Comparison of Chloroplast Genomes of English Yew (*Taxus baccata* L.) and Japanese Black Pine (*Pinus thunbergii* Parl.)

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Abstract – Previous studies based on of allozyme polymorphisms in *Taxus baccata* (English yew) have been concerned mainly with inheritance analysis and genetic differentiation within and among yew stands. Our primary goal was to find chloroplast primer restriction enzyme combinations which reveal genetic polymorphisms in the chloroplast genome of samples from Central Europe but no polymorphisms were detected. However, we were able to map location of successful primer combinations on the published chloroplast genome of Japanese black pine (*Pinus thunbergii*) and to compare the primer positions with those of two other completely sequenced conifer genomes. Yew sequences in GenBank were also placed onto our map. As we were able to amplify a number of hitherto unexplored spacers between genes, the map of chloroplast structure shows that gene order is likely to be identical between *Taxus* and *Pinus* over large parts of the chloroplast.

similarity / chloroplast / *English yew* / genetic polymorphism

Kivonat – Az európai tiszafa és a japán feketefenyő cpDNS összehasonlító vizsgálata. Korábbi tanulmányok az európai tiszafa (*Taxus baccata*) izoenzim polimorfizmus öröklődésének elemzésével, az állományokon belüli és állományok közötti genetikai differenciálódásával foglalkoztak. Vizsgálataink elsődleges célja olyan kloroplasztisz restrikciós enzimek azonosítása, amelyekkel a közép-európai minták genomjának polimorfizmusa detektálható. Sajnos e polimorfizmust nem tudtuk egyértelműen igazolni, azonban működő primerkombinációk alapján megrajzoltuk a japán feketefenyő (*Pinus thunbergii*) korábban publikált kloroplasztisz genomjára illesztett genomtérképet, amelyhez a GenBank-ban található európai tiszafa szekvenciákat is felhasználtuk. Számos, eddig még fel nem derített, nemátíródó szekvenciát tudtunk amplifikálni. Az így elkészített géntérkép azt valószínűsíti, hogy a vizsgált két faj kloroplasztiszában a gének sorrendje nagymértékben hasonló.

hasonlóság / kloroplasztisz / Európai tiszafa/ genetikai polimorfizmus

1 INTRODUCTION

English yew (*Taxus baccata* L.) has a disjunct distribution in Europe, Asia Minor, the Caucasus and North Africa, extending northward up to 61° N latitude in Scandinavia and southward to the Mediterranean, the Crimea and the Caucasus, eastward to the Baltic Sea and

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the Carpathians and westward to England and Ireland. Its altitudinal range reaches up to 2000 m elevation in Spain and 1200 m in the Alps (Atlas Florae Europeae 2008).

In Hungary, the largest natural occurrence of yew is found in the beech forest of the Bakony Mountains, near Szentgál (Majer 1980).

Previous studies based on allozyme polymorphisms have mainly been concerned with inheritance analysis and genetic differentiation within and among stands on local or regional scale (Lewandowski et al. 1992, Thoma 1992, Hertel 1996, Rajewski – Large 1997, Rajewski et al. 1999, Rajewski et al. 2000, Collins et al. 2003, Frank 2006). The levels of polymorphism were comparable to many European forest trees.

Our primary goal was to find chloroplast primer and restriction enzyme combinations to detect genetic polymorphisms in the samples from Central Europe. Additionally, we wanted to compare the results of our amplification trials with chloroplast structures of three fully sequenced conifer chloroplasts.

2 METHODS

Approximately 125 trees located in the natural yew stand in (Bakony Mts.) Szentgál in Hungary (Latitude: 47°6’31.59” N, Longitude: 17°46’59.32” E), on the territory of the Balaton Upland National Park, plus another three specimens (one male, one female, and one fastigiate "Irish yew") were sampled in the arboretum at Mariabrunn of the Federal Research Centre for Forests (BFW) in Vienna. The selection of primer pairs was based on the chloroplast PCR primer database (http://bfw.ac.at/rz/bfwcms.web?dok=977, Heinze 2007), with *Pinus* as a reference (see *Table 1* for primers).

The PCR reaction mix contained 1 µL DNA, 1x PCR buffer, 2.0 mM $MgCl_2$, 200 nM dNTPs (Invitrogen), 0.2 µM of each primer, Taq Polymerase (Invitrogen) in a total volume of 15 µL. PCR amplification comprised 10 cycles pre-amplification (94°C – 70°C), and 35 cycles of amplification at annealing temperatures of 50°C or 55°C. Five µL of PCR products were separated by electrophoresis in a 1.5% agarose gel, in 0.5% TBE buffer using a 100 base pair (bp) ladder (Invitrogen) as a size standard, and visualized by UV fluorescence with ethidium bromide staining.

PCR products were digested with the following restriction enzymes: *AluI*, *BamHI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *MspI*, *RsaI*. Ten µL of the total PCR products were digested in a total volume of 14 µL. The digested cpDNA fragments were separated on 2.5% agarose gels.

For drawing the chloroplast map in comparison to *Pinus thunbergii* (GenBank[1] Acc. No. NC_001631), we used the Circular Genome Viewer (CGView, Stothard and Wishart 2004). Fragments, that we amplified ourselves, are described as amplified in the literature, and appear in GenBank were drawn onto the map with their approximate positions.

Positions of the primer pairs that sucessfully amplified fragments in *Taxus baccata* were compared to *P. thunbergii*, *Cryptomeria japonica* (GenBank Acc. No. NC_010548) and *Keteleeria davidiana* (NC_011930) with the help of the seqmatchall procedure implemented on the mobyle@pasteur web site (http://mobyle.pasteur.fr/cgi-bin/portal.py?).

3 RESULTS

In common with previous studies, we were unable to detect any cpDNA polymorphisms in English yew samples from Central Europe. The map of chloroplast genome structure of yew

[1] GenBank is a genetic sequence database, an annotated collection of all publicly available DNA sequences. Available online: http://www.ncbi.nlm.nih.gov/genbank/

shows that we were able to amplify new fragments that were neither in NCBI (National Center for Biotechnology Information, available online: http://www.ncbi.nlm.nih.gov/), nor studied by other groups (*Figure 1*). For the spacers that we amplified, gene order is conserved between *Taxus* and *Pinus*. When comparing with the two other completely sequenced conifer chloroplasts, we were often unable to find sufficient match of the primers to the DNA sequences, or primer binding sites were so far apart or in such orientation so that amplification from these conifers (or from *Taxus*, if it had similarly arranged genes) seems unlikely. In the long run, sequencing of the total chloroplast of yew is necessary to confirm this observation (*Table 1*).

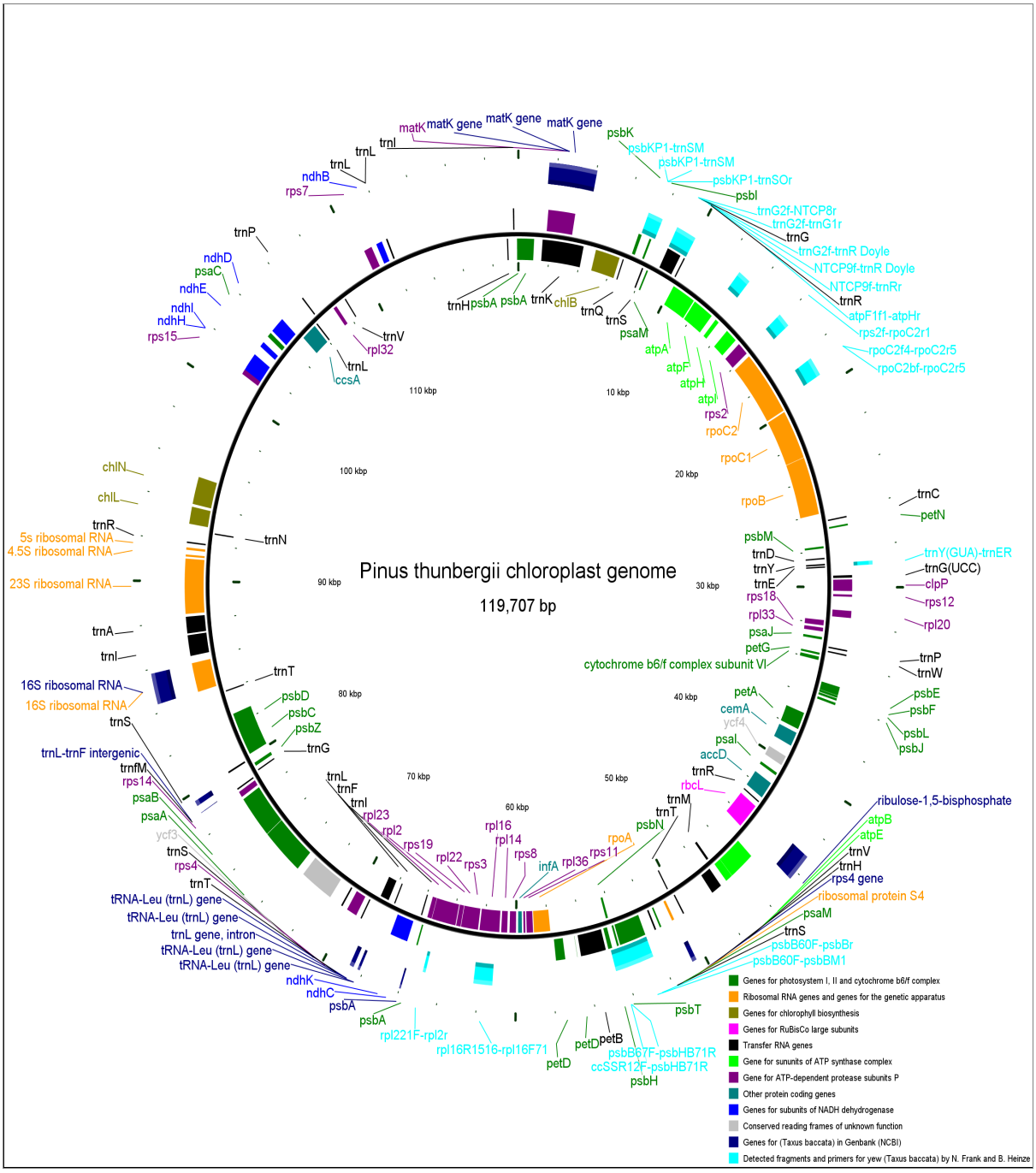

Figure 1. Structure comparison between the chloroplast of Pinus thunbergii and fragments in the Taxus baccata chloroplast

Table 1: Primers selected for PCR amplification (Heinze 2007), and their binding sites in fully sequenced conifer chloroplasts

Primer pairs (forward-reverse)	*Pinus thunbergii*		*Cryptomeria japonica*		*Keteleeria davidiana*		*Taxus baccata*
	amplification range	size (calc.)	amplification range	size (calc.)	amplification range	size (calc.)	approx. size (PCR product)
atpF1f1-atpHr	12821-13516	695	no match-78913	–	53704-no match	–	650
NTCP9f-trnR Doyle	9616-9904	288	no match-82389	–	50507-no match	–	300
trnG2f-trnR Doyle	8854-9904	1050	no match-82389	–	49685-no match	–	1000
NTCP9f-trnRr	9616-9953	337	no match-82346	–	50507-no match	–	225
trnG2f-NTCP8r	8854-9547	693	no match-82692	–	49685-no match	–	625
trnG2f-trnG1r	8854-9635	781	no match-82617	–	49685-no match	–	225
psbB60F-psbBr	52424-52774	350	4013-no match	–	91794-89390	2407	375
psbB60F-psbBM1	52424-53262	838	4013-103343	99330	91794-no match	–	750
psbB63F-psbBB68R	53105-53931	826	4709-no match	–	92490-no match	–	825
psbB67F-psbHB71R	53850-54535	685	5439-no match	–	93220-no match	–	750
ccSSR12F-psbHB71R	53908-54535	627	no match-no match	–	93288-no match	–	650
psbKP1-trnS0r	7173-7980	807	no match-84247	–	47942-91092	43150	225
psbKP1-trnSM	7173-7913	740	no match-58779	–	no match-773/91144**	–	875
rps2f-rpoC2r1	15781-16472	691	127423-128053	630	76110-no match*	–	350
rpoC2f4-rpoC2r5	18443-19366	923	no match-73345		no match-no match		950
rpoC2bf-rpoC2r5	18445-19366	921	no match-no match	–	no match-no match	–	325
rpl2-21F-rpl2r	64912-65059	147	no match-19466	–	no match-no match	–	350
rpl16R1516-rpl16F71	61262-62284	1022	64264-65444	1180	110807-no match	–	600
trnY(GUA)-trnE-R	28734-28915	181	32781-62960	30180	69749-no match	–	575

* no matches at the positions of the corresponding genes
** two matches

4 DISCUSSION

Up to now, cpDNA polymorphisms of yew were only detected in populations from the Mediterranean region (S. Gonzalez–Martinez and G.G. Vendramin, personal communication). This is in contrast to allozyme and RAPD investigations across Europe. Like many other forest tree species, *Taxus* shows typical high genetic variation within stands. Interestingly, English yew is in this respect similar to e.g. European beech (*Fagus sylvatica*), one of its strongest competitors, dominating many yew plant communities (Majer 1980, Magri et al. 2006). The absence of cpDNA polymorphism might be the consequence of a bottleneck effect. A genetic bottleneck in the past may support the view of *Taxus baccata* as a living fossil that is on the brink of extinction. The timing of such a bottleneck, however, remains highly speculative, as fossil pollen data have shown that this species was highly abundant in Central Europe in the last interglacial (Krupinski 2000, Adams 2002). Gao et al. (2007) have reported high levels of cpDNA variation in *Taxus wallichiana* in Asia; however, in a small study with material from one of these Chinese provinces, we were unable to detect variation, nor to detect differences with our Central European samples (Yafeng Wen and B. Heinze, unpublished).

Our comparison of positions of primers and amplified fragments shows that *P. thunbergii* is the best model for the *Taxus* chloroplast, among the three tested. While the taxonomic position of *Taxus* is still debated, our preliminary analysis shows that there are possibly not many gene order rearrangements in comparison to *Pinus*.

REFERENCES

ADAMS, J.M. (compiler; 2002): Europe during the last 150,000 years (web page; URL: http://www.esd.ornl.gov/projects/qen/nercEUROPE.html). In: ADAMS, J.M. – FAURE, H. (eds.): QEN members - Review and Atlas of Palaeovegetation: Preliminary land ecosystem maps of the world since the Last Glacial Maximum. Oak Ridge National Laboratory, TN, USA. http://www.esd.ornl.gov/projects/qen/adams1.html. Accessed Sept 8, 2003.

ATLAS FLORAE EUROPAEAE (2008): Atlas Florae Europaeae – Botanical Museum – FMNH - Mozilla Firefox. Available at: http://www.fmnh.helsinki.fi/english/botany/afe/. Last accessed April 28, 2009.

CAO, C-P. – LEINEMANN, L. – ZIEHE, M. – FINKELDEY, R. (2003): Study of genetic variation and diferrentiation of Yew (*Taxus baccata* L.) stands using isozyme and DNA markers [Untersuchungen zur genetischen Variation und Differenzierung von Eibenbeständen mit Hilfe von Isoenzym- und DNS-Markern. Allgemeine] Forst-und Jagdzeitung 175: 21–28

COLLINS, D – MILL R.R. – MÖLLER M. (2003): Species separation of *Taxus baccata*, *T. canadensis* and *T. cuspidata* (Taxaceae) and origins of their reputed hybrids inferred from RAPD and cpDNA data. Am J Bot 90: 175–182

FRANK, N. (2006): A szentgáli tiszafás populáció-genetikai elemzése RAPD markerrel. [Genetic analysis with RAPD marker in the yew (*Taxus baccata* L.) population of Szentgál.] MSc. Dissertation, Szent István University, Gödöllő, Hungary. (in Hungarian)

GAO, L.M. – MOLLER, M. – ZHANG, X.M. – HOLLINGSWORTH, M.L. – LIU, J. – MILL, R.R. – GIBBY, M. – LI, D.Z. (2007): High variation and strong phylogeographic pattern among cpDNA haplotypes in Taxus wallichiana (*Taxaceae*) in China and North Vietnam. Molecular Ecology: Molecular Ecology 16(22), 4684–4698.

HERTEL, H. (1996): Inheritance of isozyme markers in English yew (*Taxus baccata* L.) [Vererbung von Isoenzymgenmarkern bei Eibe (*Taxus baccata* L.)] Silvae Genet 45: 284–290. (in German)

HEINZE, B. (2007) A database of PCR primers for the chloroplast genomes of higher plants. Plant Methods 3: 4

KRUPINSKI, K.M. (2000): Palynostratigraphic correlation of deposits in the Mazovian interglacial in Poland. Memoirs of the Polish Geological Institute (Available online at: http://www.pgi.waw.pl/english/memoirs/pr169_en.php) vol. 169.

LEWANDOWSKI, A. – BURCZYK, J. – MEJNARTOWICZ, L. (1992): Inheritance and linkage in some allozyme in *Taxus baccata* L. Silvae Genetica 41: 342–347

MAJER, A. (1980): A Bakony tiszafása [Yew of the Bakony Mountains]. Akadémiai Kiadó, Budapest. (in Hungarian)

MAGRI, D. – VENDRAMIN, G.G. – COMPS, B. – DUPANLOUP, I. – GEBUREK, T. – GOMORY, D. – LATALOWA, M. – LITT, T. – PAULE, L. – ROURE, JM. – TANTAU, I. – VAN DER KNAAP, W.O. – PETIT, R.J. – DE BEAULIEU, J.L. (2006): A new scenario for the quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. New Phytologist 171: 199–221.

OTTO, K. – BRADSHAW, T. – WATSON, B. – STEWART, S. (1994): Mini DNA preparation from *Populus*. Manuscript, Dept. of Biochemistry, University of Washington, Seattle, 2 p

RAJEWSKI, M. – LANGE, S. (1997): Genetic structure in the different ontogenetic stage [Genetische Strukturen in verschieden ontogenetischen Stadien der Eibe (*Taxus baccata* L.)] Forstwissenschaftliche Diplomarbeit University of Göttingen. (in German)

RAJEWSKI, M. – LANGE, S. – HATTEMER, H.H. (1999): Genetic inference on the embryo of yew (Taxus baccata L.). Forest Genetics 6: 55–58

RAJEWSKI, M. – LANGE, S. – HATTEMER H.H. (2000): Problem of reproduction in the genetic conservation of the rare species: the example of common yew (*Taxus baccata* L.) [Reproduktion bei der Generhaltung seltener Baumarten. – Das Beispiel der Eibe (*Taxus baccata* L.).] Forest Snow Landscape Research 75: 251–266. (in German)

STOTHARD, P. – WISHART, D.S. (2005): Circular genome visualization and exploration using CGView. Bioinformatics 21: 537–539

THOMA, S, (1992): Genetische Unterschiede zwischen vier Reliktbeständen der Eibe (*Taxus baccata* L.). Forst und Holz 50: 19–24.