J For Res (2007) 12:345–349
DOI 10.1007/s10310-007-0025-9

SPECIAL FEATURE: ORIGINAL ARTICLE

Alien pests threatening biodiversity of forest ecosystems

Genetic comparison of *Ips duplicatus* (Sahlberg, 1836) (Coleoptera: Curculionidae, Scolytinae) populations from Europe and Asia

Ferenc Lakatos · Wojciech Grodzki · Qing-He Zhang · Christian Stauffer

Received: 10 February 2006 / Accepted: 19 September 2006 / Published online: 8 August 2007

Abstract The distribution of the double spined spruce engraver beetle *Ips duplicatus* ranges from Scandinavia and northeastern Europe to northern Asia. In Europe, *I. duplicatus* usually is associated with *I. typographus* on *Picea abies*, and due to morphological similarities and similar gallery constructions the damage and significance of *I. duplicatus* are often not recognised and thus underestimated. *I. duplicatus* has been recently reported from the southern part of Poland, the Czech Republic, Slovakia, northern parts of Austria and Germany; records are missing from many other central European countries (e.g., Hungary). The species became an important pest in some parts of central Europe, and continuous outbreaks of this bark beetle have been reported in Inner Mongolia, China, since the 1950s. The aim of this study was to compare *I. duplicatus* populations from Europe and Asia by genetic means using the analysis of the mitochondrial DNA. Individuals of *I. duplicatus* populations from China, Poland, the Czech Republic and Slovakia were collected, and 520 bp fragments of the *cytochrome oxidase I* (COI) gene were analysed. Four haplotypes were detected and a sequence divergence of 0.8% was found between the populations from China and Europe. These differences associate with behavioural differences in the pheromone bouquet and behavioural response of the two groups. Within Europe three haplotypes were found, but due to the small sample size no significant geographical distribution was demonstrated.

Keywords Allochthonous · Autochthonous · Genetic structure · *Ips duplicatus* · MtDNA · *Picea abies*

F. Lakatos (✉)
Institute of Forest and Wood Protection,
University of West-Hungary,
Bajcsy-Zs. u. 4, 9400 Sopron, Hungary
e-mail: flakatos@emk.nyme.hu

W. Grodzki
Department of Forest Management in Mountain Regions,
Forest Research Institute, Krakow, Poland

Q.-H. Zhang
Sterling International Inc, Spokane, WA, USA

Ch. Stauffer
Department of Forest and Soil Sciences, Boku,
University of Natural Resources and Applied Life Sciences,
Vienna, Austria

Introduction

Species of the subfamily Scolytinae (family Curculionidae) are among the most destructive forest pests in the temperate zone (for a recent review see Lieutier et al. 2004). There are many species of scolytinae that have a wide natural distribution and that have exhibited an ability to be introduced and become established in new environments and become problematic. Two recent examples are *Tomicus piniperda* and *Ips typographus* having been established in North America (Haack & Cavey 2000).

The natural distribution of the double spined spruce engraver beetle, *I. duplicatus* Sahlberg 1836 (Curculionidae, Scolytinae, Ipini), includes the Palearctic from Scandinavia through some parts of northeastern Europe to northern Asia (Pfeffer 1995; Wood and Bright 1987, 1992). In Europe, *I. duplicatus* is frequently associated with *I. typographus* on their principle host Norway spruce (*Picea abies*); however several other tree species are listed as potential hosts for

both beetle species (Pfeffer 1995). Due to morphological similarities and similar gallery constructions, the damage and significance of *I. duplicatus* are often not recognised and are thus likely underestimated (Schlyter et al. 1992). *I. duplicatus* has been reported from northern (Karpiński 1935) and southern (Grodzki 1997) Poland, the Czech Republic (Knizek and Zahradnik 1996; Holuša et al. 2003), Slovakia (Turčani et al. 2001), northern Austria (Holzschuh 1989) and Germany (Bussler and Bense 2003), but records are missing from several other central European countries where this forest pest can be expected to infest *P. abies*. The purpose of this study was to establish a rapid and clear method to distinguish between these two *Ips* species.

The importance of *I. duplicatus* in Europe is still undetermined. It is listed as a quarantine pest by the European Union and European and Mediterranean Plant Protection Organisation (Smith et al. 1996), as well as being listed on some protected species lists, too (e.g., Bussler and Bense 2003).

Local outbreaks of *I. duplicatus* have been recorded in Silesia and Moravia in Poland and the Czech Republic (Knizek and Zahradnik 1996; Grodzki 1997, 2003) and have been considered as the major damage-causing species in natural spruce forests in Inner Mongolia, China (Zhang et al. 1995, 2001; Schlyter et al. 2001a).

There have been differences shown in pheromone production and pheromone reaction behaviour of the European and Asian *I. duplicatus* populations (Schlyter et al. 2001a, b; Zhang et al. 2001).

The aim of this study was to compare *I. duplicatus* populations from central Europe with populations from northern Europe and Inner Mongolia using molecular techniques.

Materials and methods

Sample collection

Individuals of *I. duplicatus* populations were collected from China (Inner Mongolia), northern Poland (Bialowieza National Park), the Czech Republic (Sumava mountains) and Slovakia (High-Tatra mountains). Samples were placed into ethanol absolute and stored at –20°C.

Molecular analysis

Insect DNA was extracted using the GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma, USA) with slight modification of the manufacturer's protocol: Insects were put into 1.5-ml Eppendorf tubes, overlaid with 180 µl lysis solution T and homogenized with a pestle. Afterwards, 20-µl proteinase K was added, vortexed and incubated at 55°C for 2 h under constant agitation at 400 rpm. Twenty microlitres of RNase and 200 µl lysis solution C were added and the mixture incubated for 10 min at 70°C. Two hundred microliter ethanol were added and the whole mix was loaded by centrifugation at 8,000 rpm for 1 min onto a binding column. Finally, the columns were washed twice with 500 µl washing solution, and DNA was eluted in 50-µl elution buffer. The eluted DNA was stored at 4°C for up to 3 weeks, but for long-term storage DNA was kept at –20°C. The amplification of the DNA was carried out in 25 µl reactions containing 3.75 mM $MgCl_2$ 125 µM dNTPs (Fermentas, Lithuania), 0.5 µM of Dick 5′-ccaacaggaattaaaatttttagatgattagc-3′ (position: 2,410–2,441) and Pat 5′-tccattgcactaatctgccatatta-3′ (position: 3,014–3,039) (Lunt et al. 1996) and 1 U of Biotherm Taq (Genecraft, Germany). The amplifications were carried with an initial denaturation step of 3 min at 94°C, which was followed by 33 cycles of 94°C (30 s), 48°C (60 s) and 68°C (90 s) and a final extension step at 68°C (10 min). PCR was performed in a Primus 25 advanced Thermocycler (PeqLab, Germany) in 200-µl tubes (Biozym, Germany).

Statistical analysis

The mitochondrial DNA sequence alignment was performed by Clustal X (Thompson et al. 1997) using the default setting. Distance analysis was performed by the neighbour joining NJ algorithm (Saitu and Nei 1987; Studier and Keppler 1988) as it is implemented in the MEGA version 3 (Kumar et al. 2004). The distance matrix was calculated based on the Tamura–Nei substitution model (Tamura and Nei 1993), while the robustness of the topology was tested by bootstrapping with 1,000 repetitions (Felsenstein 1985, 1988). For comparison also the Genbank entries of *I. duplicatus* (submitted by Cognato A: AF113345, AF113346 and Stauffer C: U82586) and *I. typographus* were taken (submitted by Stauffer C: AF036156).

Results

Four haplotypes (HT) of the 520-bp fragment of the *cytochrome oxidase I* (COI) gene were detected, having six mutation sites, all at third codon positions (Tables 1, 2, 3). The six individuals of the three European populations represent HT1, HT2 and HT3, while the six Inner Mongolian samples expressed only HT4 (Table 1). The Inner Mongolian population showed a sequence divergence of 0.8%. *I. typographus* clearly could be distinguished from *I. duplicatus* having a sequence divergence of >12%.

Table 1 Collection sites and haplotypes of investigated *Ips duplicatus* samples

Country	Area	Individual/abbreviation	Haplotype	GenBank identifier
Poland	Bialowieza	PL-a	HT-1	DQ912411
		PL-b	HT-1	
Slovakia	High-Tatra	SK-a	HT-1	
		SK-b	HT-2	DQ912412
The Czech Republic	Sumava	CZ-a	HT-1	
		CZ-b	HT-3	DQ912413
China	Inner Mongolia	CN-a	HT-4	DQ912414
		CN-b	HT-4	
		CN-c	HT-4	
		CN-d	HT-4	
		CN-e	HT-4	
		CN-f	HT-4	

Table 2 Datasets from GenBank included in the analysis

Species	Author	Country	Abbreviation	Haplotype	GenBank identifier
Ips duplicatus	A. Cognato et al.	Russia	RU-AC	HT-1	AF113346
	A. Cognato et al.	Czech Republic	CZ-AC	HT-1	AF113345
	C. Stauffer et al.	Czech Republic	CZ-CS	HT-5	IDU82586
Ips typographus	C. Stauffer et al.	Austria	IPSTYP		AF036156

Table 3 Haplotypes of *Ips duplicatus*

Haplotypes	Mutation sites					
	75	108	315	321	348	504
HT1	G	C	G	T	G	C
HT2	A					
HT3			A			
HT4		T		C	T	T

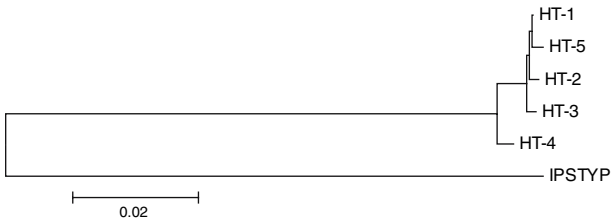

Fig. 1 Neighbour joining tree of the investigated and in the GenBank available *Ips duplicatus* COI sequences using *I. typographus* as an out-group (Kimura 2 parameter)

Discussion

In this study we have shown that *I. duplicatus* and *I. typographus* can be easily distinguished by sequence analysis (Fig. 1). This supports data of Stauffer et al. (1997) where the seven European *Ips* species were phylogenetically analysed.

The data clearly indicate that the central European areas originate most probably from the north (Fig. 2).

The sequence difference between the populations from Asia and Europe associates with the differences in the pheromone production and reaction behaviour of the two groups (Schlyter et al. 2001a, b; Zhang et al. 2001, 2007). The aggregation pheromone system of *I. duplicatus* has been intensively studied in Europe, which consisted of two components, ipsdienol (ID) and *E*-myrcenol (EM) (Bakke 1975; Byers et al. 1990; Schlyter et al. 1992). Study on the optimum proportion for behavioural attraction to these two components in different populations from Norway, the Czech Republic and Inner Mongolia, China, showed that the Inner Mongolia population has more even or reversed optimal attraction ratios (Id:EM; 1:1 to 1:9) than the European populations (Schlyter et al. 2001a), while no obvious differences in pheromone responses between European populations were found. A recent GC-EAD and GC-MS study on the Inner Mongolian population also found a third minor aggregation pheromone component, amitinol (Zhang et al. 2007). The Inner Mongolian population produces and responds significantly more to *E*-myrcenol than do the European populations. It should be noted that the Inner Mongolian samples were collected from an isolated spruce nature reserve (Zhang et al. 2001).

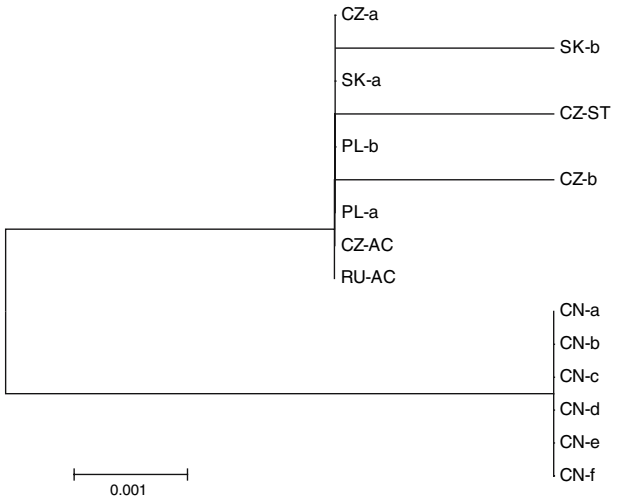

Fig. 2 Neighbour joining tree of the investigated and in the GenBank available *Ips duplicatus* COI sequences (Kimura 2 parameter) (haplotypes are in Table 1)

The small sample size and uncertainties of molecular-clock dating (reviewed by Bromham and Penny 2003) make considerations of the time of divergence of these two populations tentative, but assuming the general calculations of 2% sequence divergence per million of years, 0.4 millions of years before the present can be estimated.

Similar phylogeographic investigations of *I. typographus* and *I. cembrae* showed similar subdivisions between Asian and European species (Stauffer et al. 1999, 2001).

Acknowledgments This study was supported by the OMAA–ÖAD research grant: 942-30/2004. We thank Jaroslav Holuša and Miroslav Blazenec for providing specimens from the Czech Republic and Slovakia. QHZ would like to thank Mr. Guang-Tian Liu in Baiyinaobao Natural Reserve, Inner Mongolia, China, for his help with specimen collections in China, and Dr. Fredrik Schlyter at SLU, Sweden, for general support. A special thanks is extended to Keith Douce for the language corrections.

References

Bakke A (1975) Aggregation pheromone in the bark beetle, *Ips duplicatus* (Sahlberg). Norw J Entomol 22:67–69

Bromham L, Penny D (2003) The modern molecular clock. Nat Rev Genet 4:216–224

Bussler H, Bense U (2003) Rote Liste gefährdeter Borkenkäfer (Coleoptera: Scolytidae), Breitrüssler (Anthribidae) und Kernkäfer (Platypodidae) Bayerns. Bayer Landsamt f Umweltschutz 166:172–173

Byers JA, Schlyter F, Birgersson G, Francke W (1990) *E*-myrcenol in *Ips duplicatus*: an aggregation pheromone component new for bark beetles. Experientia 46:1209–1211

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Felsenstein J (1988) Phylogenies and quantitative characters. Ann Rev Ecol Syst 19:445–471

Grodzki W (1997) Possibilities of the control of the double-spined bark beetle *Ips duplicatus* C.R.Sahlb in the Southern Poland (in Polish). Sylwan 11:25–36

Grodzki W (2003) Distribution range of the double spined bark beetle *Ips duplicatus* C.R Sahlb (Col.: Scolytidae) in the mountain areas of southern Poland (in Polish). Sylwan 8:29–36

Haack RA, Cavey JF (2000) Insects intercepted on solid wood packing materials at United States ports-of-entry: 1985–1998. In: Proceedings of international conference on quarantine pests for the forestry sector and their effects on foreign trade, 27–28 June 2000, Concepcion, Chile. CORMA, Concepcion, Chile. 16

Holuša J, Zahradnik P, Knižek M, Drapela K (2003) Seasonal flight activity of the double-spined spruce bark-beetle *Ips duplicatus* (Coleoptera, Curculionidae, Scolytinae) in Silesia (Czech Republic). Biol Bratislava 58:935–941

Holzschuh C (1989) Wurde *Ips duplicatus* Sahlberg durch Importholz nach Österreich verschleppt? Forstschutz-Aktuell Wien 2:4

Karpiński JJ (1935) Causes limiting reproduction of the bark beetles (*Ips typographus* L i *Ips duplicatus* Sahlb.) in primeval forest. Inst Bad Pastw Rozprawy Sprawozdania, A 15:1–86

Knizek M, Zahradnik P (1996) Mass outbreak of *Ips duplicatus* Sahlberg. In: Proceedings of the XXth international congress of entomology, Firenze, Italy

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Lieutier F, Day KR, Battisti A, Grégoire J-C, Evans HF (eds) (2004) Bark and wood boring insects in living trees in Europe, a synthesis. Kluwer, Dordrecht

Lunt D, Zhang DX, Szymura JM, Hewitt GM (1996) The insect COI gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol Biol 5:153–165

Pfeffer A (1995) Zentral- und westpaläarktische Borken- und Kernkäfer (Coleoptera: Scolytidae, Platypididae). Pro Entomologia, c/o Naturhistorisches Museum, Basel

Saitu N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Schlyter F, Birgersson G, Byers JA, Bakke A (1992) The aggregation pheromone of *Ips duplicatus* and its role in competitive interactions with *I. typographus*. Chemoecology 3:103–112

Schlyter F, Svensson M, Zhang Q-H, Knizek M, Krokene P, Ivarsson P, Birgersson G (2001a) A model for peak and width of signaling windows: *Ips duplicatus* and *Chilo partellus* pheromone component proportions—does response have a wider window than production? J Chem Ecol 27:1481–1512

Schlyter F, Zhang Q-H, Liu GT, Ji LZ (2001b) A successful case of pheromone mass trapping of the bark beetle *Ips duplicatus* in a forest island, analysed by 20-year time-series data. Integr Pest Manage Rev 6:185–196

Smith IM, McNamara DG, Scott PR, Holderness M (eds) (1996) Quarantine pests for Europe, 2nd edn. CAB International, Wallingford

Stauffer C, Lakatos F, Hewitt G (1997) Phylogenetic relationships of the bark beetle species of the genus *Ips* DeGeer. Insect Mol Biol 6:233–240

Stauffer C, Lakatos F, Hewitt GM (1999) Phylogeography and postglacial colonization routes of *Ips typographus* L (Coleoptera, Scolytidae). Mol Ecol 8:763–773

Stauffer C, Kirisits T, Nussbaumer C, Pavlin R, Wingfield MJ (2001) Phylogenetic relationships between the European and Asian eight spined larch bark beetle populations (Coleoptera, Scolytidae) inferred from DNA sequence and fungal associates. Euro J Entomol 98:99–105

Studier JA, Keppler KJ (1988) A note on the neighbor-joining method of Saitou and Nei. Mol Biol Evol 5:729–731

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Thompson JD, Higgins DG, Gibbson TJ (1997) Clustal X: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673

Turčani M, Csóka Gy, Grodzki W, Zahradnik P (2001) Recent invasions of exotic forest insects in eastern central Europe. In: Protection of world forests from insect pests: advances in research. IUFRO World Series 11:99–106

Wood SL, Bright DE (1987) A catalog of Scolytidae and Platypodidae (Coleoptera), Part 1: Bibliography, Great Basin Nat Memoirs No. 11

Wood SL, Bright DE (1992) A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: taxonomic index, vol A and B. Great Basin Nat Memoirs No. 13

Zhang Q-H, Schlyter F, Liu GT (1995) Spatial distribution, mortality and sex ratio of overwintering *Ips duplicatus* in a *Picea mongolica* reserve in Inner Mongolia, China with a diffusion model In: Hain FP, Salom SS, Ravlin WF, Payne TL Raffa KF (eds) Behavior, population dynamics and control of forest insects. Ohio State University, OARDC, Wooster, pp 109–122

Zhang Q-H, Liu G-T, Schlyter F, Birgersson G, Anderson P, Valeur P (2001) Olfactory responses of *Ips duplicatus* from Inner Mongolia, China to nonhost leaf and bark volatiles. J Chem Ecol 27:955–1009

Zhang Q-H, Schlyter F, Liu GT, Sheng ML, Birgersson G (2007) Electrophysiological and behavioral responses of *Ips duplicatus* to aggregation pheromone in Inner Mongolia, China: amitinol as a potential pheromone component. J Chem Ecol 33:1303–1315. doi:10.1007/s10886-007-9320-3