Local-scale genetic structure of the spruce bark beetle *Ips typographus* in the Carpathian Mts

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Abstract

In order to quantify the degree of *Ips typographus* population differentiation 20 sites from the Carpathian Mountains were analyzed using the mitochondrial cytochrome c oxidase subunit I (COI). In total 187 individuals were studied. Three of 15 identified haplotypes were dominant in the Carpathians. *I. typographus* showed low levels of genetic variation across the sampled populations indicating weak genetic structure and strong gene flow between populations.

Introduction

The European bark beetle *Ips typographus* L. (Coleoptera, Scolytinae) is considered as a monophagous species colonizing usually weakened or freshly dead Norway spruce (*Picea abies* (L.) Karst.) in Eurasia. Exceptional climatic events initiate *I. typographus* outbreaks which may result in dramatic mortalities of spruce trees (Wermelinger 2004). *I. typographus* is a target of numerous researches due to its ability to cause serious ecological and economical damages in European spruce forests (e.g. Annila 1969; Stauffer et al. 1999; Wermelinger 2004). In the bark beetle, mitochondrial and nuclear markers have increasingly been applied to analyze genetic diversity of populations and elucidate the postglacial distribution and the evolutionary history in Europe (Stauffer et al. 1999; Sallé et al. 2007; Gugerli et al. 2008 Bertheau; et al. 2011). The Carpathians are the broadest mountain range in Europe and are usually divided into three major parts: the Western Carpathians (Czech Republic, Poland, Slovakia), the Central Carpathians (Southeastern Poland, Eastern Slovakia, Ukraine, Romania) and the Eastern Carpathians (Romania, Serbia). The Norway spruce, *P. abies*, survived during the last glaciations in the Carpathians (Tollefsrud et al. 2008) where it is now the most represented tree species. The history of contemporary geographic distribution of *I. typographus* in Europe is not well investigated. Indeed, the hypothesis that *I. typographus* and *P. abies* had shared similar glacial area during the last ice ages was evoked, the determination of this refuge is still not clear. Moreover there is still a lack of genetic data in several regions of *I. typographus* distribution area including the Carpathians. Here we present data on the genetic variation and structure of *I. typographus* populations in the Carpathians using the mitochondrial COI marker.

Data and methods

I. typographus adults were collected throughout the Carpathian region and 20 populations were sampled (see Table 1, Fig.1). Genomic DNA was extracted using Dneasy Blood and Tissue Kit (Qiagen) following manufacturers´ protocols. An 875-bp fragment of COI was amplified by Polymerase Chain Reaction (PCR) according Stauffer et al. (1999) protocol. PCR products were purified using the QIAquick PCR purification Kit (QIAGEN) and the sequencing was performed externally by Macrogene. Sequences were edited using the programs: CHROMAS 1.45, CLUSTAL W as implemented in Bioedit version 7.1.3. The statistical analyses were performed using programs implemented by Swofford (2002), Posada and Crandall (1998), Clement et al. (2000), Excoffier et al. (2005), Pons and Petit (1996) and Dupanloup et al. (2002).

Results and discussion

DNA fragments of COI from 187 individuals of *I. typographus* were amplified and sequenced. Fifteen different haplotypes were identified. Comparison with the previously known *I. typographus* haplotypes (Stauffer et al. 1999; Bertheau et al. 2011) revealed six novel haplotypes coded It30 to It35 (Fig.1). Two haplotypes corresponded to HTI and HTII defined by Stauffer et al. (1999) and seven matched with the haplotypes It1, It 4, It 5, It 7, It 18, It 19 and It 26 described by Bertheau et al. (2011).

Like in the previous studies, HTI was the most common haplotype (107 studied individuals), followed by It1, It19 and HTII with 33, 17 and 14 individuals respectively (Fig.1A). The haplotypes It18 and It5 were detected in four and three individuals respectively, and nine haplotypes (It4, It7, It26 and It30-It35) were found only once. The geographic distribution of the 15 haplotypes is shown Fig.1. The complex distribution pattern of haplotypes explains the SAMOVA analysis after grouping populations according longitude into six groups. However, concerning the distribution of genetic diversity, whatever the geographic level considered, most of the variability was found within populations. Furthermore, the similar values of indices of genetic differentiation were not significant what indicates a weak genetic differentiation. The poorly differentiated genetic structure between Western and Eastern Carpathians might be explained by the dominant distribution of HTI in each sampling site and by constitution of–haplotype network (Fig.1) where the

main haplotypes were very close to each other. *I. typographus* displays good dispersal ability and this may also explains weakly differentiated phylogeographic pattern obtained in this study

Country	Site	Code	Carpathian region	Collector
Slovakia	Tichá dolina valley	S1	IWC	Ján Ferenčík
Slovakia	Mengusovská dolina valley	S2	IWC	Ján Ferenčík
Slovakia	Pod nechcerkom	S3	IWC	Ján Ferenčík
Slovakia	Hrebienok	S4	IWC	Ján Ferenčík
Slovakia	Velická dolina valley	S5	IWC	Ján Ferenčík
Slovakia	Bielovodská dolina valley	S6	IWC	Ján Ferenčík
Slovakia	Liptovský Ján	S7	IWC	Ján Ferenčík
Slovakia	Brezno	S8	IWC	Ján Ferenčík
Slovakia	Sklené	S9	IWC	Ján Ferenčík
Slovakia	Blatnica	S10	IWC	Ján Ferenčík
Slovakia	Morskie oko	P1	IWC	Ján Ferenčík
Slovakia	Široká dolina valley	S11	OWC	Ján Ferenčík
Poland	Ždiar	S12	OWC	Ján Ferenčík
Romania	Comandau-Covasna	R1	OEC	Vasile Mihalciuc
Romania	Garcin-Brasov	R2	OEC	Vasile Mihalciuc
Romania	Vaser-Maramures	R3	IEC	Vasile Mihalciuc
Romania	Padis-Oradea	R4	WRC	Vasile Mihalciuc
Romania	Tomnatec-Cluj	R5	WRC	Vasile Mihalciuc
Romania	Gradiste-Hunedoara	R6	SC	Vasile Mihalciuc
Ukraine	Sumy reg	U1	Yampol` dis	Jurij Skrylnik

Table 1: Characteristics of *Ips typographus* sampling sites. IWC (Inner Western Carpathians), OWC (Outer Western Carpathians), OEC (Outer Eastern Carpathians), WRC (Western Romanian Carpathians), IRC (Inner Eastern Carpathians), SC (Southern Carpathians)

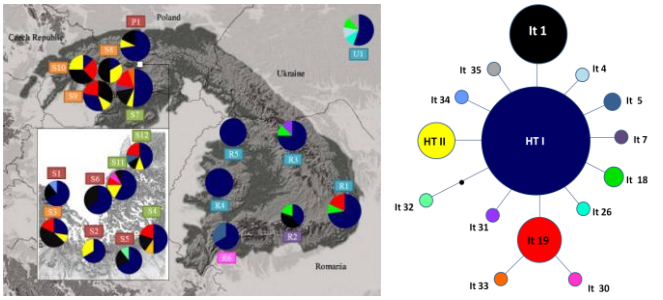

Figure 1: Haplotype distribution and haplotype network of 187 *Ips typographus* COI sequences. Left chart: Geographic distribution of the haplotypes among the 20 sampled populations. Right chart: Haplotype network of the 15 haplotypes identified in the Carpathians.

Our results furthermore suggests a high gene flow between populations what is consistent with the previous studies investigating large-scale structure of *I. typographus* populations either using the same primers (Stauffer et al. 1999) or using microsatellites (Sallé et al. 2007; Gugerli et al. 2008). The distribution of haplotypes among Carpathian populations, where the presence of several haplotypes were evident in the Western Carpathians (Poland, Slovakia) but not in the Eastern Carpathians (Romania) could help to interpret our results. Taberlet et al. (1998) suggest that northern Carpathians have two separate glacial refugia located in the Western Carpathians and the northern part of the Eastern Carpathians. Accordingly, we consider the Western Carpathians as possible refugial zone. Eventually, even the possible refugia are proposed, the geographical distributions of haplotypes and of diversity indices don't allow the inference of the existence of past refugia. Nevertheless, in order to complete the information on phylogeography of *I. typographus*, to establish its good management and to confirm unique population structure in the Carpathians, analysis of additional genetic marker is needed.

Conclusion

Our results showed that *I. typographus* has low levels of genetic variation across the Carpathians indicating weak genetic structure and provide evidence that extant gene flow are among populations. Our data suggest that historical events have had a significant effect on the distribution pattern of genetic variation at mtDNA loci.

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