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Ecophysiology and genetic structure of polar versus temperate populations of the lichen *Cetraria aculeata*

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Abstract We studied polar and temperate samples of the lichen *Cetraria aculeata* to investigate whether genetical differences between photobionts are correlated with physiological properties of the lichen holobiont. Net photosynthesis and dark respiration (DR) at different temperatures (from 0 to 30 °C) and photon flux densities (from 0 to 1,200 µmol m^{-2} s^{-1}) were studied for four populations of *Cetraria aculeata*. Samples were collected from maritime Antarctica, Svalbard, Germany and Spain, representing different climatic situations. Sequencing of the photobiont showed that the investigated samples fall in the polar and temperate clade described in Fernández-Mendoza et al. (Mol Ecol 20:1208–1232, 2011). Lichens with photobionts from these clades differ in their temperature optimum for photosynthesis, maximal net photosynthesis, maximal DR and chlorophyll content. Maximal net photosynthesis was much lower in Antarctica and Svalbard than in Germany and Spain. The difference was smaller when rates were expressed by chlorophyll content. The same is true for the temperature optima of polar (11 °C) and temperate (15 and 17 °C) lichens. Our results indicate that lichen mycobionts may adapt or acclimate to local environmental conditions either by selecting algae from regional pools or by regulating algal cell numbers (chlorophyll content) within the thallus.

Keywords Photosynthesis · Lichens · *Cetraria aculeata* · *Trebouxia jamesii* · Acclimation · Genetic adaptation

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Introduction

Lichens are highly specialised symbiotic associations of a mycobiont (the fungal partner) and an autotrophic photobiont (green algae and/or cyanobacteria) that supplies the fungus with carbohydrates. Their ability to withstand unfavourable environmental conditions in a desiccated, physiologically inactive state makes them pioneers in many ecosystems, especially in polar and alpine regions, where competition from vascular plants is low. The distributional ranges of many lichen species are generally larger than those of vascular plants and often extend over several biomes.

The wide ecological amplitude of many lichens is probably facilitated by their ability to display physiological adaptations to different climatic parameters such as temperature, moisture availability and light intensity. For example, the temperature optima for photosynthesis may

vary greatly between polar and temperate lichen species (Green 2009) or between individuals of the same species sampled in different biomes. These intraspecific differences may affect light compensation points (Sonesson et al. 1992), or photosynthetic rates, chlorophyll content and the number of photobiont cells (Sancho et al. 2000). The different physiological properties in geographically distinct individuals may be due to acclimation effects, i.e. the same genotype exhibiting different photosynthetic properties when grown under different climatic regimes. Many authors have also reported annual acclimation effects (Lange and Green 2005; Vráblíková et al. 2006, among others). The acclimation ability of a species is genetically fixed (Pigliucci 2001). Kershaw (1985) introduced the idea that lichens may adopt a wide range of acclimation strategies, for example a change of photobiont unit size, photobiont unit density, changes in energy transduction and alteration of enzymatic rates.

Another possible acclimation strategy of lichens has recently gained more attention among lichenologists. Lichen mycobionts are able to form symbioses with genetically different photobionts, either different species or different evolutionary lineages of the same species, sometimes even within the same thallus (Piercey-Normore 2005; Fernández-Mendoza et al. 2011; Casano et al. 2011; Henskens et al. 2012). Several recent studies have indicated an influence of environmental factors on the photobionts associated with lichen mycobionts (Opanowicz and Grube 2004; Blaha et al. 2006; Yahr et al. 2006; Muggia et al. 2008; Werth and Sork 2010; Fernández-Mendoza et al. 2011; Peksa and Škaloud 2011; Henskens et al. 2012). These observations, together with ecophysiological studies on specimens of the same lichen species collected in different climatic zones (Sonesson et al. 1992; Schipperges et al. 1995; Sancho et al. 2000), support the notion that photobiont switches could result in ecotypic differentiation of lichens. One of the best studied examples of ecological adaptation by symbiont switching is the endosymbiosis between corals and dinoflagellates of the genus *Symbiodinium*, in which corals may change their symbiont type as an adaptive response to temperature-driven bleaching events (Baker et al. 2004; Jones et al. 2008). Recently published results also indicate that genetically different photobionts found in single lichen thalli display different physiological properties (Casano et al. 2011). Del Hoyo et al. (2011) suggested that switches in photobionts as well as up- and down-regulation of photobiont populations within the thallus may enable lichens to react to environmental stress. The different observations on acclimation or ecotypic differentiation in combination with symbiont switches in various symbioses have culminated in a number of different hypotheses, among them the "community adaptation hypothesis" (Friedmann and Sun 2005), which predicts that lichens adjust their temperature optima by changing the ratio of producers (photobionts) to consumers (mycobionts). The hologenome theory of evolution (Zilber-Rosenberg and Rosenberg 2008) even states that, in symbiotic systems, selection acts on the "hologenome", the genome of all partners involved in a symbiosis, and not only on each of the genomes separately.

It is plausible to assume that the adaptive physiological differences observed between lichen populations can at least partly be attributed to photobiont switches and/or up- and down-regulation of photobiont abundance. However, studies combining genetical and physiological methods, which could corroborate, e.g., the hologenome theory for lichens, are largely lacking. Fernández-Mendoza et al. (2011) have recently demonstrated that polar and temperate photobionts of the lichen *Cetraria aculeata* belong to two different haplotype groups of *Trebouxia jamesii* and that the genetic structure of the photobiont is best explained by climatic differences between sampling localities. *Cetraria aculeata* is a small fruticose lichen with a remarkable distribution and ecological niche. It occurs in polar tundra throughout the Arctic and Antarctic and in the (sub)alpine zone of most high mountain ranges, including the European Alps, the Rocky Mountains, the Andes and Afro-alpine Highlands. At the same time, it is found in open vegetation sites (e.g. steppes, maritime dunes, open woodlands) in temperate lowlands, where competition from vascular plants is low.

In this study, we investigate whether the physiological behaviour of *Cetraria* populations from different origins is correlated with the selection of a different algal partner and/or with the regulation of its relative abundance within the lichen. In particular, we compare photosynthetic temperature optima, maximal photosynthetic rates, and respiration rates, as well as chlorophyll contents, Chl *a/b* ratios and photobiont amounts, in thalli from four different temperate and polar populations. We try to find out whether there is any reason to assume that photobiont manipulation by the mycobiont, i.e. photobiont switching and up- and down-regulation of photobiont populations within the thallus, might increase the adaptive or acclimation potential of *C. aculeata*.

Materials and methods

Sampling

Lichen samples of *Cetraria aculeata* were collected at four different sites (Table 1): in the maritime Antarctic, Spain, Germany and on Svalbard. Samples from Antarctica were taken in December 2009, samples from Germany and Svalbard in July 2010, and samples from Spain in October

Table 1 Populations, individuals per population and collection data of *Cetraria aculeata* used in this study

Population	*n*	Latitude/longitude	Locality, year and collector	Genbank accession numbers (mycobiont)	Genbank accession numbers (photobiont)
Antarctica	7	62.24′370″S/58.66′676″W	Antarctica, King George Island, 2009, S. Domaschke and F. Fernández-Mendoza	JN243337	JN243330
Svalbard	7	78°10′07.7″N/15°56′30.9″E	Spitsbergen, Svalbard, Longyearbyen, 2010, S. Domaschke	JN243332	JN243328
				JN243334	JN243329
				JN243338	JN243330
Spain	8	41°39′25.53″N/3°32′06.20″W	Spain, Burgos, San Juan del Monte, 2010, S. Pérez-Ortega	JN243335	JN243325
				JN243336	JN243326
				JN243339	
Germany	7	49°58′18.3″N/9°04′48.7″E	Germany, Bavaria, Stockstadt (Main), 2010, S. Domaschke	JN243331	JN243327

2010. Except for the Antarctic individuals, material was collected 4–6 days before starting the laboratory measurements and kept frozen at −20 °C until 1 h before reactivation. Individuals from Antarctica were air-dried and frozen at −80 °C immediately after sampling and kept at this temperature until the day of the reactivation for the gas exchange measurements. Previous studies have shown that a comparable low temperature treatment is of no harm for dried lichen individuals (Larson 1978; Meyer et al. 1988).

CO_2 exchange measurement

Before using the lichen thalli for gas exchange measurements, they were carefully washed with mineral water (mineral content: HCO_3 299 mg/l, SO_4 133 mg/l, Cl 53.8 mg/l, Ca 93.6 mg/l, Mg 39.7 mg/l). Visible parts of other lichen species were removed. The preconditioning of the thalli was performed in a climate chamber (light intensity 100 µmol m^{-2} s^{-1} in a cycle of 12 h light/12 h dark, temperature 15 °C, moistening every 24 h with mineral water) for the duration of 72 h. The lichen thalli were fixed in wire-mesh baskets (3.8 cm diameter, 1–1.5 cm high, mesh size 1.6 mm) to avoid losing lichen material during the measurements.

Laboratory CO_2 measurements were performed with a compact minicuvette system (CMS 400; WALZ, Effeltrich, Germany) under controlled temperature, light and humidity conditions. For each population, 7–8 replicates were investigated. Air flow inside the cuvette was always 600 ml min^{-1}. Prior to all measurements, samples were sprayed with mineral water for 20 min to ensure full hydration of thalli. Optimal water contents were determined for four individuals per population, by studying the change of the photosynthetic performance during the desiccation process of the lichen at a constant temperature of 15 °C and photon flux densities of 0 and 400 µmol m^{-2} s^{-1}. The response of net CO_2 exchange was measured at optimal water content at temperatures between 0 and 30 °C and photosynthetic flux density (PPFD) of 0, 25, 50, 100, 200, 400, 800 and 1,200 µmol m^{-2} s^{-1}. Each sample was weighed on an electronic scale (Sartorius BP 2215) immediately before and after each CO_2 measurement. With these data, thallus water content was calculated on a dry weight basis. After finishing the CO_2 gas exchange measurement and the chlorophyll measurements, thallus weight at air humidity was determined with an electronic balance ABS 120-4 (KERN, Germany). For the calculation of absolute dry weight, thalli with the same population origin were weighed at air humidity which was measured with the alarm hygrometer 608-H2 (Testo, Germany), and after drying the samples for 24 h at 70 °C. With the percental weight loss after drying for these samples, the absolute dry weight for the other samples was calculated. PPFD response curves were analysed by statistical fitting of a Smith function (Lange et al. 1991; Green et al. 1997), using SigmaPlot 10.0 (Chicago, USA). Temperature optima were calculated by fitting temperature response curves to second degree polynomial functions.

Chlorophyll determination

Chlorophyll quantification of the lichen material followed Barnes et al. (1992). Six replicates of 20 mg for each sample were first rinsed six times in 100 % $CaCO_3$ saturated acetone to remove lichen acids and then extracted in $CaCO_3$ saturated DMSO for 40 min at 65 °C. Absorbance of the extracts was measured with a Jasco V-630 spectrophotometer (Japan).

Photobiont quantification

Photobionts were isolated from lichen thalli using the adapted micromethod described by Gasulla et al. (2010).

For every sample, three replicate isolations were used. Samples of 20 mg dried lichen material were gathered between the first and second apical branch. The material was homogenised with mortar and pestle in isotonic buffer (0.3 M sorbitol, 50 mM HEPES, pH 7.5) and filtered through muslin. Further steps followed the isolation protocol without sonicating the resuspended material with Tween 20. The pellet was diluted in 200 µl isotonic buffer.

To count the number of photobiont cells for the different populations, a Fuchs-Rosenthal chamber with a volume of 0.0125 mm^3 was used. Before counting, samples were diluted four to eight times depending on the density of algal cells. Four squares of each of the two grids were counted with an optical microscope. The number of photobiont cells per µl isolate was calculated using the formula: $N_P = (N \times D_f)/(A \times D)$, where N is the number of algal cells counted, A is the area of all counted squares, D is the depth of the Fuchs-Rosenthal chamber and D_f is the dilution factor.

Significance tests

Significance tests for the photosynthetic parameters, the chlorophyll contents and the photobiont cell counting were performed by one-way general ANOVAs using the program R (R Development Core Team 2009). Differences between groups and/or populations were considered significant for $p < 0.05$. Comparisons of means were performed using Tukey's (HSD) test.

DNA sequencing

In order to ascertain to which haplotype group (polar or temperate; see Fernández-Mendoza et al. 2011), the symbionts investigated in this study belonged, we sequenced the internal transcribed spacer 1 and 2 (ITS 1 and 2) regions of the nuclear ribosomal DNA repeat of the photobiont and mycobiont. Thallus fragments of seven individuals from Antarctica, Germany and Svalbard and eight from Spain were checked for fungal infections. DNA was extracted from uninfected lobes using the DNeasy™ Plant Mini Kit (QIAGEN) following the manufacturer's protocol. PCR reactions were performed with 5 µl of DNA extract in 25 µl PCR reactions together with 1 µl of the 5′- and 3′- primers (ITS1T and ITS4T developed by Kroken and Taylor (2000) for the photobiont and ITS1f and ITS4 developed by Gardes and Bruns (1993) for the mycobiont) using PCR-PuReTaq Ready-to-Go Beads© (GE Healthcare) and the following cycling conditions: initial denaturation of 94 °C (5′), five cycles of 94 °C (30″), 54 °C (30″), 72 °C (1′), 33 cycles of 94 °C (30″), 48 °C (30″), 72 °C (1′), and final extension of 72 °C (10′).

PCR products were purified by electrophoretic separation on agarose gels using the QIAquick Gel Extraction Kit (QIAGEN) to isolate DNA from the cut out bands. Purified DNA was labelled with the BigDye© Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) and cycle sequenced at 94 °C (30″), and 29 cycles of 95 °C (15″), 45 °C (15″), 60 °C (4′). Sequences were determined on an ABI 3730 DNA Analyzer (Applied Biosystems). A total of 27 photobiont sequences and 24 mycobiont sequences were edited and aligned with 200 photobiont and 173 mycobiont ITS haplotypes that were previously published by Fernández-Mendoza et al. (2011) using the program Geneious v.4.7 (Drummond et al. 2009). Alignment settings were the following: cost matrix with 65 % similarity (5.0/−4.0), gap opening penalty 12 and gap extension penalty 3.

We reconstructed the phylogenetic relationships between the investigated photobionts and mycobionts by calculating haplotype networks with the program TCS v.1.21 (Clement et al. 2000). Gaps were treated as 5th character state.

Results

Sequencing

Of the 29 thalli used in this study, 27 yielded in photobiont ITS sequences with lengths between 572 and 714 bp. Two individuals from Svalbard did not amplify and hence were not included in the genetic analysis. The sequencing of the mycobiont resulted in 24 ITS sequences with lengths between 543 and 564 bp. One individual each from Antarctica, Svalbard and Spain and two from Germany did not amplify.

The haplotype networks (Fig. 2, and Supplementary material, Fig. S1) show that all our photobiont sequences belong to the previously identified haplotype groups. The haplotype network of the photobiont shows a group of arctic/antarctic samples containing individuals from Antarctica and Svalbard and a group of temperate individuals with German and Spanish samples (Fig. 2). The haplotype network of the mycobiont (Supplementary material, Fig. S1) shows a slightly different structure, mainly because the distinction between polar and temperate haplotypes is less pronounced. Haplotypes from the northern hemisphere (polar and temperate) appear more closely related to each other, as witnessed, e.g., by the temperate haplotype in the otherwise exclusively arctic haplotype group at the bottom of the network.

Ecophysiological response

Figure 1 shows the response of CO_2 exchange to temperature at 1,200 µmol m^{-2} s^{-1} and DR averaged over all individuals within each population. The response of net photosynthesis to temperature and light intensity differed

strongly between polar and temperate samples. Temperature optima varied between 11 °C for the Antarctic population and the one collected from Svalbard, 15 °C for samples from Spain and 16.96 °C for German samples (Table 3). Also, the maximal net photosynthesis (NP_{max}) differed strongly between temperate and polar populations (Table 3). At 5 °C, it is six times higher in German samples (3.1 µmol CO_2 kg DW^{-1} s^{-1}) than in Antarctic samples (0.53 µmol CO_2 kg DW^{-1} s^{-1}) (Table 3). This difference is even more pronounced at 20 °C. Here, the Antarctic samples have a maximal net photosynthesis of only 0.15 µmol CO_2 kg DW^{-1} s^{-1}, while German samples show a 50 times higher net photosynthesis value of 7.77 µmol CO_2 kg DW^{-1} s^{-1}. In Spanish samples, NP_{max} rate reaches 5.11 µmol CO_2 kg DW^{-1} s^{-1} at 20 °C. The different maximal net photosynthesis rates between polar and temperate populations are only partly explained by the higher chlorophyll content in the latter ones, as shown in Fig. 1. Differences become smaller when rates are expressed on a Chl content basis, but they are still significant (results of ANOVAS not shown). Hence, although the polar samples have lower chlorophyll contents than the temperate ones, the chlorophyll level cannot be the only limiting factor for the polar lichen samples. The four graphs in Fig. 2 shows the temperature-dependent net photosynthesis at various irradiance levels separately for the four investigated populations and the phylogenetic position of the investigated photobionts and mycobionts.

Dark respiration rates ranged between −0.68 µmol CO_2 kg DW^{-1} s^{-1} (0 °C) and −2.62 µmol CO_2 kg DW^{-1} s^{-1} (30 °C) in Antarctic samples and from −1.09 µmol CO_2 kg DW^{-1} s^{-1} (0 °C) to −4.86 µmol CO_2 kg DW^{-1} s^{-1} (30 °C) in German samples. There was no visible difference between the respiration rates in samples from

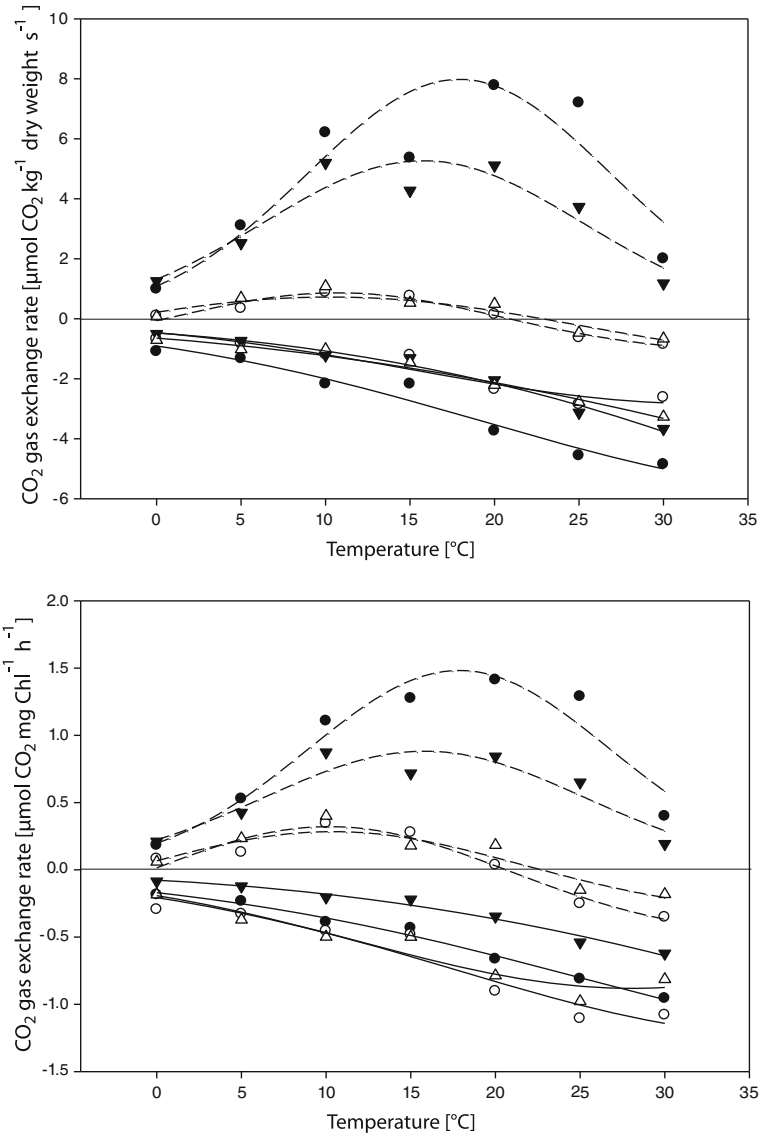
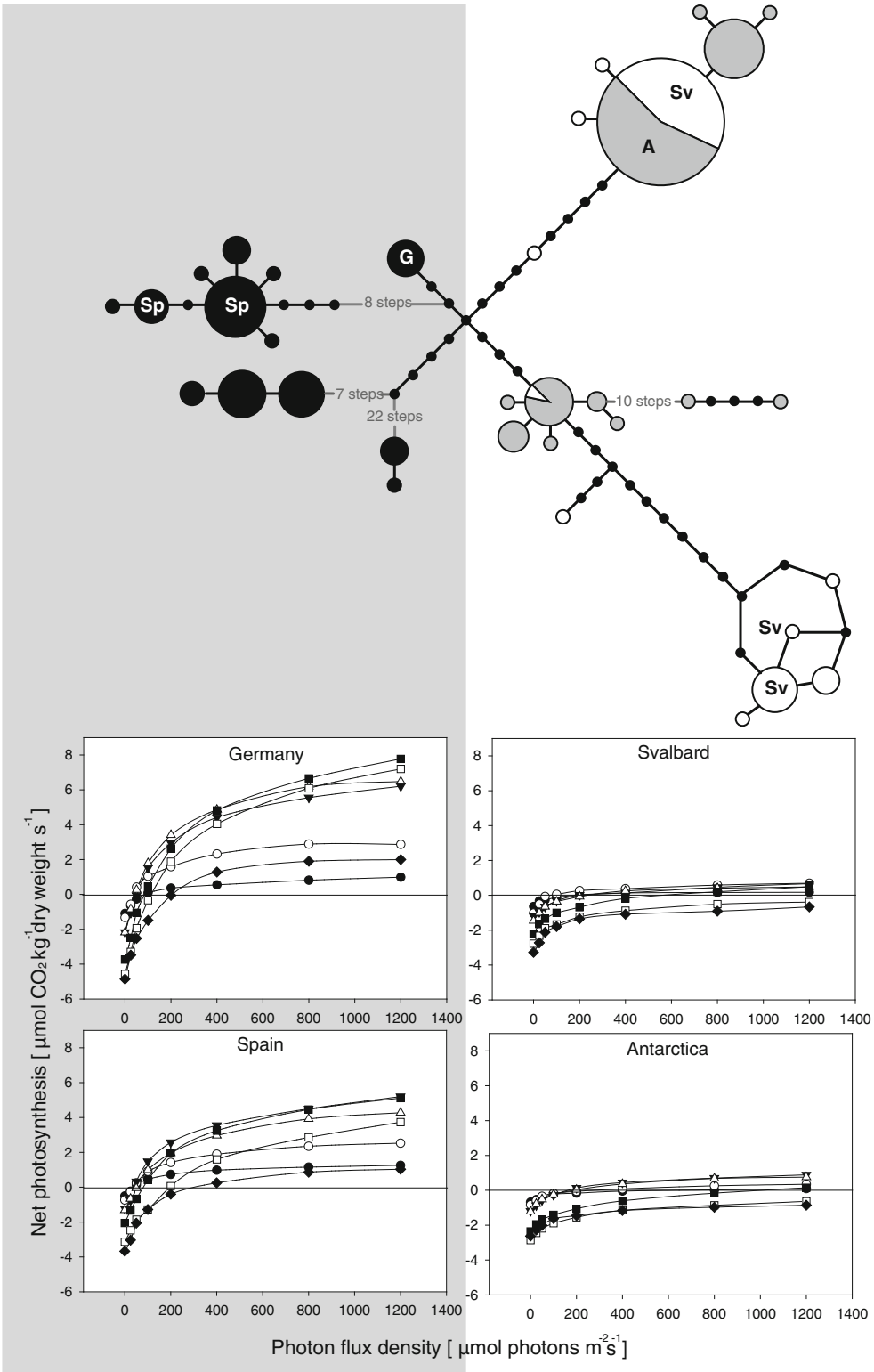


Fig. 1 Comparison of the net photosynthesis (NP) for all localities with NP calculated per dry weight (*top*), and per chlorophyll content (*bottom*). For the calculation of net photosynthesis rates, seven replicates for populations from Antarctica, Germany and Svalbard and eight replicates for the Spanish population were used. *Dashed lines* display NP curves for the maximal light intensity of 1,200 µmol photons/m^{-2} s^{-1}, *continuous lines* indicate curves for DR with 0 µmol photons/m^{-2} s^{-1}. Geographical origin is coded by symbol colours and forms: *black dot* (Germany), *black triangle* (Spain), *white dot* (Antarctica) and *white triangle* (Svalbard)



Antarctica, Svalbard and Spain. But individuals from Germany showed a significantly higher respiration rate at 10–30 °C, when calculated on a dry weight basis (Table 3).

Chlorophyll measurement and photobiont counting

Compared to the temperate populations, the polar ones have very low amounts of chlorophyll in their thalli

◀**Fig. 2** Dependency of net CO_2 exchange on temperatures at various photon flux densities (PPFD) and haplotype network of the photobiont of *Cetraria aculeata*. *Circles* in the haplotype networks represent haplotypes, with the size being proportional to the number of individuals sharing this haplotype. *Lines* between *circles* indicate mutation steps. *Black lines* indicate a posterior probability of >0.95, *grey lines* a posterior probability of <0.95. *Small black dots* indicate potential haplotypes not present in this study. Geographical origin of the individuals is coded by colour of the circles: *white* (Arctic), *grey* (Antarctic) and *black* (temperate). *Different symbols* in the gas exchange graphs indicate different temperatures: *black circle* (0 °C), *white circle* (5 °C), *black triangle* (10 °C), *white triangle* (15 °C), *black square* (20 °C), *white square* (25 °C) and *black diamond* (30 °C). The position of the ecophysiologically investigated samples is indicated by the following *letters* in the haplotype network: *A* Antarctica, *Sv* Svalbard, *Sp* Spain and *G* Germany. The *grey background* frame marks the position of temperate haplotypes and gas exchange curves in the figure. Haplotypes and gas exchange curves for polar samples are displayed with *white background*

(Table 2). Chl *a*/*b* ratio differs as well between climatic regions: average values for samples from Antarctica (1.82) and Svalbard (2.37) are significantly lower than those for German (5.83) and Spanish (5.01) populations. The photobiont cell counts also differed strongly among the populations (Table 2). With an average number of 4.969 cells per mg dry weight, Antarctic samples have roughly 20 % of the algal cells of the German population (average: 24.559). Individuals from Svalbard (7.141) and Spain (13.242) have intermediate numbers, but the difference between polar and temperate samples is highly significant ($p = 0.001$ in a paired t test). The chlorophyll content per photobiont cell is within a narrow range for the investigated populations (Table 2). Only German samples show a significantly lower chlorophyll content (p ranged between 0.0438 for difference between Germany and Svalbard and 0.0007 for difference between Germany and Antarctica in a paired t test) per photobiont cell when compared with the other populations.

Discussion

The data presented in this study provides support to the theory of photosynthetic acclimation of *Cetraria aculeata* via a two-level-control of its photobiont population. The first level of control is the selection of a genetically adapted photobiont partner via photobiont switching. This is reflected by the genetically different photobiont populations in polar and temperate samples in our study. Similar ideas have been introduced by Lücking et al. (2009) and Piercey-Normore and Deduke (2011) among others. The second level of control is a regulation of the population size within the thallus. In our study, this is shown by different numbers of photobiont cells in polar and temperate thalli of *Cetraria aculeata* and supports the idea of physiological characters influenced by a regulation of the producer/consumer ratio as advocated by Sun and Friedmann (2005).

Ecophysiological response

The most important difference observed between our samples concerns the temperature optima and maxima of positive net photosynthesis. The optimum of 11 °C for the samples collected in Antarctica and Svalbard (Table 3) fits the ones reported for other polar and alpine lichens (Green 2009). Lichens from these populations did not show positive net photosynthesis above 20 °C and hence meet the general definitions of psychrophilic bacteria and fungi given by Morita (1975) and Robinson (2001). The temperature optima of samples collected from Spain and Germany (15 and 17 °C) are comparable to those of mesophilic lichen species from mountainous or lower altitude habitats of various climates (Del Prado and Sancho 2000; Green 2009). Thalli from both populations showed positive net photosynthesis even at 30 °C, the highest temperature measured by us. In a meta-analysis, Lechowicz (1982) found a similar latitudinal trend of optimum temperatures for net photosynthesis in 42 published datasets on different lichen species.

Maximal net photosynthesis rates were much higher in the temperate populations than in polar ones (absolute photosynthetic maxima between 0.91 and 1.08 μmol CO_2 kg DW^{-1} s^{-1}), but also differed considerably between the two temperate populations (5.11 μmol CO_2 kg DW^{-1} s^{-1} for Spain, 7.77 for Germany). The values are again within the range of NP_{max} measured for other arctic and alpine (Green et al. 2008; Reiter et al. 2008; Green 2009, among

Table 2 Chlorophyll content, chlorophyll *a*/*b* ratio, number of algal cells per mg dry weight and chlorophyll content per photobiont cell (mean ± standard deviation)

Population	Chl content (mg g^{-1} DW)	Chl *a*/*b* ratio	Photobiont cells mg^{-1} DW	Photobiont cell size (μm)	Chl/photobiont cell (ng per cell)
Antarctica	0.43 ± 0.11[a]	2.83[a]	4,969 ± 823[a]	18.41 ± 4.76[a]	0.083 ± 0.005[a]
Svalbard	0.41 ± 0.22[a]	1.93[a]	7,141 ± 294[a]	19.78 ± 4.78[a]	0.066 ± 0.007[a]
Spain	0.84 ± 0.12[b]	4.02[b]	13,242 ± 1370[c]	18.25 ± 5.26[a]	0.073 ± 0.005[a]
Germany	0.90 ± 0.12[b]	5.84[b]	24,559 ± 1258[b]	15.90 ± 4.01[b]	0.033 ± 0.003[b]

Different superscript letters indicate significant differences for $p < 0.05$ calculated with a one-way general ANOVA

Table 3 Temperature optimum (T_{opt} in °C), maximal net photosynthesis at 0, 5, 10, 15, 20, 25 and 30 °C (NP_{max} in μmol CO_2 kg DW^{-1} s^{-1}) and respiration rates at each temperature (R in μmol CO_2 kg DW^{-1} s^{-1}) in *Cetraria aculeata*

Parameter	Antarctica	Svalbard	Spain	Germany
T_{opt}	10.85	11.02	15.41	16.96
R0	−0.68 ± 0.43	−0.71 ± 0.46	−0.51 ± 0.22	−1.09 ± 0.74
R5	−0.83 ± 0.32	−1.02 ± 0.32	−0.73 ± 0.11	−1.33 ± 0.55
R10	−1.19 ± 0.36^{a}	−1.02 ± 0.69^{a}	−1.22 ± 0.22^{a}	−2.17 ± 0.33^{b}
R15	−1.21 ± 0.38^{a}	−1.38 ± 0.42^{a}	−1.31 ± 0.1^{a}	−2.12 ± 0.49^{b}
R20	−2.36 ± 0.75^{a}	−2.21 ± 0.55^{a}	−2.05 ± 0.51^{a}	−3.74 ± 0.57^{b}
R25	−2.86 ± 0.95^{a}	−2.81 ± 0.68^{a}	−3.13 ± 0.55^{ab}	−4.57 ± 1.14^{b}
R30	−2.62 ± 0.8^{a}	−3.27 ± 0.59^{ab}	−3.67 ± 0.62^{ab}	−4.86 ± 1.05^{b}
$NP_{max}0$	0.23 ± 0.3^{a}	0.07 ± 0.3^{a}	1.26 ± 0.29^{b}	0.97 ± 0.26^{b}
$NP_{max}5$	0.53 ± 0.51^{a}	0.69 ± 0.58^{a}	2.53 ± 0.21^{b}	3.1 ± 0.61^{b}
$NP_{max}10$	0.91 ± 0.6^{a}	1.08 ± 1.25^{a}	5.2 ± 0.96^{b}	6.21 ± 1.18^{b}
$NP_{max}15$	0.61 ± 0.45^{a}	0.53 ± 0.53^{a}	4.27 ± 0.13^{b}	5.36 ± 1.56^{b}
$NP_{max}20$	0.15 ± 0.39^{a}	0.49 ± 0.92^{a}	5.11 ± 1.42^{c}	7.77 ± 2.08^{b}
$NP_{max}25$	−0.63 ± 0.38^{a}	−0.47 ± 0.88^{a}	3.73 ± 0.04^{c}	7.2 ± 2.39^{b}
$NP_{max}30$	−0.86 ± 0.5^{a}	−0.66 ± 0.22^{a}	1.18 ± 0.47^{b}	2 ± 0.93^{b}

Mean ± standard deviation. Different superscript letters indicate significant differences for $p < 0.05$ calculated with a one-way general ANOVA

others) or mesophilic lichens (Sancho et al. 1997; Del Prado and Sancho 2000). NP_{max} is considerably higher in German samples (7.2 ± 2.39 at 25 °C) than in the Spanish ones (3.73 ± 0.04 at 25 °C). This difference is maintained when rates are related to chlorophyll content instead of dry weight, because both populations have very similar chlorophyll contents (Fig. 1 bottom; Table 2). Dark respiration rates do not differ significantly for the samples from Antarctica, Svalbard and Spain, but are significantly higher in the German samples between 10 and 30 °C. This could be related to a more active metabolism in the German samples, suggesting that the German sampling site has the most favourable climatic conditions for growth and development of *C. aculeata* of all investigated locations. Körner and Larcher (1988) and Murtagh et al. (2002) reported higher respiration rates of polar lichens and plants than for temperate samples. On the other hand, several studies have shown that DR rates increase with increasing mean annual temperature (Lechowicz et al. 1980, 1982; Friedmann and Sun 2005, among others). Interestingly, when we relate DR to chlorophyll content, the difference between the localities disappears (data not shown), and if DR is calculated per algal cell, we find indeed the highest rates in the polar samples (e.g. at 25 °C, Germany: 1.86×10^{-4}; Spain: 2.36×10^{-4}; Svalbard: 3.93×10^{-4}; and Antarctica: 5.7×10^{-4} μmol CO_2 algal $cell^{-1}$ s^{-1}). This means that the respiration, a mainly fungal process (Quispel 1960; Sundberg et al. 1999), is highest in relation to algal proportion in Antarctica. Because photosynthetic yield levels off at higher temperatures while respiration continues to increase, temperate populations need higher numbers of algae to compensate for fungal respiration. Moreover, German samples have probably higher numbers of metabolically active cells per mg dry weight than samples from the other sites. This explanation is supported by the much higher number of photobiont cells in their thalli (Table 2), as mycobiont demands have been suggested to regulate the amount and/or activity of the photobiont (Palmqvist 2000). Due to their lower chlorophyll content (smaller number of photobiont cells), polar samples also have higher light compensation points than temperate samples.

Chlorophyll content and photobiont cell counts

Chlorophyll contents in thalli from temperate populations are twice as high as in polar populations. A similar result could be shown by Schipperges et al. (1995). They found that the chlorophyll content of *Cetraria nivalis* decreased with latitude between Central Europe and the Arctic. Sonesson et al. (1992) found a similar connection along an altitudinal gradient. Chlorophyll contents in a subalpine population of *Nephroma arcticum* were twice as high as in an alpine one. In *C. aculeata*, the differences in chlorophyll content are largely due to varying numbers of photobiont cells in the lichen thallus and not to changing chlorophyll contents per algal cell (Table 2). A dependency of photobiont cell content on latitude or climatic parameters has been shown for various lichen species (Kärenlampi and Pelkonen 1971; Kunkel 1980; Larcher and Vareschi 1988; Schipperges et al. 1995; Sancho et al. 2000) and has been explained as an adaptation of lichens to an increase of respiration rates with temperature (Sun and Friedmann

2005). If mean annual temperature (MAT) were the major factor explaining the number of photobiont cells in lichen thalli, one would expect more or less equal numbers in thalli from the German site (MAT 10.5 °C) and in Spain (MAT 10.1 °C). Instead, we observe an almost two times higher number in the thalli from Germany. A possible explanation could be a more equal distribution of precipitation and hence longer periods of activity in the German site resulting in higher growth rates and a higher proportion of active tissue. Alternatively, differences in nitrogen and/or phosphorus content between the localities could account for the differing photobiont numbers, as these factors can limit the size of the photobiont population (Johansson et al. 2011). At any rate, it is likely that the different numbers of photobiont cells between polar and temperate samples are due to a regulation of the photobiont population by the mycobiont.

The Chl *a/b* ratios measured in the temperate populations are remarkably high. The values reported for most vascular plants and algae range between 1.5 and 4.2 (Humbeck et al. 1988; Johnson et al. 1993), while Chl *a/b* ratio in the German population is 5.8 and hence more similar to values found in the shade tolerant algae *Euglena gracilis* (4.1–7.6) by Beneragama and Goto (2010). These authors concluded that the high values were due to an increased size or number of Photosystem I units under low light conditions. However, our samples from Antarctica and Svalbard display a much lower ratio of chlorophyll *a* to chlorophyll *b* than the temperate ones. Chlorophyll *b* is known to be responsible for photosynthesis at low light intensities (Dale and Causton 1992; Harper et al. 2004). Its concentration generally decreases with increasing irradiance which leads to a higher Chl *a/b* ratio under high light regimes. Apparently, *Cetraria aculeata* displays signs of adaptation to low light conditions in polar localities.

Genetic structure

The ecophysiological and anatomical differences outlined above are not only correlated with the geographical origin of our samples but also with genetic differences between photobiont populations. As shown in Fig. 2, the photobionts of *C. aculeata* investigated by us belong to the polar and temperate haplotype groups that were previously identified by Fernández-Mendoza et al. (2011). The relationship between ecophysiological response and genetic structure of the mycobiont (Supplementary material, Fig. S1) is less obvious. Two closely related fungal haplotypes from Germany and Svalbard show very different ecophysiological characteristics. If there is any genetic background at all (see below), this suggests that it is the photobiont (and its population size) that mainly influences the ecophysiological response to light and temperature in lichens. In that respect, it is conceivable that the mycobiont chooses the best adapted algae from the local pool, which allows its growth and development under various climatic conditions. This interpretation would be in line with recently published evidence that symbioses behave as microbial communities rather than as interacting organisms, and respond to environmental changes by adjusting their symbiotic relationships (Friedmann and Sun 2005; Sun and Friedmann 2005; Gilbert et al. 2010; Rodriguez et al. 2008; Zilber-Rosenberg and Rosenberg 2008). While Sun and Friedmann, in their "community adaptation" hypothesis, only consider up- and down-regulation of photobiont numbers as acclimation mechanisms in lichens, the concepts of "habitat-adapted symbiosis" (Rodriguez et al. 2008), "outsourcing of stress responses" (Gilbert et al. 2010) and the hologenome theory of evolution (Zilber-Rosenberg and Rosenberg 2008) consider association with different symbionts as adaptational strategies. In the case of lichens, this would not only include the photobionts but also the associated bacterial communities (Cardinale et al. 2012; Printzen et al. 2012). An impact of photobiont preferences on the composition of the associated microbiome has recently been shown by Hodkinson et al. (2012). Disentangling the contributions of all three symbiotic partners to the adaptation and acclimation potential of widely distributed lichens such as *C. aculeata* is therefore getting ever more complex.

Outlook

Ultimately, common garden experiments, ecophysiological studies of isolated photobionts and investigations involving the associated microbiome will have to show to what degree the observed physiological differences are attributable to photobiont genotype or to the regulation of photobiont populations by the mycobiont. An aspect that we have not systematically considered here is a possible seasonal effect on the photosynthetic response in *C. aculeata*. We have partly accounted for this parameter by collecting lichen specimens in what we considered the optimal growth season: early summer in Spain, Germany and Svalbard and mid-summer in Antarctica. However, seasonal variations of photosynthetic response in *C. aculeata* should also be investigated in controlled experiments.

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