levels, we found significant changes both for fungal and *Nostoc* candidate genes. The study identified two gene groups with contrasting patterns, one showing a decrease in mRNA levels associated with a temperature shift from 5°C to 15°C and another characterized by increased levels after a switch from 15°C to 25°C. The mRNA levels of cyanobacterial DNA repair genes and the fungal *lec2* gene were downregulated already at 15°C compared to 5°C whereas most fungal heat shock genes, DNA repair genes and genes involved in transcriptional regulation of stress responses were upregulated at 25°C. Gene expression also differed between sites with differences showing correlation with increasing sea distance and elevation above sea level. However, the site effect was by far smaller than the temperature effect. For *lec2* and the cyanobacterial DNA repair genes, the temperature response in gene expression also differed significantly between sites. This might be an indication that increasing temperatures can affect lichens from higher elevations in a different way than lichens from lower elevations. This finding contributed to answering the second research question asking whether lichens from different sites differ in temperature responses.

4.1 Temperature responses

A temperature increase from 5°C to 15°C and further to 25°C strongly affected the expression of genes involved in transcriptional regulation of fungal stress responses as well as of several fungal and cyanobacterial genes encoding heat-shock (protein refolding) and DNA repair proteins. A subset of the fungal candidate genes (*mot1, hhk2, msn2*) is known to play an important role in stress responses through transcriptional regulation of heat stress-responsive genes (heat shock genes, DNA repair genes) (Dasgupta et al. 2007; Gasch 2007; Liu et al. 2013; Lopez-Maury et al. 2008; Schmoll 2008). Hhk2 and Msn2 are part of the Hog1 pathway, pivotal in inducing transcription of stress-response element containing genes (Enjalbert et al. 2006; Gasch 2007; Liu et al. 2013; Martínez-Pastor et al. 1996; Schmoll 2008; Schüller et al. 1994; Stock et al. 2000). As a member of the Swi/Snf2 family, Mot1 plays a role in transcriptional regulation of stress responsive TATA-box containing genes (Dasgupta et al. 2007; Lopez-Maury et al. 2008; Sikorski and Buratowski 2009; Yasuhira et al. 1999; Zanton and Pugh 2004). The upregulated expression of these genes at 25°C indicates that this temperature represented a heat stress condition for the investigated samples of *P. membranacea*. In addition to transcriptional regulators of stress response, the study also revealed increased expression of specific candidate genes involved in heat shock and DNA

repair. As members of a broad heat-shock response network, the heat shock proteins Hsp88 and Hsp98 function in refolding and preventing aggregation of denatured proteins and have been shown to be upregulated in response to temperature stress (Doyle and Wickner 2009; Plesofsky-Vig and Brambl 1998; Vassilev et al. 1992; Wang et al. 2007). The signal peptide peptidase Spp belongs to a network of membrane proteases and is thought to play a role in protein quality control by collecting damaged membrane protein aggregates for later disposal (Dalbey et al. 2012). The Swi/Snf2 family member Swi10 has been shown to be involved in nucleotide excision repair under UV-stress (Yasuhira et al. 1999) and UCRNP2_806 possibly contributes to base-excision DNA repair (http://www.uniprot.org/uniprot/R1GVL6; accessed 10.07.2015). In line with previous findings, the upregulation of these genes indicates that a temperature of 25°C causes protein and DNA damage in *P. membranacea* calling for repair.

For the *Nostoc* photobiont, the results also showed upregulation of candidate genes involved in cyanobacterial heat stress response processes (*dnaJ, hsp90*) and DNA repair (*Npun_F4482*). Hsp90 prevents non-native signal transduction proteins from forming unproductive protein aggregates (Hartl 1996; Wiech et al. 1992) and contributes to general protein refolding under heat stress, similar to the Hsp40 protein DnaJ which is part of the Hsp70/Hsp40 cycle (Hartl 1996; King-Chuen and Wai 1998; Langer et al. 1992; Young 2001). Studies of fish, plants and cyanobacteria revealed increased gene expression of both *hsp90* and *dnaJ* under heat and oxidative stress (Hossain and Nakamoto 2003; Reddy et al. 2011). Confirming these findings, the temperatures applied in our study (15°C and 25°C) apparently call for protein and DNA repair in the *Nostoc* photobiont. However, expression of cyanobacterial SOS DNA repair genes (*radA, recF, recN* and *recO*) and of the heat shock gene *groEL* was significantly downregulated at increased temperatures, in contrast to other studies in bacteria (Melkani et al. 2005; Mendoza et al. 1996; Odsbu and Skarstad 2014; Rajaram and Apte 2010; Rostas et al. 1987; Sargentini and Smith 1986). In *Eschericia coli,* the RecN protein is recruited for repair of DNA double strand breaks followed by the recruitment of RecF and RecO and further RadA (Odsbu and Skarstad 2014; Sargentini and Smith 1986). Together with GroES, GroEL is part of the Hsp60/Hsp10 complex for protein damage repair (Goloubinoff et al. 1997; Melkani et al. 2005). The drop in *groEL* expression makes sense, in that the expression of the *groEL* repressor gene *hrcA* was upregulated at increased temperatures. The expression drop of the *Nostoc* SOS DNA repair genes could be explained by that the gene expression quantification after three hours of exposure may have coincided with a second stress response phase characterized by decreased expression after an initial increase. Sampling at several timepoints during exposure to thermal stress might

resolve this question, but this was beyond the scope of the present investigation. Interestingly, the correlation analysis revealed that the drop of cyanobacterial DNA repair gene expression was positively correlated with reduced expression of the fungal lectin gene *lec2* at 15°C. The expression drop of *lec2* is similar to the results of a study on coral larvae where a mannose-binding C-type lectin in the coral was downregulated at increased temperatures (Rodriguez-Lanetty et al. 2009). Fungal lectins such as *lec2* are thought to be involved in symbiont communication of plant-microbial and fungal-microbial symbioses (Antonyuk and Evseeva 2006; McCowen et al. 1986; Miao et al. 2012; Sharon and Lis 2004). In a wheat symbiosis with *Azospirillum brasilense,* different wheat lectin concentrations were shown to affect a range of metabolic processes such as nitrogen fixation in the rhizobacterial symbionts (Antonyuk and Evseeva 2006). Therefore, the correlated downregulation might indicate an interplay between the two symbionts at increased temperatures, providing an alternative explanation for the unexpected expression decrease of *Nostoc* DNA repair genes at 15°C. However, the connection between the fungal *lec2* and the cyanobacterial genes needs to be investigated further, e.g. by a comparison with the expression of genes in pure *Nostoc* cultures, in order to clarify the role of *lec2* in establishing and maintaining, or changing the symbiosis and to elucidate possible other roles.

A study on the cyanolichen *Peltigera scabrosa* (MacFarlane and Kershaw 1980) determined that 25°C represents a thermal stress condition for the lichen, reflected in altered nitrogenase activity, photosynthesis and respiration rate. In this study, the photobiont showed an overall lower stress tolerance than the mycobiont. Our results, that photobiont genes showed a stress response at 15°C but mycobiont genes at 25°C, are in accordance with the findings of MacFarlane and Kershaw (1980).

4.2 Habitat differences

In addition to the expression changes detected with increasing temperature, gene expression also differed significantly between sampling sites, especially the inland sites at Uxahryggir (UX4) and Hrauneyjar (LL3). It remains to be determined whether these site-related expression differences are associated with genetic variation or rather with long-term physiological acclimatization of individuals to local environmental conditions.

Site-specific expression differences were more strongly associated with increasing elevation above sea level than with habitat categories (sea-exposed vs. inland). A relationship between gene expression and elevation has been found for several species. For example, one study showed differential expression of nearly 200 transcripts between high-altitude and low-

494 altitude populations of the rufous-collared sparrow (*Zonotrichia capensis*), which were
495 mainly due to physiological acclimatization (Cheviron et al. 2008). In plants, expression of
496 flowering-inducing genes has been shown to differ significantly along an elevation gradient
497 (Suter et al. 2014). Our results underlined the complexity of the relation between expression
498 variation and the environment. In our system, other abiotic and biotic factors not assessed in
499 this study, such as soil properties, topology, nutrient availability or salt content might also be
500 correlated with gene expression differences between sites.

501 Additionally, for the majority of fungal stress-response genes, the temperature response
502 differed between sites, and a set of *Nostoc* DNA repair genes and fungal *lec2* further differed
503 in temperature response with increasing elevation. Such differences in response to various
504 stresses have been documented for a variety of species. One study on the Mediterranean shrub
505 *Atriplex halimus* (Ben Hassine et al. 2008) found a differential response to salt stress in
506 coastal vs. inland populations. Being more exposed to salt spray, coastal populations showed
507 a greater ability to produce glycinebetaine – an osmolyte facilitating water uptake for
508 lowering the osmotic potential. In brown trout (*Salmo trutta*), expression differences of
509 common heat-shock and osmoregulative genes were found between individuals from different
510 populations, correlating with local adaptation to salinity on a regional scale (Larsen et al.
511 2008). A study on the epiphytic lichen *Ramalina menziesii* revealed distinct differences in
512 photosynthetic and respirational responses at increased temperatures for lichens from
513 locations with different levels of temperature variation in California (Larson 1989). In that
514 study, individuals from locations with naturally higher temperature stress (inland sites: greater
515 temperature amplitude) also showed a lower stress response in the temperature treatments
516 compared to individuals from cool coastal rainforest areas. A recent study indicated that
517 coastal and inland populations of *R. menziesii* differ genetically (Sork and Werth 2014). In
518 line with these results, our current study indicates that long-term physiological acclimatization
519 or genetic differences affect the temperature response of lichens at the gene expression level.

520 # 5. Conclusion

521 Our study indicated that temperatures increases of 5°C to 15°C and 25°C represent heat
522 stress conditions for cold-acclimated individuals of the lichen forming fungus *P.*
523 *membranacea* and for their *Nostoc* photobionts. Increased temperatures led to altered mRNA
524 levels for genes involved in transcriptional induction of diverse fungal stress response
525 pathways and in differential expression of genes directly involved in protein refolding and
526 DNA damage repair in both symbionts. Most fungal stress response genes reacted to a

temperature increase to 25°C whereas in *Nostoc*, most such genes responded already at 15°C. Drop of the mRNA levels of a set of photobiont SOS DNA repair genes was strongly correlated with an mRNA drop of the fungal *lec2* gene at 15°C, indicating a possible interplay between symbionts. The study also revealed site-specific expression differences that could either be due to long-term acclimatization or to genetic differences among sites. For a large number of fungal and cyanobacterial genes, these differences had a strong correlation with environmental gradients of sea distance and elevation showing the incremental nature of the stress response. In conclusion, this study contributed to revealing molecular responses of *P. membranacea* lichens at moderately increased temperatures and therefore provides a solid base for further studies, including those of the interplay among symbionts.

6. Acknowledgements

We thank Zophonias O. Jónsson for contributing to planning the project and Ehsan P. Ahi for helpful advice when we conducted the experiments. We gratefully acknowledge Andrey Gagunashvili for providing *Nostoc* sequencing data, and Kristen M. Westfall and Hörður Guðmundsson for additional work on genomic data. Martin A. Mörsdorf kindly supported the statistical analyses. This work was funded by the Icelandic Research Fund (IRF) of The Icelandic Centre for Research (RANNÍS) (grants 120247021 and 141102-051 to SW and OSA). SW was supported by the European Commission within FP7 (Marie Curie Actions, IEF, 'LICHENOMICS', grant # 302589).

7. References

Ahi EP, Guethbrandsson J, Kapralova KH, Franzdottir SR, Snorrason SS, Maier VH, Jonsson ZO, 2013. Validation of reference genes for expression studies during craniofacial development in Arctic Charr. *PLoS One* **8**: e66389.

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S, 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* **1**: 95-111.

Albright R, Mason B, Miller M, Langdon C, 2010. Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 20400-20404.

Antonyuk LP, Evseeva NV, 2006. Wheat lectin as a factor in plant-microbial communication and a stress response protein. *Microbiology* **75**: 470-475.

Apte SK, Fernandes T, Badran H, Ballal A, 1998. Expression and possible role of stress-responsive proteins in *Anabaena*. *Journal of Biosciences* **23**: 399-406.

Aptroot A, van Herk CM, 2007. Further evidence of the effects of global warming on lichens, particularly those with *Trentepohlia* phycobionts. *Environmental Pollution* **146**: 293-298.

Athukorala SN, Piercey-Normore MD, 2015. Recognition- and defense-related gene expression at 3 resynthesis stages in lichen symbionts. *Canadian Journal of Microbiology* **61**: 1-12.

Ben Hassine A, Ghanem ME, Bouzid S, Lutts S, 2008. An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. *Journal of Experimental Botany* **59**: 1315-1326.

Burkle LA, Souza L, Genung MA, Crutsinger GM, 2013. Plant genotype, nutrients, and G × E interactions structure floral visitor communities. *Ecosphere* **4**: 1-20.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT, 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* **55**: 611-622.

Che S, Song W, Lin X, 2013. Response of heat-shock protein (HSP) genes to temperature and salinity stress in the antarctic psychrotrophic bacterium *Psychrobacter* sp. G. *Current Microbiology* **67**: 601-608.

581 Cheng Z, Caillet A, Ren B, Ding H, 2012. Stimulation of *Escherichia coli* DNA damage
582 inducible DNA helicase DinG by the single-stranded DNA binding protein SSB. *FEBS*
583 *Letters* **586**: 3825-3830.
584 Cheviron ZA, Whitehead A, Brumfield RT, 2008. Transcriptomic variation and plasticity in
585 rufous-collared sparrows (*Zonotrichia capensis*) along an altitudinal gradient.
586 *Molecular Ecology* **17**: 4556-4569.
587 Dalbey RE, Wang P, van Dijl JM, 2012. Membrane proteases in the bacterial protein
588 secretion and quality control pathway. *Microbiology and Molecular Biology Reviews*
589 **76**: 311-330.
590 Dasgupta A, Sprouse RO, French S, Aprikian P, Hontz R, Juedes SA, Smith JS, Beyer AL,
591 Auble DT, 2007. Regulation of rRNA synthesis by TATA-binding protein-associated
592 factor Mot1. *Molecular Cell Biology* **27**: 2886-2896.
593 Davis MBS, R. G.; Etterson, J. R., 2005. Evolutionary responses to changing climate. *Ecology*
594 **86**: 1704-1714.
595 del Campo EM, Catala S, Gimeno J, del Hoyo A, Martinez-Alberola F, Casano LM, Grube
596 M, Barreno E, 2013. The genetic structure of the cosmopolitan three-partner lichen
597 *Ramalina farinacea* evidences the concerted diversification of symbionts. *FEMS*
598 *Microbiology Ecology* **83**: 310-323.
599 di Pasqua R, Mauriello G, Mamone G, Ercolini D, 2013. Expression of DnaK, HtpG, GroEL
600 and Tf chaperones and the corresponding encoding genes during growth of *Salmonella*
601 Thompson in presence of thymol alone or in combination with salt and cold stress. *Food*
602 *Research International* **52**: 153-159.
603 Diaz EM, Vicente-Manzanares M, Sacristan M, Vicente C, Legaz ME, 2011. Fungal lectin of
604 *Peltigera canina* induces chemotropism of compatible *Nostoc* cells by constriction-
605 relaxation pulses of cyanobiont cytoskeleton. *Plant Signaling and Behavior* **6**: 1525-
606 1536.
607 Diffenbaugh NS, Field CB, 2013. Changes in ecologically critical terrestrial climate
608 conditions. *Science* **341**: 486-491.
609 Doyle SM, Wickner S, 2009. Hsp104 and ClpB: protein disaggregating machines. *Trends in*
610 *Biochemical Sciences* **34**: 40-48.
611 Ellis C, Coppins B, Dawson T, 2007a. Predicted response of the lichen epiphyte *Lecanora*
612 *populicola* to climate change scenarios in a clean-air region of Northern Britain.
613 *Biological Conservation* **135**: 396-404.

614 Ellis CJ, Coppins BJ, Dawson TP, Seaward MRD, 2007b. Response of British lichens to
615 climate change scenarios: Trends and uncertainties in the projected impact for
616 contrasting biogeographic groups. *Biological Conservation* **140**: 217-235.
617 Enjalbert B, Smith DA, Cornell MJ, Alam I, Nicholls S, Brown AJP, Quinn J, 2006. Role of
618 the Hog1 stress-activated protein kinase in the global transcriptional response to stress
619 in the fungal pathogen *Candida albicans*. *Molecular Biology of the Cell* **17**: 1018-1032.
620 Gasch AP, 2007. Comparative genomics of the environmental stress response in ascomycete
621 fungi. *Yeast* **24**: 961-976.
622 Goloubinoff P, Diamant S, Weiss C, Azem A, 1997. GroES binding regulates GroEL
623 chaperonin activity under heat shock. *FEBS Letters* **407**: 215-219.
624 Hartl FU, 1996. Molecular chaperones in cellular protein folding. *Nature* **381**: 571-580.
625 Hawksworth DLR, F., 1970. Qualitative scale for estimating sulphur dioxide air pollution in
626 England and Wales using epiphytic lichens. *Nature* **227**: 145-148.
627 Honegger R, 2012. The symbiotic phenotype of lichen-forming ascomycetes and their endo-
628 and epibionts. In: Hock B (ed), *Fungal Associations*. Springer, Berlin, pp. 288-326.
629 Hossain MM, Nakamoto H, 2003. Role for the cyanobacterial HtpG in protection from
630 oxidative stress. *Current Microbiology* **46**: 70-76.
631 Joneson S, Armaleo D, Lutzoni F, 2011. Fungal and algal gene expression in early
632 developmental stages of lichen-symbiosis. *Mycologia* **103**: 291-306.
633 King-Chuen C, Wai LT, 1998. Overexpression of dnaK/dnaJ and groEL confers freeze
634 tolerance to *Escherichia coli*. *Biochemical and Biophysical Research Communications*
635 **253**: 502-505.
636 Langer T, Chi L, Echols H, Flanagan J, Hayer MK, Hartl FU, 1992. Successive action of
637 DnaK, DnaJ and GroEL along the pathway of chaperone-mediated protein folding.
638 *Nature* **356**: 683-689.
639 Larsen PF, Nielsen EE, Koed A, Thomsen DS, Olsvik PA, Loeschcke V, 2008.
640 Interpopulation differences in expression of candidate genes for salinity tolerance in
641 winter migrating anadromous brown trout (*Salmo trutta* L.). *BMC Genetics* **9**: 12.
642 Larson DW, 1989. Differential heat sensitivity amongst four populations of the lichen
643 *Ramalina menziesii* Tayl. *New Phytologist* **111**: 73-79.
644 Legaz M-E, Fontaniella B, Millanes A-M, Vicente C, 2004. Secreted arginases from
645 phylogenetically far-related lichen species act as cross-recognition factors for two
646 different algal cells. *European Journal of Cell Biology* **83**: 435-446.

Liu Q, Ying SH, Li JG, Tian CG, Feng MG, 2013. Insight into the transcriptional regulation of Msn2 required for conidiation, multi-stress responses and virulence of two entomopathogenic fungi. *Fungal Genetics and Biology* **54**: 42-51.

Lopez-Maury L, Marguerat S, Bahler J, 2008. Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. *Nat Rev Genet* **9**: 583-593.

Lutzoni F, Miadlikowska J, 2009. Lichens. *Current Biology* **19**: R502-R503.

MacFarlane JD, Kershaw KA, 1980. Physiological-environmental interactions in lichens. IX. Thermal stress and lichen ecology. *New Phytologist* **84**: 669-685.

Manel S, Poncet BN, Legendre P, Gugerli F, Holderegger R, 2010. Common factors drive adaptive genetic variation at different spatial scales in *Arabis alpina*. *Molecular Ecology* **19**: 3824-3835.

Manoharan SS, Miao VP, Andrésson ÓS, 2012. LEC-2, a highly variable lectin in the lichen. *Symbiosis* **58**: 91-98.

Martínez-Pastor MT, Marchler G, Schüller C, Marchler-Bauer A, Ruis H, Estruch F, 1996. The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress-response element (STRE). *The EMBO Journal* **15**: 2227-2235.

McCowen SM, MacArthur L, Gates JE, 1986. Azolla fern lectins that specifically recognize endosymbiotic cyanobacteria. *Current Microbiology* **14**: 329-333.

Melkani GC, Zardeneta G, Mendoza JA, 2005. On the chaperonin activity of GroEL at heat-shock temperature. *The International Journal of Biochemistry and Cell Biology* **37**: 1375-1385.

Mendoza JA, Warren T, Dulin P, 1996. The ATPase activity of chaperonin GroEL is highly stimulated at elevated temperatures. *Biochemical and Biophysical Research Communications* **229**: 271-274.

Miadlikowska J, Lutzoni F, 2004. Phylogenetic classification of Peltigeralean fungi (Peltigerales, Ascomycota) based on ribosomal RNA small and large subunits. *American Journal of Botany* **91**: 449-464.

Miao VPW, Manoharan SS, Snæbjarnarson V, Andrésson ÓS, 2012. Expression of lec-1, a mycobiont gene encoding a galectin-like protein in the lichen *Peltigera membranacea*. *Symbiosis* **57**: 23-31.

679 Molina MC, Vicente C, 2000. Purification and characterization of two isolectins with arginase
680 activity from the lichen *Xanthoria parietina*. *Journal of Biochemistry and Molecular*
681 *Biology* **33**: 300-307.
682 Nagashima K, Kubota Y, Shibata T, Sakaguchi C, Shinagawa H, Hishida T, 2006.
683 Degradation of *Escherichia coli* RecN aggregates by ClpXP protease and its
684 implications for DNA damage tolerance. *The Journal of Biological Chemistry* **281**:
685 30941-30946.
686 Nimis PLF, F.; Bizzotto, A.; Codogno, M.; Skert, N., 2002. Bryophytes as indicators of trace
687 metals pollution in the River Brenta (NE Italy). *The Science of the Total Environment*
688 **286**: 233-242.
689 Odsbu I, Skarstad K, 2014. DNA compaction in the early part of the SOS response is
690 dependent on RecN and RecA. *Microbiology* **160**: 872-882.
691 Parmesan C, 2006. Ecological and evolutionary responses to recent climate change. *Annual*
692 *Review of Ecology, Evolution, and Systematics* **37**: 637-669.
693 Penuelas J, Sardans J, Estiarte M, Ogaya R, Carnicer J, Coll M, Barbeta A, Rivas-Ubach A,
694 Llusia J, Garbulsky M, Filella I, Jump AS, 2013. Evidence of current impact of climate
695 change on life: a walk from genes to the biosphere. *Global Change Biology* **19**: 2303-
696 2338.
697 Pinto F, Pacheco CC, Ferreira D, Moradas-Ferreira P, Tamagnini P, 2012. Selection of
698 suitable reference genes for RT-qPCR analyses in cyanobacteria. *PLoS One* **7**: e34983.
699 Plesofsky-Vig N, Brambl R, 1998. Characterization of an 88-kDa heat shock protein of
700 *Neurospora crassa* that interacts with Hsp30. *The Journal of Biological Chemistry* **273**:
701 11335–11341.
702 Rajaram H, Apte SK, 2010. Differential regulation of groESL operon expression in response
703 to heat and light in *Anabaena*. *Archives of Microbiology* **192**: 729-738.
704 Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O, Liss P, Riebesell U, Shepherd J,
705 Turley C, Watson A, 2005. *Ocean acidification due to increasing atmospheric carbon*
706 *dioxide*. The Royal Society, London.
707 Reddy PS, Thirulogachandar V, Vaishnavi CS, Aakrati A, Sopory SK, Reddy MK, 2011.
708 Molecular characterization and expression of a gene encoding cytosolic Hsp90 from
709 *Pennisetum glaucum* and its role in abiotic stress adaptation. *Gene* **474**: 29-38.
710 Rodriguez-Lanetty M, Harii S, Hoegh-Guldberg O, 2009. Early molecular responses of coral
711 larvae to hyperthermal stress. *Molecular Ecology* **18**: 5101-5114.

712 Rostas K, Morton SJ, Picksley SM, Lloyd RG, 1987. Nucleotide sequence and LexA
713 regulation of the *Escherichia coli* recN gene. *Nucleic Acids Research* **15**: 5041-5049.
714 Sargentini NJ, Smith KC, 1986. Quantitation of the involvement of the recA, recB, recC,
715 recF, recJ, recN, lexA, radA, radB, uvrD, and umuC genes in the repair of X-ray-
716 induced DNA double-strand breaks in *Escherichia coli*. *Radiation Research* **107**: 58-72.
717 Sato S, Ikeuchi M, Nakamoto H, 2008. Expression and function of a groEL paralog in the
718 thermophilic cyanobacterium *Thermosynechococcus elongatus* under heat and cold
719 stress. *FEBS Letters* **582**: 3389-3395.
720 Schmoll M, 2008. The information highways of a biotechnological workhorse - signal
721 transduction in *Hypocrea jecorina*. *BMC Genomics* **9**: 430.
722 Schröter DC, W.; Leemans, R.; Prentice, I. C., B. Araújo, M. B.; W. Arnell, N. W.; Bondeau,
723 A.; Bugmann, H.; Carter, T. R.; Gracia, C. A.; de la Vega-Leinert, A. C.; Erhard, M.;
724 Ewert, F.; Glendining, M.; House, J. I.; Kankaanpää, S.; Klein, R. J. T.; Lavorel, S.;
725 Lindner, M.; Metzger, M. J.; Meyer, J.; Mitchell, T. D.; Reginster, I.; Rounsevell, M.,
726 2005. Ecosystem service supply and vulnerability to global change in Europe. *Science*
727 **310**: 1333-1337.
728 Schüller C, Brewster JL, Alexander MR, Gustin MC, Ruis H, 1994. The HOG pathway
729 controls osmotic regulation of transcription via the stress response element (STRE) of
730 the *Saccharomyces cerevisiae* CTT1 gene. *The EMBO Journal* **13**: 4382-4389.
731 Sharon N, Lis H, 2004. History of lectins: from hemagglutinins to biological recognition
732 molecules. *Glycobiology* **14**: 53R-62R.
733 Sikorski TW, Buratowski S, 2009. The basal initiation machinery: beyond the general
734 transcription factors. *Current Opinion in Cell Biology* **21**: 344-351.
735 Sork VL, Davis FW, Westfall R, Flint A, Ikegami M, Wang H, Grivet D, 2010. Gene
736 movement and genetic association with regional climate gradients in California valley
737 oak (*Quercus lobata* Née) in the face of climate change. *Molecular Ecology* **19**: 3806-
738 3823.
739 Sork VL, Werth S, 2014. Phylogeography of *Ramalina menziesii*, a widely distributed lichen-
740 forming fungus in western North America. *Molecular Ecology* **23**: 2326-2339.
741 Stock AM, Robinson VL, Goudreau PN, 2000. Two-component signal transduction. *Annual*
742 *Review of Biochemistry* **69**: 183-215.
743 Suter L, Ruegg M, Zemp N, Hennig L, Widmer A, 2014. Gene regulatory variation mediates
744 flowering responses to vernalization along an altitudinal gradient in *Arabidopsis*. *Plant*
745 *Physiology* **166**: 1928-1942.

746 Timmusk S, Wagner EGH, 1999. The plant growth promoting Rhizobacterium *Paenibacillus*
747 *polymyxa* induces changes in *Arabidopsis thaliana* gene epression: A possible
748 connection between biotic and abiotic stress responses. *Molecular Plant-Microbe*
749 *Interactions* **12**: 951-959.
750 Tukey JW, 1949. Comparing individual means in the analysis of variance. *Biometrics* **5**: 99-
751 114.
752 Vassilev AO, Plesofsky-Vig N, Brambl R, 1992. Isolation, partial amino acid sequence, and
753 cellular distribution of heat-shock protein hsp98 from *Neurospora crassa*. *Biochimica et*
754 *Biophysica Acta* **1156**: 1-6.
755 Voloshin ON, Vanevski F, Khil PP, Camerini-Otero RD, 2003. Characterization of the DNA
756 damage-inducible helicase DinG from *Escherichia coli*. *The Journal of Biological*
757 *Chemistry* **278**: 28284-28293.
758 Wang X-Y, Easton DB, Subjeck JR, 2007. Large mammalian hsp70 family proteins, hsp110
759 and grp170, and their roles in biology and cancer therapy. *Protein Reviews* **7**: 178-205.
760 Wang Y-Y, Liu B, Zhang X-Y, Zhou Q-M, Zhang T, Li H, Yu Y-F, Zhang X-L, Hao X-Y,
761 Wang M, Wang L, Wei J-C, 2014. Genome characteristics reveal the impact of
762 lichenization on lichen-forming fungus *Endocarpon pusillum* Hedwig (Verrucariales,
763 Ascomycota). *BMC Genomics* **15**.
764 Wang Y-Y, Zhang X-Y, Zhou Q-M, Zhang X-L, Wei J-C, 2015. Comparative transcriptome
765 analysis of the lichen-forming fungus *Endocarpon pusillum* elucidates its drought
766 adaptation mechanisms. *Science China. Life Sciences* **58**: 89-100.
767 Whitman DW, Agrawal AA, 2009. What is phenotypic plasticity and why is it important? In:
768 Whitman DW, Ananthakrishnan TN (eds), *Phenotypic plasticity of insects: mechanisms*
769 *and consequences*. Science Publishers, Enfield, NH, pp. 1-63.
770 Wiech H, Buchner J, Zimmermann R, Jakob U, 1992. Hsp90 chaperones protein folding *in*
771 *vitro*. *Nature* **358**: 169-170.
772 Wright K, 2013. Package 'corrgram'. Website http://cran.r-
773 project.org/web/packages/corrgram/.
774 Yahr R, Vilgalys R, Depriest PT, 2004. Strong fungal specificity and selectivity for algal
775 symbionts in Florida scrub *Cladonia* lichens. *Molecular Ecology* **13**: 3367-3378.
776 Yasuhira S, Morimyo M, Yasui A, 1999. Transcription dependence and the roles of two
777 excision repair pathways for UV damage in fission yeast *Schizosaccharomyces pombe*.
778 *The Journal of Biological Chemistry* **274**: 26822-26827.

779 Young JC, 2001. Hsp90: a specialized but essential protein-folding tool. *The Journal of Cell*
780 *Biology* **154**: 267-274.
781 Zahrl D, Wagner A, Tscherner M, Koraimann G, 2007. GroEL plays a central role in stress-
782 induced negative regulation of bacterial conjugation by promoting proteolytic
783 degradation of the activator protein TraJ. *Journal of Bacteriology* **189**: 5885-5894.
784 Zanton SJ, Pugh BF, 2004. Changes in genomewide occupancy of core transcriptional
785 regulators during heat stress. *Proceedings of the National Academy of Sciences of the*
786 *United States of America* **101**: 16843-16848.
787

788 # 8. Tables and Figures

789

790 **Table 1.** Sampling site information; Table includes location name, habitat, distance to the sea
791 [km] and elevation above sea level [m].

Site	Location	Habitat	Sea distance [km]	Elevation [m]
RY10	Strandakirkja	Sea-exposed	0.0	10
RY9	Garður	Sea-exposed	0.0	7
RY7	Grindavík	Sea-exposed	0.0	30
HF2	Kjalarnes	Sea-exposed	0.1	31
UX4	Uxahryggir	Inland	20.0	415
LA1	Gjábakkaheiði	Inland	30.0	184
HV3	Árnes	Inland	40.0	68
LL3	Hrauneyjar	Inland	70.0	285

792

793

794 **Table 2.** Reference and candidate genes for *Peltigera membranacea* and *Nostoc*. Table
795 includes gene names and functional descriptions.

796

Gene	Function
***Pmem* reference**	
gpd1	Glyceraldehyde-3-phosphate dehydrogenase
tub2	Beta-tubulin
***Pmem* target**	
lec1	Galectin/galactose-binding lectin protein Lec1
lec2	Galectin/galactose-binding lectin protein Lectin-2
msn2	Zinc finger protein Msn2 (C2H2 type)
hsp88	Molecular chaperone Hsp88
hsp98	Molecular chaperone Hsp98/Hsp104/ClpA
mot1	TATA-binding protein-associated factor Mot1
swi10	Mating-type switching protein Swi10
spp	Peptidase A22B, signal peptide peptidase
hhk2	Histidine kinase HHK2p
UCRNP2_806	HhH-GPD family base excision DNA repair protein
***Nostoc* reference**	
rnpB	RNase P subunit B
secA	Preprotein translocase subunit SecA
***Nostoc* target**	
groEL	Molecular chaperone GroEL/Cpn60
radA	DNA repair protein RadA
hsp90	Molecular chaperone Hsp90 (ATPase domain protein)
Npun_F4482	Helicase domain protein (DNA phosphorothioation system restriction enzyme)
dnaJ	Molecular chaperone DnaJ
hrcA	Heat-inducible transcription repressor HrcA
recO	DNA replication and repair protein RecO
recF	DNA replication and repair protein RecF (recombinase RecF)
recN	DNA recombination/repair protein RecN

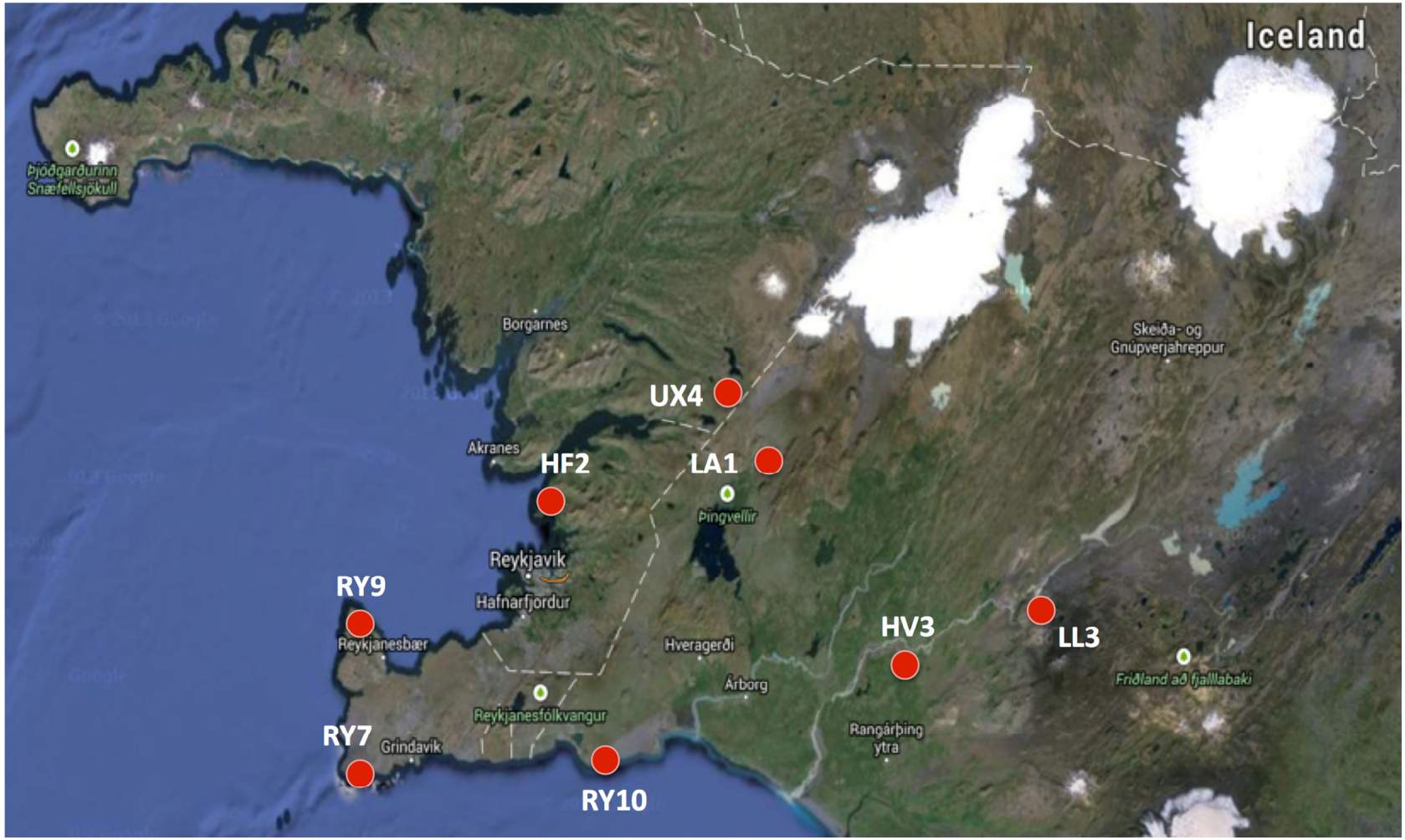
797

Table 3. Significance (p-values) of multivariate ANOVA for PC1-3. Column 2-4: ANOVA results of linear mixed-effects model using temperature and site as fixed and individual as random factor. Column 5-6: ANOVA results of linear mixed-effects model using temperature and habitat as fixed and site and individual within site as random factors. Column 7-8: temperature and sea-distance as fixed and site and individual as random factors. Column 9-10: temperature and elevation as fixed and site and individual as random factors. T: temperature, S: site, H: habitat, D: sea distance, E: elevation. Significant effects and/or interactions are marked in bold.

PC	Temp.	Site	T x S	Habitat	T x H	Sea distance	T x D	Elevation	T x E
1	**<0.001**	**<0.001**	0.572	**0.014**	0.093	**0.014**	0.072	**<0.001**	**0.017**
2	**<0.001**	**<0.001**	**0.016**	0.464	0.367	0.121	0.111	0.488	0.108
3	0.051	**<0.001**	0.998	0.169	0.697	0.485	0.656	**0.019**	0.729

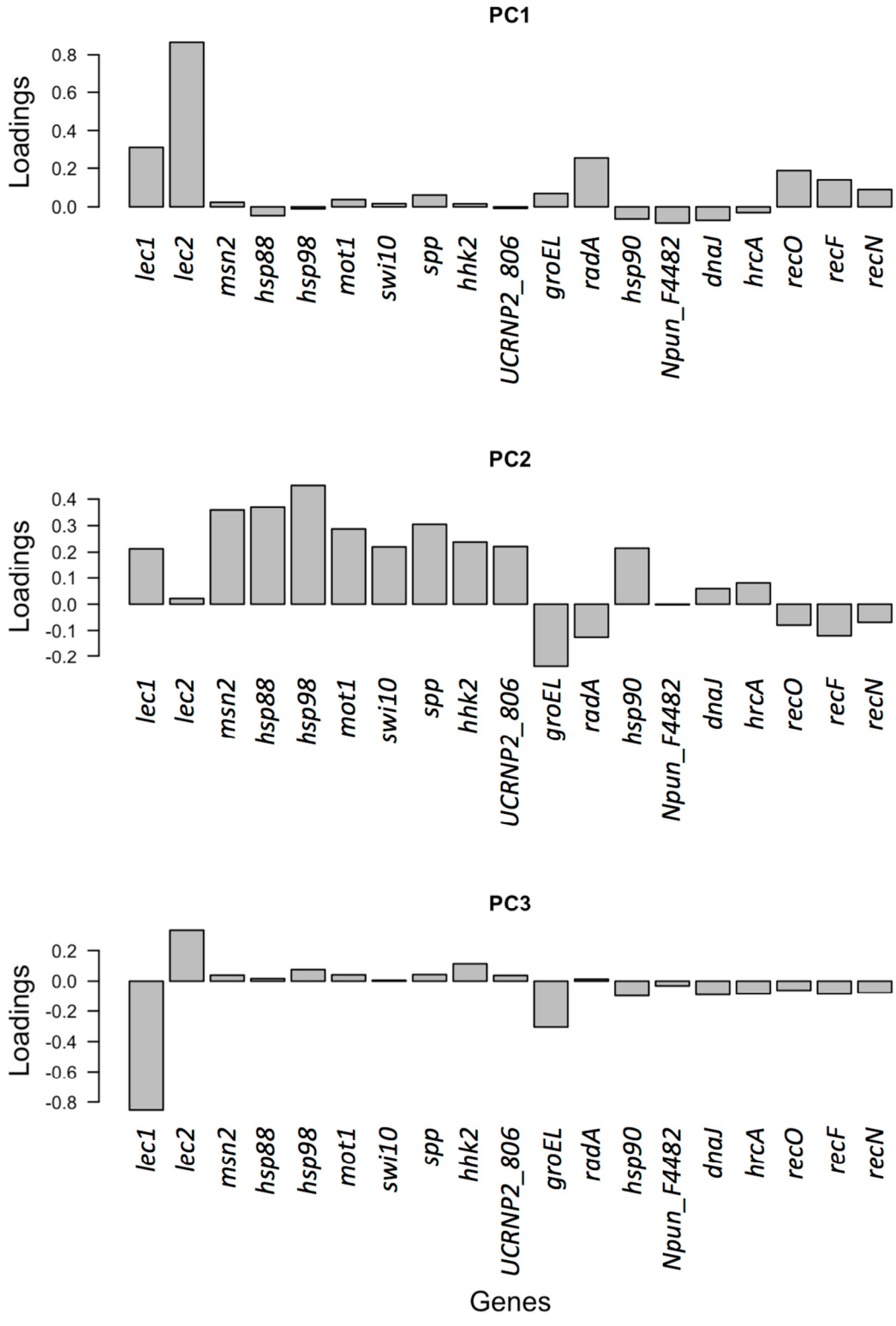
Table 4: Significance (p-values) of Tukey´s honest significant difference test for the effect of the three temperature treatments (5°C vs. 15°C, 5°C vs. 25°C and 15°C vs. 25°C) in principal components PC1, PC2, and PC3 (linear mixed-effects model: temperature and site = fixed factors, individual = random factor). Significant values are marked in bold letters.

PC	5°C vs. 15°C	5°C vs. 25°C	15°C vs. 25°C
1	**<0.001**	**<0.001**	0.419
2	0.252	**<0.001**	**<0.001**
3	**0.046**	0.210	0.757



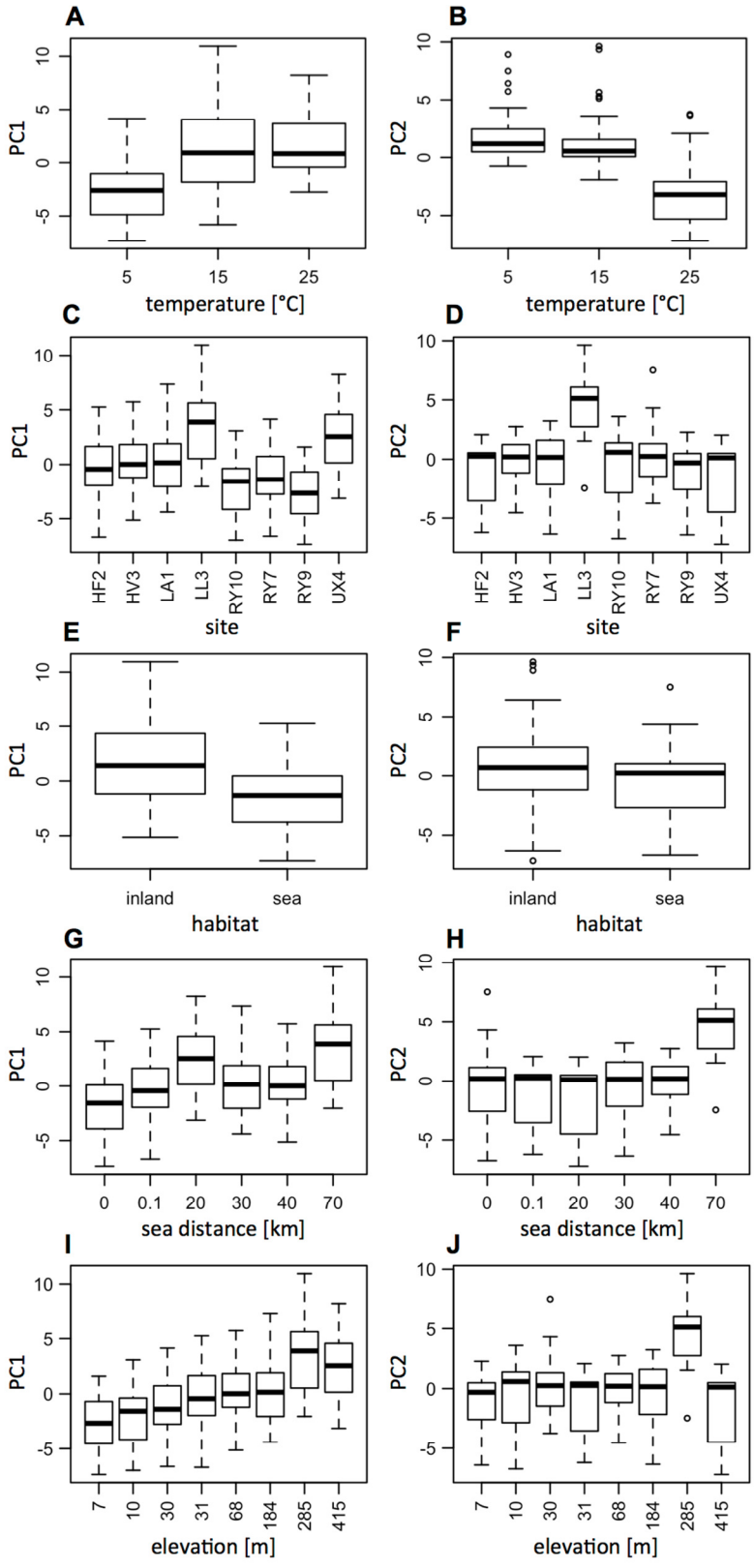
818

819 Figure 1. Map of SW-Iceland showing chosen sampling site locations. Located close to the sea were RY10 (Strandakirkja), RY9 (Garður), RY7
820 (Grindavík), and HF2 (Kjalarnes). Inland sites were UX4 (Uxahryggir), LA1 (Gjabakkaheiði), HV3 (Árnes), and LL3 (Hrauneyjar).



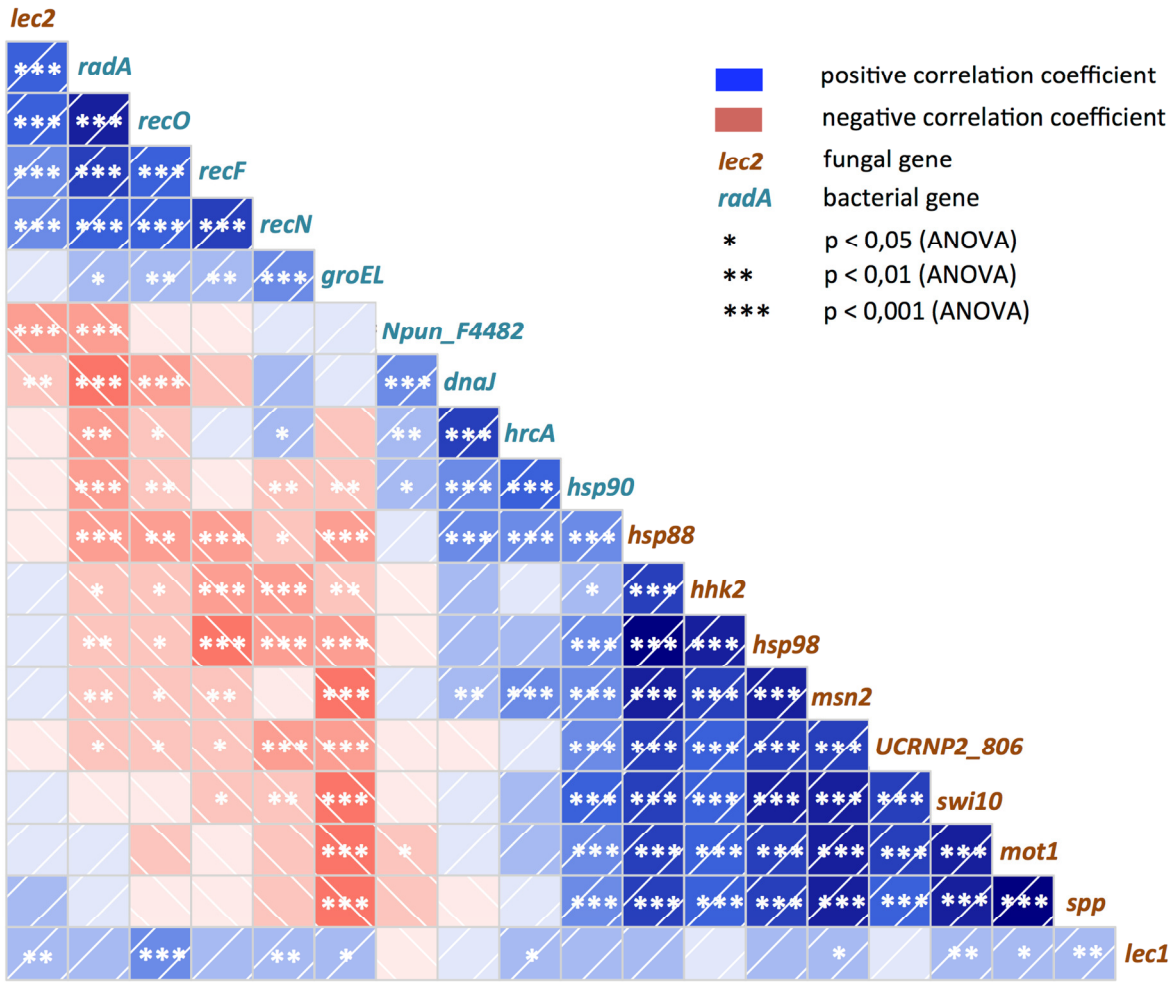
821

822 Figure 2. Axis loadings of the first three principle components, PC1 through PC3. The figure
823 shows to what extent individual fungal and cyanobacterial genes contribute to each principle
824 component. Positive or negative loadings represent the direction of the contribution relative to
825 the PC vector.



826

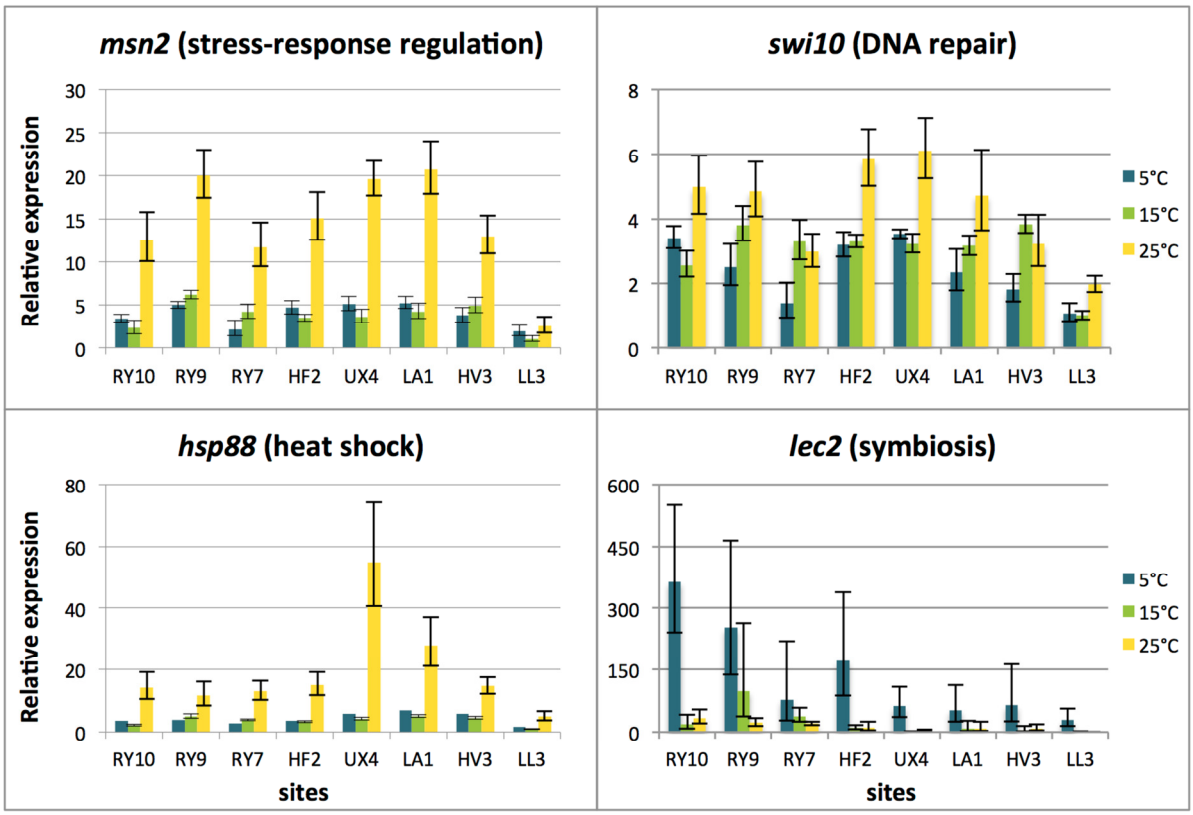
827 Figure 3. Boxplots of differences in gene expression between temperature, sampling sites,
828 habitats, along sea distance and elevation gradients for principle components PC1 and PC2.
829 (A-B) Comparison of differences in PC1 and PC2 between temperatures (5°C, 15°C, 25°C),
830 (C-D) between sampling sites, (E-F) between habitats, (G-H) with sea distance and (I-J) with
831 elevation above sea level. Y-axis: axis scores (eigenvalues) of PC1 and PC2. Boxes enclose
832 upper and lower quartile around the median (fat line).



833

834 Figure 4. Correlation analysis for fungal and cyanobacterial candidate genes; shown are
835 positive (blue) or negative (red) correlations in gene expression patterns for all selected
836 mycobiont and photobiont (*Nostoc*) genes, including interspecific correlations; genes were
837 grouped according to correlation. The color-shading scheme is based on correlation
838 coefficients (for values see Electronic Supplement, Table S10); stars represent significant p-
839 values.

840



841

842 Figure 5. Relative gene expression (RQ) of fungal heat shock genes *msn2*, *swi10*, *hsp88* and
843 *lec2*, plotted for all sampling sites at 5°C, 15°C and 25°C; the sample with lowest expression
844 over all sites and temperatures was set as reference (RQ = 1). Presented genes were chosen as
845 representative genes for stress response regulation (*msn2*), DNA repair (*swi10*), heat shock
846 (*hsp88*) and symbiosis (*lec2*). Sampling sites were arranged from sea-exposed (RY10) to
847 inland (LL3).

848

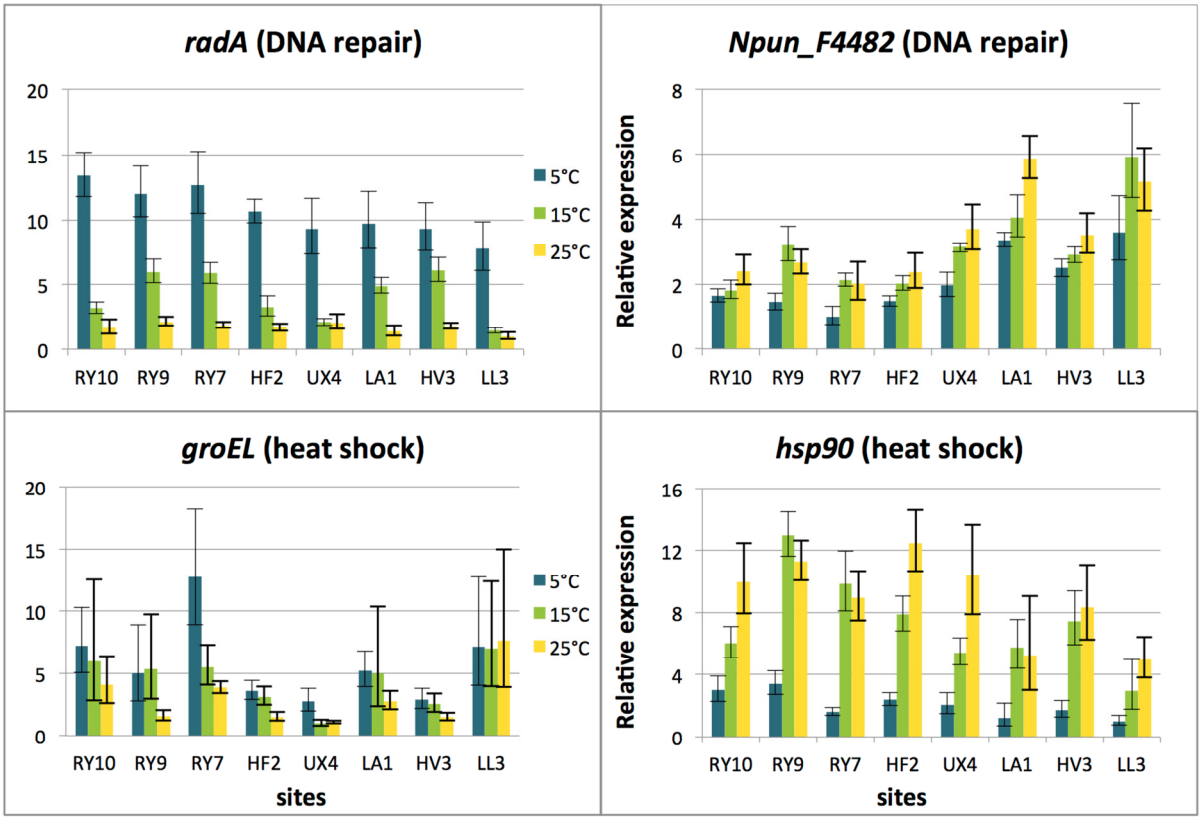


Figure 6. Relative gene expression (RQ) of cyanobacterial genes *radA*, *Npun_F4482*, *groEL* and *hsp90* for all sampling sites at 5°C, 15°C and 25°C; the sample with lowest expression was set as reference (RQ = 1); *radA* was chosen to represent the downregulated DNA repair gene set (*radA*, *recF*, *recN* and *recO*) and *hsp90* to represent upregulated heat-shock genes *hsp90* and *dnaJ*. For further information, see legend of Fig. 5.

Electronic Supplement to Steinhäuser, Andrésson, Pálsson & Werth (2016): *Fungal and cyanobacterial gene expression in a lichen symbiosis: Effect of temperature and location*

Supplementary Figures

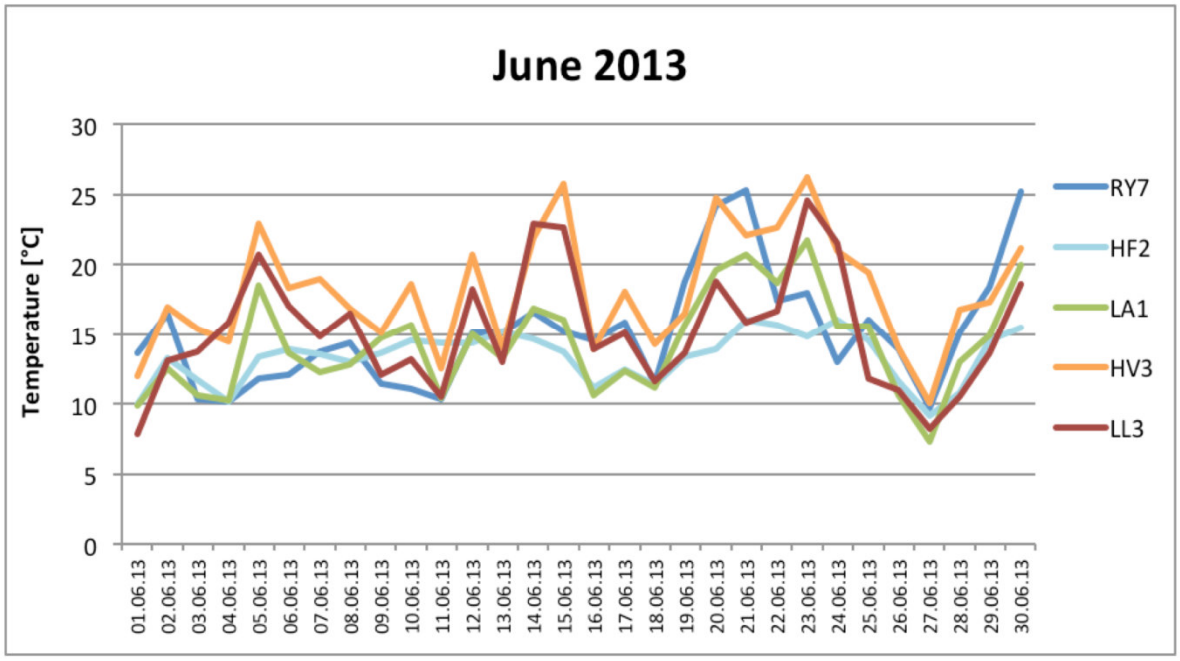


Figure S1. Daily mean temperatures at the sampling sites RY7 and HF2 (sea-exposed), as well as at LA1, HV3, and LL3 (inland) in June 2013. Mean temperature was calculated for every day based on hourly measurements.

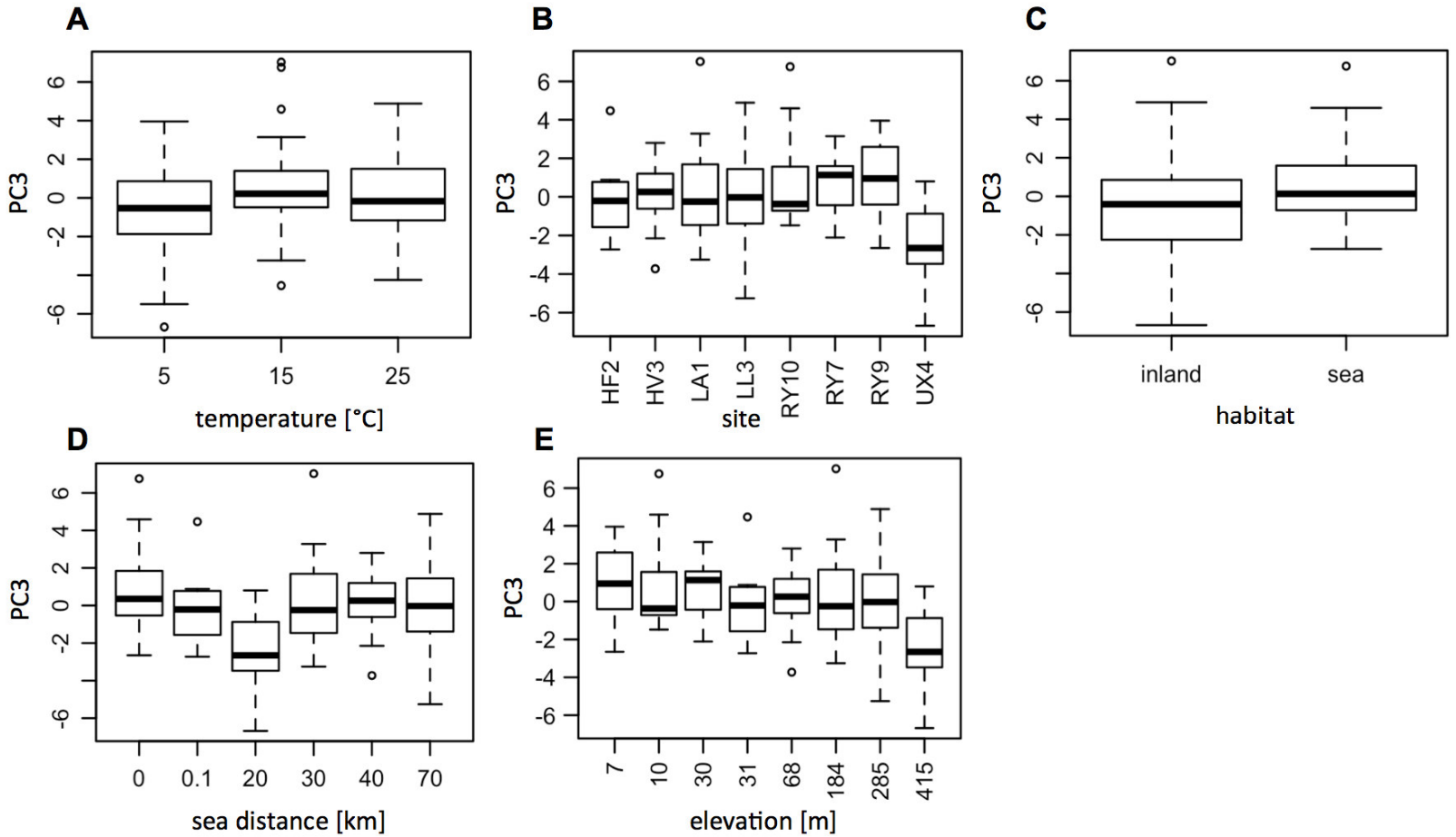


Figure S2. Differences in gene expression between temperature, sampling sites, habitats, along sea distance and elevation gradients for principle component PC3. (A) Comparison of differences in PC3 between temperatures (5°C, 15°C, 25°C), (B) between sampling sites, (C) between habitats, (D) with sea distance and (E) with elevation above sea level. Y-axis: axis scores (eigenvalues) of PC3. Boxes enclose upper and lower quartile around the median (fat line); upper and lower whiskers represent 95% of data (outliers shown as circles).

Supplementary Tables

Table S1. Reference gene variation in *Peltigera membranacea* and its *Nostoc* cyanobacterial photobiont. ANOVA results for linear mixed effects model with "site" (coastal; inland) and "temperature" (5°C, 15°C, 25°C) as fixed and "individual" as random factor for fungal and cyanobacterial reference genes.

Ref. gene	Factor	Estimate	SE	t-value	p-value
gpd1	(Intercept)	20.0226	0.3203	62.5040	**<2e-16**
	temp2	0.3256	0.4530	0.7190	0.4738
	temp3	1.0283	0.4530	2.2700	**0.0251**
	site2	-0.6298	0.4530	-1.3900	0.1672
	temp2:site2	-0.4229	0.6407	-0.6600	0.5106
	temp3:site2	0.0116	0.6407	0.0180	0.9856
tub2	(Intercept)	20.8417	0.4046	51.5130	**<2e-16**
	temp2	-0.2612	0.5722	-0.4560	0.6490
	temp3	0.7529	0.5722	1.3160	0.1910
	site2	-0.9204	0.5722	-1.6090	0.1100
	temp2:site2	-0.0748	0.8092	-0.0920	0.9270
	temp3:site2	0.1360	0.8092	0.1680	0.8670
rnpB	(Intercept)	16.1908	0.5023	32.2330	**<2e-16**
	temp2	0.5047	0.7104	0.7110	0.4790
	temp3	-0.0923	0.7104	-0.1300	0.8970
	site2	-0.5116	0.7104	-0.7200	0.4730
	temp2:site2	-0.5134	1.0046	-0.5110	0.6100
	temp3:site2	0.0480	1.0046	0.0480	0.9620
secA	(Intercept)	21.6534	0.4218	51.3400	**<2e-16**
	temp2	1.0067	0.5965	1.6880	0.0942
	temp3	0.6977	0.5965	1.1700	0.2445
	site2	-0.3897	0.5965	-0.6530	0.5148
	temp2:site2	-0.4176	0.8435	-0.4950	0.6216
	temp3:site2	0.0665	0.8435	0.0790	0.9374

Table S2. MANOVA results for the factors temperature, site, habitat, sea distance and elevation, showing the factor, the degrees of freedom (df), the multivariate test statistic Pillai's trace (Pillai), the approximate F-statistic, the degrees of freedom used for determining the value of the approximate F-statistic (num DF, den DF), and the p-value.

Factor	df	Pillai	approx F	num df	den df	p-value
temp	2	1.756	29.924	38	158	<0.001
site	7	3.510	4.446	133	588	<0.001
temp:site	14	2.648	1.117	266	1274	0.116
temp	2	1.684	27.255	38	194	<0.001
habitat	1	0.756	15.627	19	96	<0.001
temp:habitat	2	0.257	0.753	38	194	0.850
temp	2	1.686	27.414	38	194	<0.001
distance	1	0.740	14.411	19	96	<0.001
temp:distance	2	0.404	1.291	38	194	0.135
temp	2	1.694	28.235	38	194	<0.001
elevation	1	0.684	10.958	19	96	<0.001
temp:elevation	2	0.423	1.369	38	194	0.089

Table S3. Proportional contribution of principle components (PC1-19) to the total variance.

PC	SD	Proportion of variance	Cumulative proportion
PC1	3.589	0.324	0.324
PC2	3.348	0.282	0.606
PC3	2.322	0.136	0.741
PC4	1.477	0.055	0.796
PC5	1.417	0.050	0.847
PC6	1.218	0.037	0.884
PC7	1.089	0.030	0.914
PC8	0.886	0.020	0.934
PC9	0.721	0.013	0.947
PC10	0.683	0.012	0.958
PC11	0.584	0.009	0.967
PC12	0.550	0.008	0.975
PC13	0.472	0.006	0.980
PC14	0.437	0.005	0.985
PC15	0.425	0.005	0.990
PC16	0.375	0.004	0.993
PC17	0.339	0.003	0.996
PC18	0.322	0.003	0.999
PC19	0.239	0.001	1.000

Table S4. Significance (p-values) of multivariate ANOVAs for 10 fungal genes of the lichen *Peltigera membranacea*. Column 2-4: ANOVA results of linear mixed-effects model using temperature and site as fixed and lichen individual as random factor. Column 5-6: ANOVA results of linear mixed-effects model with temperature and habitat as fixed and site and individual within site as random factors. Column 7: temperature and sea-distance as linear factors (linear model). Column 8: temperature and elevation as linear factors (linear model). Significant effects on gene expression (ΔCt values) are marked in bold. For the latter two models, interaction terms were omitted because none were significant.

Fungal gene	Temp.	Site	T × S	Habitat	T × H	Sea distance	Elevation
hsp88	**<0.0001**	**<0.0001**	**0.0069**	0.7411	0.1085	**0.0037**	0.5120
hsp98	**<0.0001**	**<0.0001**	**0.0290**	0.5699	0.0796	**<0.0001**	0.9940
UCRNP2_806	**<0.0001**	**<0.0001**	**0.0037**	0.6022	0.5349	**<0.0001**	0.6880
msn2	**<0.0001**	**<0.0001**	0.0602	0.6704	0.2155	**<0.0001**	0.1091
mot1	**<0.0001**	**<0.0001**	**0.0261**	0.6044	0.3893	**<0.0001**	**0.0369**
hhk2	**<0.0001**	**0.0002**	**0.0095**	0.4847	0.3800	**0.0121**	0.9680
spp	**<0.0001**	**<0.0001**	0.6052	0.4749	0.9782	**<0.0001**	**0.0280**
swi10	**<0.0001**	**<0.0001**	0.1218	0.4512	0.9640	**<0.0001**	0.1670
lec1	0.5903	**<0.0001**	0.9046	**0.0353**	0.1855	**<0.0001**	**<0.0001**
lec2	**<0.0001**	**<0.0001**	0.8560	**0.0074**	0.1991	**<0.0001**	**<0.0001**

Table S5. Significance (p-values) of Tukey´s honest significant difference tests for the effect of three temperature treatments (5°C vs. 15°C, 5°C vs. 25°C and 15°C vs. 25°C) on gene expression (ΔCt values) for fungal candidate genes of *Peltigera membranacea* (linear mixed-effects model: temperature and site = fixed factors, lichen individual = random factor). Significant values are marked with bold print.

Fungal gene	5°C vs. 15°C	5°C vs. 25°C	15°C vs. 25°C
hsp88	0.1665	**<0.0001**	**<0.0001**
hsp98	**0.0383**	**<0.0001**	**<0.0001**
UCRNP2_806	**0.0358**	**<0.0001**	**0.0011**
msn2	0.6539	**<0.0001**	**<0.0001**
mot1	0.3001	**<0.0001**	**0.0001**
hhk2	**<0.0001**	**<0.0001**	**<0.0001**
spp	0.4230	**<0.0001**	**0.0004**
swi10	**0.0263**	**<0.0001**	**0.0004**
lec1	0.9559	0.7561	0.5789
lec2	**<0.0001**	**<0.0001**	0.9954

Table S6. Significance (p-values) of multivariate ANOVAs for cyanobacterial genes of the lichen *Peltigera membranacea*. Column 2-4: ANOVA results of linear mixed-effects model using temperature and site as fixed and lichen individual as random factor. Column 5-6: ANOVA results of linear mixed-effects model with temperature and habitat as fixed and site and individual within site as random factors. Column 7: temperature and sea-distance as linear factor (linear model). Column 8: temperature and elevation as linear factor (linear model). Significant effects on gene expression (ΔCt values) are marked with bold letters. For the latter two models, interaction terms were omitted because none were significant.

Nostoc gene	Temp.	Site	T × S	Habitat	T × H	Sea distance	Elevation
radA	**<0.0001**	**<0.0001**	**0.0254**	0.1662	0.7230	**<0.0001**	**0.0001**
recO	**<0.0001**	0.1023	**0.0380**	0.1462	0.7478	0.2400	**0.0074**
recN	**<0.0001**	**<0.0001**	0.4531	0.8699	0.5734	0.7980	**0.0032**
recF	**<0.0001**	**<0.0001**	0.9828	0.9404	0.6719	0.2200	**0.0356**
groEL	**0.0014**	**<0.0001**	0.9073	0.4896	0.2738	0.4460	0.0738
dnaJ	**<0.0001**	**<0.0001**	0.3926	0.8768	0.0645	0.0514	0.1100
dnaK	**<0.0001**	**0.0001**	0.8684	**0.0339**	0.7788	**<0.0001**	**0.0019**
Npun_F4428	**<0.0001**	**<0.0001**	0.7440	**0.0060**	0.4919	**<0.0001**	**<0.0001**
hrcA	**<0.0001**	**0.0001**	**0.0297**	0.3894	0.3752	0.1075	**0.0051**

Table S7. Significance (p-values) of Tukey's honest significant difference tests for the effect of the three temperature treatments (5°C vs. 15°C, 5°C vs. 25°C and 15°C vs. 25°C) on gene expression (ΔCt values) for fungal candidate genes (linear mixed-effects model: temperature and site = fixed factors, lichen individual = random factor). Significant values are marked with bold letters.

Nostoc gene	5°C vs. 15°C	5°C vs. 25°C	15°C vs. 25°C
radA	**<0.0001**	**<0.0001**	**<0.0001**
recO	**<0.0001**	**<0.0001**	**0.0024**
recN	**<0.0001**	**<0.0001**	0.3365
recF	0.4232	**<0.0001**	**<0.0001**
groEL	0.3238	**0.0010**	0.0627
dnaJ	0.1905	**<0.0001**	**<0.0001**
dnaK	**<0.0001**	**<0.0001**	0.2062
Npun_F4428	**<0.0001**	**<0.0001**	0.4940
hrcA	**0.0020**	**<0.0001**	**<0.0001**

Table S8: Primer sequences, amplicon length, melting temperature, and primer efficiency (±SE).

Gene	Forward Primer 5‘-3‘	Reverse Primer 5‘-3‘	Amplicon [bp]	T_M [°C]		Efficiency [%]
				F	R	
***Peltigera membranacea* reference**						
gpd1	CGCCGTGTTCTTGATCTCCTA	CGCCCCTCCATTCGTCATT	139	59,9	60,2	77,2 ± 0,04
tub2	CTGACACCACAGATTAGCCCTAG	CGCCAGCACCATCTAGTCC	144	60,2	60,2	79,0 ± 0,05
***Peltigera membranacea* target**						
lec1	ATCCTACGGCTTGAATAAGGGC	CGACGGATACAGTGGGAGGT	104	60,2	61,0	82,0 ± 0,04
lec2	CTCCAACATACACACCTTCACCA	GCGTCATAGGGCAGATTCACT	164	60,5	60,2	78,4 ± 0,02
msn2	CATATCTCATCGTGTCAAACCGC	GGATGGGTGAATCGCCTC	172	60,1	57,5	80,0 ± 0,04
hsp88	CAGACACGGAAGATAAGAAGAACGA	TGTAGCATCGTCGCCTTCG	167	60,6	60,2	75,1 ± 0,04
hsp98	GCTACTCCCCCGCATACG	CACCACAATCCTTCCATCGTCT	146	60,0	60,4	79,2 ± 0,03
mot1	GGACACCGTGATTTTCGTGG	GAAGAGGTCAAGGATTTGGTCTG	241	59,5	59,0	78,0 ± 0,06
swi10	CTCCAGCAGCAGCATCTGT	CGAATCAGAGCGAGAATTGGGTT	157	60,1	61,2	83,5 ± 0,03
spp	CGTCAAAGCAGAATACATCACTG	TCCATTACTGCCAGGGTTGTAA	174	58,1	59,4	80,2 ± 0,06
hhk2	ATGTTGTTTCCTTGGACATGGC	GATGTGAGCCTCCGTGGTA	185	59,7	58,8	83,2 ± 0,05
UCRNP2_806	CGCCACTTTCTTCCAACAAC	CGATCTTTTCCTCCAAGTCAAC	216	57,9	57,3	81,7 ± 0,04

Gene	Forward Primer 5‘-3‘	Reverse Primer 5‘-3‘	Amplicon [bp]	T_M [°C]		Efficiency [%]
				F	R	
***Nostoc* sp. reference**						
rnpB	GTGAGGATAGTGCCACAGAAA	CGCCTCTAGCGGCTCT	239	57,7	58,0	69,1 ± 0,06
secA	ATTACATCGTCCGCAATGGGG	GCTAGAGTTTGAGTTTCTGGCTG	145	60,8	60,0	81,7 ± 0,05
***Nostoc* sp. target**						
groEL	TGGTACAACTCTGGCTCACC	ACAGCACCATTCTGACCGG	153	59,3	60,0	79,2 ± 0,05
radA	TTTAGGCAACCGTGACGATCC	AACCCACCCGCAGAAGCA	237	60,7	61,9	71,5 ± 0,05
hsp90	CGATGCGGGGTGTGATTGAT	GCGGAAAAGTTCTTTGAGGCG	137	60,8	60,7	70,8 ± 0,05
Npun_F4482	TACCAGGATGCCCAAGAATGC	TACCACTACCAGTAGCCATTTTCAG	120	60,4	60,3	79,8 ± 0,03
dnaJ	CCTGGGTGTCTCTCGTGAC	GCGGGTTTCGGGTTCTGA	160	59,4	60,0	78,7 ± 0,02
hrcA	GGAACCAGAACCAGAGGATGC	CCCCAAAACTCCCACACTTCC	148	60,7	61,1	76,5 ± 0,05
recO	CTGTCACAACCAGAGATAATGC	TCAAGGTAGCAGAGCGAATAG	124	57,1	57,3	70,8 ± 0,04
recF	CTATTAACCAGACACCCGCTC	CGGGATAAATCTAGTTCGGCAAGA	160	58,2	60,5	77,2 ± 0,03
recN	TTGGATGCCATTGATGCCG	GCTGTGATTTCTCGGCTAATGA	182	58,9	58,8	76,4 ± 0,05

Table S9: Reference to Bioproject accessions and locus tags of *Nostoc* sp. (N) and to GenBank accessions of *Peltigera membranacea* (P).

Locus	**Protein**	**Accession**	**Locus_tag**	**Biont**
secA	Preprotein translocase subunit SecA	PRJNA279350	NPM_3413	N
rnpB	RNA component of RNaseP rnpB	PRJNA279350	NPM_r001	N
groEl	Molecular chaperone GroEL/Cpn60	PRJNA279350	NPM_6376	N
dnaJ	Molecular chaperone DnaJ	PRJNA279350	NPM_5294	N
Npun_F4482	Helicase domain protein (DNA phosphorothioation system restriction enzyme)	PRJNA279350	NPM_1098	N
recN	DNA recombination/repair protein RecN	PRJNA279350	NPM_0418	N
radA	DNA repair protein RadA	PRJNA279350	NPM_0979	N
recF	DNA replication and repair protein RecF (recombinase RecF)	PRJNA279350	NPM_4340	N
hrcA	Heat-inducible transcription repressor HrcA	PRJNA279350	NPM_4350	N
recO	DNA replication and repair protein RecO	PRJNA279350	NPM_1329	N
hsp90	Molecular chaperone Hsp90	PRJNA279350	NPM_5363	N
gpd1	Glyceraldehyde 3-phosphate dehydrogenase	AFJ45057		P
tub2	Beta-tubulin	AFJ45056		P
lec1	Galectin/galactose-binding lectin protein Lec1	AGC12988		P
lec2	Galectin/galactose-binding protein lectin-2	AGC54757		P
hsp98	Molecular chaperone Hsp98	KU904448		P
hhk2	histidine kinase Hhk2p	KU904449		P
UCRNP2_806	HhH-GPD family base excision DNA repair protein	KU904450		P
spp	Peptidase A22B, signal peptide peptidase	KU904451		P
swi10	Mating type switch protein Swi10	KU904452		P

msn2	Zink finger protein Msn2 (C2H2 type)	KU904453		P
hsp88	Molecular chaperone Hsp88	KU904454		P
mot1	TATA-binding protein-associated factor Mot1	KU904455		P

Table S10: Correlation in gene expression among loci. Above diagonal: correlation coefficients; below diagonal: significance of the correlation (p-value).

	lec1	**lec2**	**hsp90**	**Npun_F4482**	**hsp88**	**hsp98**	**UCRNP2_806**	**msn2**	**mot1**	**hhk2**	**spp**	**swi10**	**dnaJ**	**groEL**	**hrcA**	**radA**	**recF**	**recN**	**recO**
lec1		0.2514	0.1581	-0.1333	0.1614	0.1719	0.1245	0.2302	0.2013	0.0326	0.2400	0.2497	0.0723	0.2221	0.1891	0.1729	0.1722	0.2477	0.3101
lec2	0.0056		-0.1380	-0.3349	-0.0871	0.0186	-0.0063	0.0990	0.0992	0.1331	0.1598	0.0581	-0.2581	0.0527	-0.1275	0.5688	0.3139	0.3333	0.5293
hsp90	0.0845	0.1329		0.1993	0.3298	0.3062	0.3629	0.4116	0.3981	0.2060	0.4217	0.5222	0.3964	-0.2450	0.4912	-0.3704	-0.0869	-0.2348	-0.2373
Npun_F4482	0.1467	0.0002	0.0291		0.1365	-0.0395	-0.1316	0.0660	-0.2070	-0.0332	-0.1792	-0.0141	0.3454	0.0053	0.2731	-0.3439	-0.1002	0.0445	-0.0993
hsp88	0.0781	0.3443	0.0002	0.1372		0.8719	0.5740	0.8405	0.6657	0.7066	0.6345	0.6702	0.3377	-0.3731	0.3557	-0.3323	-0.3333	-0.2043	-0.2956
hsp98	0.0604	0.8401	0.0007	0.6680	0.0000		0.6371	0.7774	0.6355	0.7995	0.6802	0.7532	0.1617	-0.4005	0.1490	-0.2809	-0.4494	-0.3605	-0.2095
UCRNP2_806	0.1753	0.9457	0.0000	0.1519	0.0000	0.0000		0.6182	0.7069	0.5184	0.5642	0.6817	-0.0092	-0.3594	0.1180	-0.2204	-0.1955	-0.3534	-0.2013
msn2	0.0114	0.2819	0.0000	0.4738	0.0000	0.0000	0.0000		0.7403	0.6691	0.7171	0.7220	0.2486	-0.5071	0.3986	-0.2462	-0.2367	-0.1234	-0.2171
mot1	0.0275	0.2811	0.0000	0.0233	0.0000	0.0000	0.0000	0.0000		0.5299	0.8695	0.7975	0.0004	-0.4481	0.1478	0.0001	-0.0111	-0.1687	-0.1461
hhk2	0.7233	0.1472	0.0240	0.7188	0.0000	0.0000	0.0000	0.0000	0.0000		0.4760	0.5282	0.1721	-0.2509	0.1218	-0.2283	-0.3920	-0.3261	-0.2084
spp	0.0083	0.0813	0.0000	0.0502	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.8086	-0.0383	-0.4784	0.0892	0.0178	-0.0953	-0.1709	-0.0269
swi10	0.0060	0.5288	0.0000	0.8787	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0303	-0.4450	0.1575	-0.0913	-0.2044	-0.2627	-0.0326
dnaJ	0.4324	0.0044	0.0000	0.0001	0.0002	0.0777	0.9204	0.0062	0.9961	0.0602	0.6783	0.7422		0.0772	0.6400	-0.4351	-0.1496	0.1440	-0.3417
groEL	0.0147	0.5672	0.0070	0.9540	0.0000	0.0000	0.0000	0.0000	0.0000	0.0057	0.0000	0.0000	0.4018		-0.1778	0.2215	0.2612	0.3416	0.2551
hrcA	0.0385	0.1653	0.0000	0.0025	0.0000	0.1043	0.1993	0.0000	0.1073	0.1849	0.3324	0.0857	0.0000	0.0520		-0.2941	0.0580	0.1908	-0.1893
radA	0.0590	0.0000	0.0000	0.0001	0.0002	0.0019	0.0156	0.0067	0.9995	0.0121	0.8471	0.3212	0.0000	0.0151	0.0011		0.6542	0.5243	0.7417
recF	0.0600	0.0005	0.3454	0.2761	0.0002	0.0000	0.0324	0.0092	0.9038	0.0000	0.3006	0.0252	0.1030	0.0040	0.5294	0.0000		0.6490	0.4437
recN	0.0064	0.0002	0.0098	0.6293	0.0252	0.0000	0.0000	0.1794	0.0655	0.0003	0.0620	0.0037	0.1167	0.0001	0.0369	0.0000	0.0000		0.4615
recO	0.0006	0.0000	0.0091	0.2805	0.0010	0.0217	0.0274	0.0172	0.1114	0.0224	0.7706	0.7234	0.0001	0.0049	0.0384	0.0000	0.0000	0.0000	

Table S11. Canonical correlation analysis of ten *Peltigera membranacea* variables (gene expression of *lec1, lec2, msn2, hsp88, hsp98, mot1, swi10, spp, hh2,* and *UCRNP2_806*) with nine *Nostoc* sp. variables (gene expression of *groEL, radA, hsp90, Npun_F4482, dnaJ, hrcA, recO, recF,* and *recN*). The analysis tests the null hypothesis that the canonical correlations in the current row and all that follow are zero. The table reports the canonoical correlation coefficient (CanR), the coefficient of determination of the canonical correlation (CanRSQ), the eigenvalue (Eigen), the percent of variance explained by the model (Percent), the cumulative percentage of variance (Cum%), Wilk's lambda (WilksL), the F value, the degrees of freedom (df), and the statistical significance level (p-value) of the test.

	CanR	CanRSQ	Eigen	Percent	Cum%	WilksL	F	df1	df2	p-value
1	0.8163	0.6664	1.9974	35.86	35.86	0.0249	5.5913	90	695	<0.0001
2	0.7663	0.5871	1.4221	25.53	61.39	0.0746	4.6416	72	628	<0.0001
3	0.6630	0.4395	0.7843	14.08	75.47	0.1807	3.7391	56	560	<0.0001
4	0.6430	0.4134	0.7048	12.65	88.13	0.3225	3.1916	42	491	<0.0001
5	0.4759	0.2265	0.2928	5.26	93.38	0.5498	2.2693	30	422	0.0002
6	0.4089	0.1672	0.2008	3.61	96.99	0.7108	1.9112	20	353	0.0111
7	0.3501	0.1226	0.1397	2.51	99.50	0.8535	1.4574	12	283	0.1398
8	0.1482	0.0220	0.0224	0.40	99.90	0.9727	0.5015	6	216	0.8069
9	0.0739	0.0055	0.0055	0.10	100.00	0.9945		2		