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Detection of paralogous polyketide synthase genes in *Parmeliaceae* by specific primers

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Abstract: A first assessment of paralogy in non-reducing polyketide synthases of *Parmeliaceae* is presented. Primers which are specific to the keto-acyl synthase domain were used to amplify gene fragments of putative non-reducing polyketide synthases from various representatives of the family. The corresponding sequences were analysed together with a selection of known polyketide synthase genes from other fungi, including lichenized fungi. The results suggest that genes from *Parmeliaceae* represent at least 6 paralogs. Their different positions in the tree partly correlate with the variable presence of spliceosomal introns at particular positions in the gene fragments. Because only one paralog could be unambiguously detected in each species by direct sequencing of PCR products with this approach, we tested the applicability of clade-specific primers, designed by using orthologous signature sequences. With these primers more paralogs could be detected from the same DNA extract in a number of species, but certain paralogs were consistently not amplified in these species. The paralog-specific primer approach can potentially be used for a rapid screening of PKS genes from a broader range of lichen fungi.

Key words: lichens, paralogy, *Parmeliaceae*, polyketide synthase genes, secondary metabolites

Introduction

Lichen mycobionts are outstanding in their richness of distinctive secondary metabolites that are expressed in diverse phylogenetic lineages. More than 800 different secondary metabolites have so far been characterized from lichenized fungi (Huneck & Yoshimura 1996). Some of these can be produced in considerable quantities in the thalli to serve diverse functions: lichen compounds can filter particular wavelengths of light for the symbiotic algae, they can chelate ions to assist the hyphal penetration of rocky substrata, or are involved in the defence against feeders or parasites (Rundel 1978; Rikkinen 1995; Lawrey 1986). Irrespective of their functions, the patterns of compound have been used as important characters at different hierarchic levels in the systematics of lichens (Hawksworth 1976; Lumbsch 1998), yet, lengthy discussions have arisen about their taxonomic significance in certain groups. To understand chemical differences better, it might be helpful to understand how the production of a substance is regulated, what genes are involved, and how these are related.

The genetic background of lichen metabolite synthesis is still poorly known. So far, it is known that the genes for fungal secondary metabolite pathways are sequentially arranged in gene clusters (e.g. Yu *et al.* 1995; Brown *et al.* 1996). All of the gene clusters of the pathway leading to polyketides, including most classes of lichen compounds, contain polyketide synthase genes (PKS, e.g. Hopwood 1997). Polyketide synthases build the initial structure of compounds, which can then be modified in subsequent steps. In non-lichenized fungi, for example, PKSs are involved in the assembly of the precursors of potent carcinogens such as aflatoxins, of metabolic regulators such as lovastatin, or a

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range of fungal pigments, including melanin precursors. Polyketide synthases are related to fatty acid synthases, and similarly condensate C2-units iteratively involving the action of a keto-acyl synthase domain. The sequence of that domain can be obtained by PCR and sequencing using conserved primers (Bingle *et al.* 1999; Miao *et al.* 2001; Lee *et al.* 2001; Sauer *et al.* 2002; Grube & Blaha 2003). When the resulting sequences are phylogenetically analysed, this reveals the relationship of PKS genes from different organismal lineages and indicates the presence of several paralogous genes.

The conserved primers, which are currently used for this purpose are of limited use when a high diversity of paralogous genes is expected in genomes of fungi, as is the case with the class of polyketide synthases (Varga *et al.* 2003; Kroken *et al.* 2003; Schmitt *et al.* 2005). We suspect that any primer pair will amplify only a subset of related paralogs, and sample among these with varying efficiency. As a consequence PCR products may contain mixtures of related paralogs in different concentrations. This may lead to more or less ambiguous sequencing results, for example, if closely related paralogs are equally well amplified, or when many poorly amplified paralogs distort the signal of one efficiently amplified fragment. Cloning of the PCR products will in these cases help to obtain clean sequences of the involved paralogs. However, sequences of many clones are sometimes required for a representative estimate of amplifiable paralogs. If particular paralogs are amplified with rather high efficiency, even a higher number of analysed clones may fail to detect the presence of particular paralogs of interest (J. Rankl, unpublished results). The application of primers which are specific to such paralogs may represent an alternative.

In this contribution, we present a first assessment of paralogy in PKS genes from the *Parmeliaceae*, which is used to develop paralog-specific primers. With these primers we then assess whether other paralogs are present in the genomes of the species, which were not detected by the initial PCR with conserved primers. As the *Parmeliaceae* are rich in diverse phenolic compounds, a high number of paralogous PKS genes for the expression of non-reducing polyketide synthases could thus be expected to be present in the genomes.

Materials and Methods

Voucher material of the specimens analysed from the *Parmeliaceae* is listed in Table 1, and deposited in the herbarium of the Institute of Plant Sciences, Karl-Franzens-University Graz (GZU). Further details are available from the authors on request. Prior to the extraction, the samples were carefully inspected for the presence of lichenicolous fungi, to avoid the amplification of miscellaneous fungal PKS genes. Total DNA was extracted from individual thalli according to a modified CTAB method (Cubero *et al.* 2000), or alternatively, with the Plant DNeasy extraction kit (Qiagen, Vienna).

Amplifications of PKS genes were carried out using the primers LC1 (5′-GAT CCI AGI TTT TTT AAT ATG-3′), LC2c (5′-GT ICC IGT ICC GTG CAT TTC-3′), of Bingle *et al.* (1999). 50 µl PCR mix (10 mM Tris pH 8·3/50 mM KCl/1·5 mM $MgCl_2$/ 50 µg gelatine) contained 1.25 units of Taq polymerase (Amersham), 0·2 mM of each of the four dNTPs, 0·5 µM of each primer and *c.* 10–50 ng genomic DNA. Annealing conditions were 53°C or alternatively 60–54°C touchdown during the first 7 cycles. Products were either PEG precipitated or cleaned using QIAGEN quick spin columns (Qiagen, Vienna). Both complementary strands were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applera, Vienna) according to the manufacturer's instructions. Sequences were run on an ABI310 automated sequencer (Applera).

The PKS DNA sequences were assembled using the BioEdit software (BioEdit 5.0.9; Hall 1999) and aligned using ClustalW as embedded in BioEdit. The mRNA introns in the PKS alignment were excluded prior to phylogenetic analyses. The DNA-sequence alignment was subject to a Bayesian analysis using MrBAYES Version 3 (available from http://morphbank.ebc.uu.se/mrbayes/; Huelsenbeck & Ronquist 2001). The GTR model was used with gamma distribution of rates and invariable sites, as suggested by the Akaike information criterion in MrModeltest (Nylander 2002). 1 000 000 generations were run (8 chains), every 100th tree was sampled, and the first 100 000 generations were discarded as burn-in. The outgroup in the tree was a gene from *Aspergillus terreus* (AB072444). A consensus phylogram showing mean branch lengths was calculated with the sumt command in MrBAYES. The tree with posterior probabilities of topologies higher than 50% is presented in Fig. 1.

The phylogenetic tree was used to delineate well-supported groups of related sequences (representing paralogs). These were grouped in the alignment and

TABLE 1. *Specimens of* Parmeliaceae *sequenced in this study, with locality, detail of reference collection and Genbank accession numbers*

Specimen	Origin	Collector and year of collection	GenBank Accession No.
Alectoria ochroleuca AO1	Austria, Styria, Koralpe.	*M. Grube*, 2003	DQ174479
Arctocetraria nigricascens AN1	Russia, Severnaya Zemlya, Bol'shevik Island.	*M. Zhurbenko* (96150), 1996	DQ174480
Brodoa intestiniformis BI1	Austria, Styria, Koralpe.	*M. Grube*, 2003	DQ174481
Cetrelia cetrarioides C1	Austria, Styria, Schladminger Tauern.	*H. Mayrhofer* (14061), 2001	DQ174502
Cetraria chlorophylla CCH1	Austria, Styria, Rabenwald.	*M. Grube*, 2003	DQ174483
C. islandica 22IA	Austria, Styria, Alp Mts, Handalpe.	*M. Opanowicz*, 2002	DQ174482
Cornicularia normoerica CN1	Austria, Styria, Triebener Tauern.	*J. Hafellner* (58390), 2002	DQ174484
Evernia divaricata ED1	Austria, Styria, Rabenwald.	*M. Grube*, 2003	DQ174486
E. prunastri EP1	Austria, Styria, Seckauer Tauern.	*J. Hafellner* (58628), 2002	DQ174485
Flavocetraria cucullata 13CA	Austria, Styria, Alp Mts, Handalpe.	*M. Opanowicz*, 2002	DQ174490
F. nivalis 30P	Poland, Tuchola Forest.	*M. Opanowicz*, 2001	DQ174487
F. nivalis 32P	Poland, Mierzeja Wiślana.	*M. Opanowicz*, 2001	DQ174488
F. nivalis 1S	Sweden, Öland Island, Gösslunda Hutterstad.	*T. Johansson*, 2002	DQ174489
Flavoparmelia caperata FCA2	USA, New Jersey, Cumberland County.	*J. C. Lendemer* 523 & *L. H. Smith II*, 2002	DQ174491
Hypogymnia tubulosa HT1	Austria, Styria, Rabenwald.	*M. Grube*, 2003	DQ174492
Letharia vulpina LV1	Turkey, Prov. Yozgat, Büyüknalbent Dağı.	*V. John* (10098), 2001	DQ174493
Melanelia sp. ME1	Austria, Styria, Rabenwald.	*M. Grube*, 2003	DQ174494
Parmelia omphalodes PO2	Austria, Styria, Schladminger Tauern.	*W. Obermayer* (9122), 2002	DQ174495
Protoparmelia badia U167	Austria, Styria, Handalpe.	*M. Grube*, 1997	DQ174496
Pseudevernia furfuracea Pfu1	Austria, Styria, Rabenwald.	*M. Grube*, 2003	DQ174497
Usnea sp. U2	Costa Rica, Cerro de la Muerte.	*M. Grube*, 2003	DQ174500
Usnea rigida Uri1	Austria, Styria, Glashütten.	*M. Grube*, 1997	DQ174498
U. rubicunda UR1	Costa Rica, Cerro de la Muerte.	*M. Grube*, 2003	DQ174499
Xanthoparmelia conspersa XC2	Austria, Styria, Fischbacher Alpen.	*J. Hafellner* (50921), 2000	DQ174501

TABLE 2. *Sequences retrieved from Genbank included in the phylogenetic analysis*

Species	Gene	GenBank accession no.
Aspergillus fumigatus	*alb1*	AF025541
A. nidulans	*wA*	X65866
A. nidulans	*pksST*	L39121
A. parasiticus	*pksL1*	L42765
A. terreus	*at5*	AB072497
A. terreus	*at4*	AB072445
A. terreus	*at1*	AB072444
Colletotrichum lagenarium	*pks1*	D83643
Dermatocarpon miniatum	*ks1*	AY398713
Exophiala dermatitidis	*pks1*	AF130309
Gibberella moniliformis	*pks3*	AF414140
G. moniliformis	*pks4*	AJ278141
Glarea lozoyensis	*pks1*	AF549411
Lecanora rupicola	*ks1*	AY398724
Monascus purpureus	*pks1*	AF414729
Nodulisporium sp.	*pks1*	AF151533
Penicillium patulum	*wA*	AJ132274
Tephromela atra	*ks1*	AY398729
Xanthoria parietina	*ks1*	AY398730

manually searched for conserved sequence stretches that may serve as binding sites for specific primers. We concentrated our search at the 5′-end of the LC1–LC2c fragment. Primers for 3 different paralogs were designed and used in combination with primer LC2c: PAR1 (5′-TCAAGTTCAAAGGGCCGAG-3′), PAR4 (5′-CTACTTCATACCAGGTGGG-3′), and PAR6 (5′-CCTATTTCATCACTGCCGGAG-3′). Expected sizes for the products were about 450 bp for PAR1, 500 bp for PAR4, and 570 bp (including introns 1 and 4) for PAR6.

Results

Using the primer pair LC1-LC2C of Bingle *et al.* (1999) we usually obtained only a single sequence in several species of the *Parmeliaceae* tested, while samples of several other species of the family tested failed with these primers (including *Cetraria ericetorum*, *C. muricata*, *C. sepincola*, *Cetrelia chicitae*, *C. alaskana*, *Hypogymnia farinacea*, *H. physodes*, *Melanelia disjuncta*, *Parmelia tiliacea*, *P. sulcata*, *Platismatia glauca*, *Usnea filipendula*, *U. subfloridana*, and *Vulpicida pinastri*) or resulted in ambiguous sequences due to the presence of more than one PKS type (data not shown). The sequences for which we could generate clean sequences were aligned. The alignment revealed the presence of four mRNA intron positions. The lengths of the introns are variable in the *Parmeliaceae* (intron 1: 47–55 bp, intron 2: 51 bp, intron 3: 55 bp, intron 4: 52–61 bp). The introns at homologous positions 1 and 4 show a high degree of sequence variation and could be aligned without ambiguity only among closely related species.

The Bayesian tree (analysed after pruning of mRNA intron sequences) had an estimated marginal likelihood of −11 949·45 (arithmetic mean). The parameters of the analysis had the following mean values (variance): r(GT)=1·00 (0·00), r(CT)=5·55 (0·60), r(CG)=1·14 (0·03), r(AT)=1·58 (0·07), r(AG)=4.64 (0.52), r(AC)=1.56 (0.06), π(A)=0·21 (<0·01), π(C)=0·31 (<0·01), π(G)=0·20 (<0·01), π(T)=0·27 (<0·01), alpha parameter of gamma distribution=0·52 (<0·01), portion of invariable sites=0·07 (<0·01).

The PKS sequences of the members of the *Parmeliaceae* analysed contribute to six clades with high support (Fig. 1), which often do not contain genes of this family alone. One clade (clade 1) contains two species of *Parmeliaceae*, i.e. *Cornicularia normoerica* and *Flavocetraria nivalis*, in which none of four possible introns was detected. A second clade (clade 2) is represented by a sample of *Letharia vulpina* which groups together with *Trapelia involuta* (*Trapeliaceae*). In this group, introns were also lacking. However, another group (clade 3) includes a sequence of *Usnea* sp. which contains two introns (named intron 2 and 3). The exon sequence grouped together with an ortholog from *Aspergillus terreus* (*Eurotiaceae*). A gene fragment from *Alectoria ochroleuca* has an unsupported position at the basis of this clade and lacks any introns. The paralogous clade 4 (lacking introns) is represented by two cetrarioid lichens (*Flavocetraria cucullata* and *Cetraria islandica*). The paralogous clade 5, is represented by genes from *Parmelia omphalodes* and *Lecanora rupicola* (*Lecanoraceae*). Both contain intron 1, while *Protoparmelia badia* which forms an unsupported basal branch,

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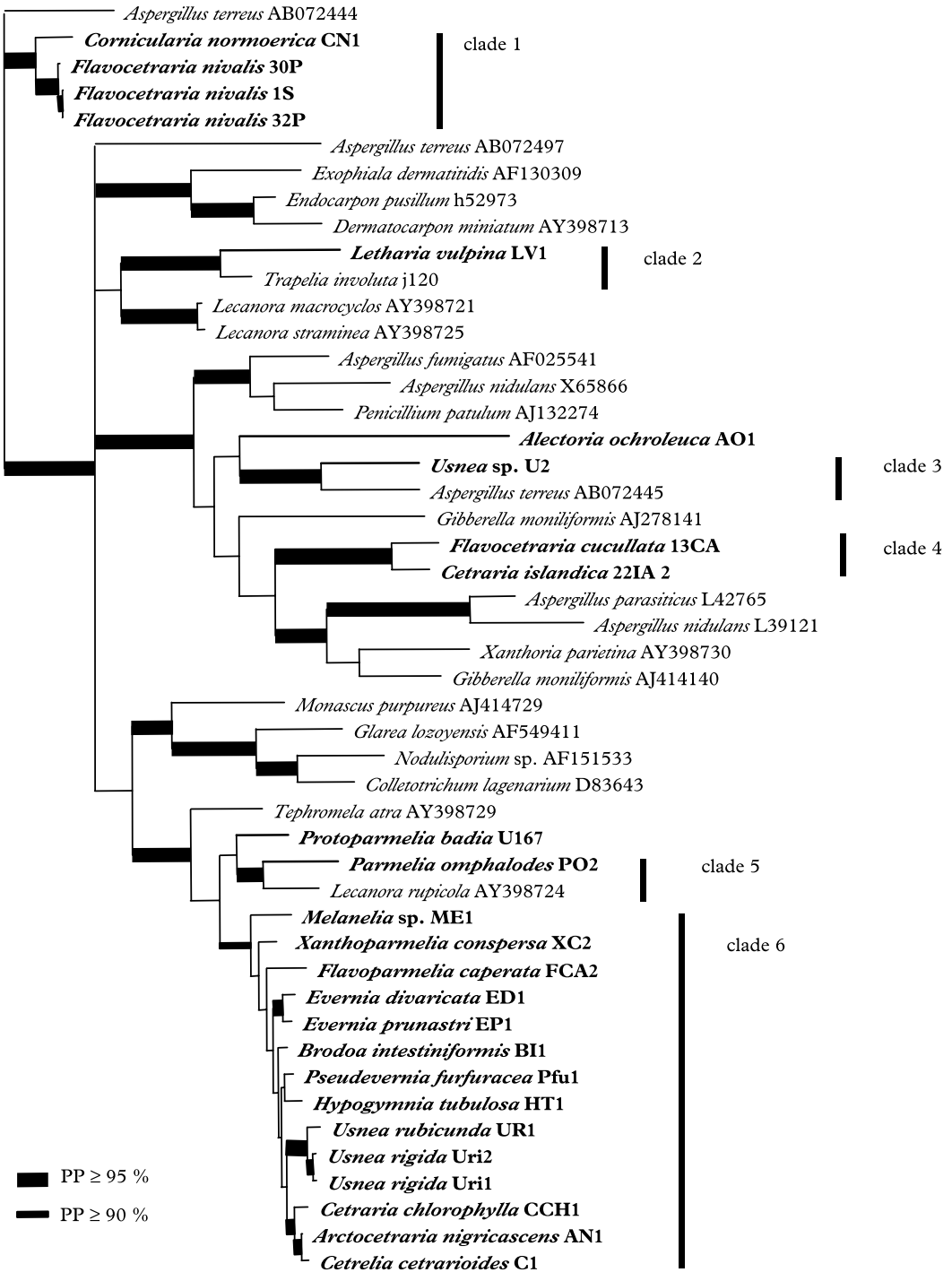

FIG. 1. Phylogenetic tree of gene fragments of non-reducing polyketide synthases, including members of *Parmeliaceae*. Explanation in text. Posterior probability support is indicated by varying thickness of the branches.

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TABLE 3. *Amplification experiments with paralog-specific primers. '+' denotes a successful amplification of a fragment, whereas the amplification failed in case of a '−'*

Species	PAR1-LC2c	PAR4-LC2c	PAR6-LC2c
Alectoria ochroleuca	+	−	+
Arctocetraria nigricascens	+	+	+
Brodoa intestiniformis	+	+	+
Cetraria chlorophylla	+	−	+
Cetrelia cetrarioides	+	+	+
Cetraria islandica	+	+	+
Cornicularia normoerica	+	−	+
Evernia divaricata	+	−	+
Evernia prunastri	+	−	+
Flavocetraria cucullata	+	+	+
Flavocetraria nivalis	+	−	−
Flavoparmelia caperata	−	−	+
Hypogymnia physodes	−	−	+
Hypogymnia tubulosa	+	−	+
Letharia vulpina	+	−	−
Parmelia omphalodes	+	−	−
Parmelia sulcata	−	−	+
Pseudevernia furfuracea	+	−	+
Usnea rubicunda	+	−	+
Usnea rigida	−	−	+
Xanthoparmelia conspersa	+	−	+

contains also intron 4. Both intron 1 and 4 are present throughout clade 6. This paralog was frequently detected in *Parmeliaceae* (i.e. amplified from *Arctocetraria nigricascens*, *Brodoa intestiniformis*, *Cetrelia cetrarioides*, *Cetraria chlorophylla*, *Evernia divaricata*, *Evernia prunastri*, *Flavoparmelia caperata*, *Hypogymnia tubulosa*, *Melanelia* sp., *Pseudoevernia furfuracea*, *Usnea rigida*, *Usnea rubicunda*, *Usnea* sp. and *Xanthoparmelia conspersa*).

This phylogenetic hypothesis was then used to design 3 new primers for specific amplification of paralogs, as represented by well-supported clades which include different *Parmeliaceae* species. The primers were designed to match orthologous 'signature' sequences close to the 5′-end of the LC1-LC2c sequences. The primers were then named PAR1, PAR4, PAR6 according to the numbers of paralogous clades (in fig. 1) for which they should be specific. The results of PCRs using these primers in combination with LC2c are summarized in Table 3. Sequencing confirmed the similarity of the sequences with those of the corresponding paralogs (data not shown). We have not aimed at including the paralogs detected with clade-specific primers in a further phylogenetic analysis, primarly due to the shorter length of their sequences (except for *Usnea rigida* Uri 2). Both the paralogs (of clade) 1 and 6 are present in 14 species, whereas in 7 species either paralog 1 or 6 could be detected. Paralog 4 was detected only in 5 of the 21 isolations tested.

Discussion

Fungal PKSs can be assigned to various main groups according to their products, for example PKSs that contribute to the production of reduced, or of non-reduced polyketides, or of methyl-salicylic acid in fungi (Sauer *et al.* 2002; Kroken *et al.* 2003). In this study we have focused on the ketoacyl synthase domains of PKSs that are involved in the production of non-reducing polyketides, as predicted by the primer pair LC1-LC2C and similarity with sequences of PKS with known functions in non-lichenized fungi. At least 6 paralogs of this

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group have been detected so far in *Parmeliaceae* and it remains to be evaluated whether the genes from *Alectoria ochroleuca* (basal to clade 3) or from *Protoparmelia badia* (basal to clade 5) repesent additional clades.

We are only beginning to understand the evolution of these paralogous genes, as orthologous variants still need to be sampled from the species investigated. The paralog-specific primers presented here show that certain paralogs have a widespread occurrence in *Parmeliaceae*. At present it is not entirely clear, whether corresponding orthologs are missing in the genomes when the PCR using paralog-specific primers fail to amplify fragments, or whether the absence of products is eventually due to mutations at the primer site of the conserved downstream primer. We are convinced that more paralogous clades will be discovered by a more detailed exploratory analysis of the *Parmeliaceae*, for example by thorough screening of clones from products obtained with conserved primers, or by the application of alternative heterologous primer approaches, namely the CODEHOP strategy (Rose *et al.* 1998).

Any speculation about the function of a particular gene is rather premature and there is no apparent correlation of paralogs with secondary compounds. We could still have missed certain genes that produce major substances in the species, but the fate of a polyketide is determined by post-PKS processing. This may involve chain shortening of the initially synthesized structure, as has recently been demonstrated for the precursor of melanin in *Aspergillus* (e.g. Tsai *et al.* 2001). It could therefore be an additional complication, if an ortholog maintains other functions in different organisms. The PKS1 gene of *Colletotrichum* is known to be involved in the production of dihydroxynaphthalene (DHN), a precursor of melanin, but heterologous expression in *Aspergillus* shows that the tetraketide orsellinic acid was synthesized as a side product (Fuji *et al.* 1999).

The large group of monophyletic genes in clade 6 (Fig. 1) from *Parmeliaceae* could be involved in the production of DHN-derived melanin, because genes from non-lichenized fungi (*Monascus purpureus*, *Colletotrichum lagenarium*, *Glarea lozoyensis*, *Nodulisporium* sp.) known to be involved in this function, form a sister clade to clades 5 and 6. However, the differences in the occurrences of the intron in these clades, which also includes *Lecanora rupicola* (*Lecanoraceae*) and *Tephromela atra* (*Mycoblastaceae*) suggest either the presence of further paralogs with an unknown function, or the presence of several closely related paralogs involved in DHN synthesis. Clearly, the functional assignment of PKS paralogs would require additional experimental strategies, for example the comparison of detected compounds with the transcription patterns of PKS genes.

It is generally assumed that new genes arise mainly by duplication of already existing genes (Ohno 1970; Long *et al.* 2003). According to the timing of the duplication events, Sonnhammer & Koonin (2002) delineated two types of paralogy, that is, inparalogs as genes resulting from duplications after the evolution of an organismal lineage, and outparalogs as those already present before a lineage became independent. Outparalogs can readily be detected in a phylogenetic tree as they should group with sequences from other organismal lineages. So far, all of the paralogs detected in our data seem to represent outparalogs, which were already present before the diversifications of the *Parmeliaceae*. It remains to be addressed, whether additional duplications occurred within the *Parmeliaceae*, or whether there is comprehensible evidence for the degeneration of certain paralogs in *Parmeliaceae*, and how many paralogs of non-reducing PKS are traceable in the related families of the *Lecanorales*. The paralog-specific primer approach may be useful also for the latter purpose and for a rapid screening of PKS genes from a broader range of lichen fungi, because the primers for the detected paralogs in *Parmeliaceae* may also amplify the same paralogs in other unrelated mycobionts.

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References

Bingle, L. E. H., Simpson, T. J. & Lazarus, C. M. (1999) Ketosynthese domain probes identify two subclasses of fungal polyketide synthase genes. *Fungal Genetics and Biology* **26:** 209–223.

Brown, D. W., Yu, J.-H., Kelkar, H. S., Fernandes, M., Nesbitt, T. C., Keller, N. P., Adams, T. H. & Leonard, T. J. (1996) Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*. *Proceedings of the National Academy of Sciences USA* **92:** 1418–1422.

Cubero, O. F., Crespo, A., Fatehi, J. & Bridge, P.D. (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution* **216:** 243–249.

Fuji, I., Mori Y., Watanabe, A., Kubo, Y., Tsuji, G. & Ebizuka, Y. (1999) Heterologous expression and product identification of *Colletrotrichum lagenarium* polyketide synthase encoded by the PKS1 gene involved in melanin biosynthesis. *Biosciences and Biotechnological Biochemistry* **63:** 1445–1452.

Grube, M. & Blaha J. (2003) On the phylogeny of some polyketide synthase genes in the lichenized genus *Lecanora*. *Mycological Research* **107:** 1–8.

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.

Hawksworth, D. L. (1976) Lichen chemotaxonomy. In *Lichenology: Progress and Problems* (D. H. Brown, D. L. Hawksworth & R. H. Bailey, eds): 139–184. London: Academic Press.

Hopwood, D. A. (1997) Genetic contributions to understanding polyketide synthases. *Chemical Reviews* **97:** 2465–2497.

Huelsenbeck, J. P. & Ronquist, F. (2001) MrBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Huneck, S. & Yoshimura, I. (1996) *Identification of Lichen Substances*. Berlin: Springer.

Kroken, S., Glass, N. L., Taylor, J. W., Yoder, O. C. & Turgeon, B. G. (2003) Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. *Proceedings of the National Academy of Sciences USA* **100:** 15670–15675.

Lawrey, J. D. 1986. Biological role of lichen substances. *Bryologist* **89:** 111–122.

Lee, T., Yun, S-H., Hodge, K. T., Humber, R. A., Krasnoff, S. B., Turgeon, G. B., Yoder, O. C. & Gibson, D. M. (2001) Polyketide synthase genes in insect- and nematode-associated fungi. *Applied Microbiology and Biotechnology* **56:** 181–187.

Long, M., Betrán, E., Thornton, K. & Wang, W. (2003) The origin of new genes: glimpses from the young and old. *Nature Reviews Genetics* **4:** 865–875.

Lumbsch, T. (1998) The use of metabolic data in lichenology at the species and subspecific levels. *Lichenologist* **30:** 347–367.

Miao, V., Coeffet-LeGal, M-F., Brown, D., Sinneman, S., Donaldson, G. & Davies, J. (2001) Genetic approaches to harvesting lichen products. *Trends in Biotechnology* **19:** 349–355.

Nylander J. A. A.(2002) *MrModeltest*. Version 1.1b. http://morphbank.ebc.uu.se/MrBayes.

Ohno, S. (1970) *Evolution by Gene Duplication*. New York: Springer.

Rikkinen, J. (1995) What's behind the pretty colours? A study on the photobiology of lichens. *Bryobrothera* **4:** 1–239.

Rose, T. M., Schultz, E. R., Henikoff, J. G., Pietrokovski, S., McCallum, C. M., Henikoff, S. (1998) Consensus-degenerate hybrid oligonucleotide primers for amplification of distantly related sequences. *Nucleic Acids Research* **26:** 1628–1635.

Rundel, P. W. (1978) The ecological role of secondary lichen substances. *Biochemical and Systematic Ecology* **6:** 157–170.

Sauer, M., Lu, P., Sangari, R., Kennedy, S., Polishook, J., Bills, G. & An, Z. (2002) Estimating polyketide metabolic potential among non-sporulating fungal endophytes of *Vaccinium macrocarpum*. *Mycological Research* **106:** 460–470.

Schmitt, I., Martin, M., Kautz, S. & Lumbsch, T. (2005) Diversity and evolution of secondary metabolite encoding genes in the Pertusariaceae. *Phytochemistry* **66:** 1241–1253.

Sonnhammer, E. L. L. & Koonin, E. V. (2002) Orthology, paralogy and proposed classification for paralog subtypes. *Trends in Genetics* **18:** 619–620.

Tsai, H.F., Fujii, I., Watanabe, A., Wheeler, M. H., Chang, Y. C., Yasuoka, Y., Ebizuka, Y. & Kwon-Chung, K. J. (2001) Pentaketide-melanin biosynthesis in *Aspergillus fumigatus* requires chain-length shortening of a heptaketide precursor. *Journal of Biological Chemistry* **276:** 29292–29298.

Varga, J., Rigo, K., Kocsube, S., Farkas, B. & Pal, K. (2003) Diversity of polyketide synthase gene sequences in *Aspergillus* species. *Research in Microbiology* **154:** 593–600.

Yu, J., Chang, P-K., Cary, J. W., Wright, M., Bhatnagar, D., Cleveland, T. E., Payne, G. A. & Linz, J. E. (1995) Comparative mapping of aflatoxin pathway gene clusters in *Aspergillus parasiticus* and *Aspergillus flavus*. *Applied and Environmental Microbiology* **61:** 2365–2371.

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