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Comparative multilocus phylogeography of two Palaearctic spruce bark beetles: influence of contrasting ecological strategies on genetic variation

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Abstract

While phylogeographic patterns of organisms are often interpreted through past environmental disturbances, mediated by climate changes, and geographic barriers, they may also be strongly influenced by species-specific traits. To investigate the impact of such traits, we focused on two Eurasian spruce bark beetles that share a similar geographic distribution, but differ in their ecology and reproduction. *Ips typographus* is an aggressive tree-killing species characterized by strong dispersal, whereas *Dendroctonus micans* is a discrete inbreeding species (sib mating is the rule), parasite of living trees and a poor disperser. We compared genetic variation between the two species over both beetles' entire range in Eurasia with five independent gene fragments, to evaluate whether their intrinsic differences could have an influence over their phylogeographic patterns. We highlighted widely divergent patterns of genetic variation for the two species and argue that the difference is indeed largely compatible with their contrasting dispersal strategies and modes of reproduction. In addition, genetic structure in *I. typographus* divides European populations in a northern and a southern group, as was previously observed for its host plant, and suggests past allopatric divergence. A long divergence time was estimated between East Asian and other populations of both species, indicating their long-standing presence in Eurasia, prior to the last glacial maximum. Finally, the strong population structure observed in *D. micans* for the mitochondrial locus provides insights into the recent colonization history of this species, from its native European range to regions where it was recently introduced.

Keywords: comparative phylogeography, *Dendroctonus micans*, *Ips typographus*, life history traits, nuclear marker

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Introduction

One primary goal of phylogeography is to identify the historical events that have shaped the current genetic variation within species (Avise 2000). Comparative phylogeography of codistributed species based on multiple genes has emerged as a powerful tool to infer general

patterns of evolutionary history associated with specific regions (Bermingham & Moritz 1998; Avise 2008).

Many such studies have involved comparisons of species sharing similar life history traits to identify climatic events that had a major and similar impact on the range of multiple species, for example, for those adapted to temperate climate in Europe (Taberlet *et al.* 1998; Hewitt 2000) or eastern North America (Bernatchez & Wilson 1998; Soltis *et al.* 2006). An alternative approach is to compare codistributed species that differ only in one or a few major trait(s) to investigate their impact on the distribution of genetic variation. Highlighting the impact of specific life history traits on genetic variation has a large potential for unravelling observed phylogeographic patterns. However, to the best of our knowledge, relatively few studies implementing this approach have been conducted so far (e.g. Turner *et al.* 1996; Michaux *et al.* 2005; Duminil *et al.* 2007).

Here, we examine genetic variation in two codistributed bark beetle species, the Eurasian spruce bark beetle *Ips typographus* (L., tribe Ipini) and the great spruce bark beetle *Dendroctonus micans* (Kug., tribe Hylurgini). Both species are Palaearctic Scolytinae (Coleoptera, Curculionidae) and are specific phloeophagous pests on spruces, although they can occasionally shift to other hosts such as Scots pine (*Pinus sylvestris* L.) (Voolma 1978; Kolomiets & Bogdanova 1999; Bertheau *et al.* 2009). In Europe, their main host is the Norway spruce, *Picea abies* [L.] Karst., naturally present in northern regions and in the mountain ranges of central and southern Europe (Schmidt-Vogt 1977). While the two species share the same host plant and feeding mode, they differ widely in their dispersal strategies and mating behaviour (Table 1). Our goal was to determine whether these differences have a measurable impact on genetic variation within the two species.

I. typographus is a virulent tree-killing species responsible for heavy losses in spruce stands (Grégoire & Evans 2004) across Europe. At low population density, *I. typographus* is restricted to freshly fallen trees. However, after a strong increase in population density, for example after a clear cut or a storm, the species also becomes capable of overcoming defences of healthy trees, often leading to

Table 1 Main biological features of *D. micans*, *I. typographus* and *P. chalcographus*

	Dendroctonus micans	*Ips typographus*	*Pityogenes chalcographus**
Host plant	Mainly *Picea abies*	Mainly *Picea abies*	*Picea abies* and other Pinaceae species
Feeding mode	Phloephagous	Phloephagous	Phloephagous
Sex ratio	Largely female-biased sex ratios, from 5:1 to 48:1 Grégoire (1988)	Overall sex ratio of emerging insects from 50% Faccoli & Buffo (2004) to 62% Lindelöw & Weslien (1986)	On average 74% and stable over time Hedgren (2004)
Mating behaviour	Sib mating Grégoire (1988); some possibilities of outbreeding Fraser *et al.* (2014)	Outbreeding Wermelinger (2004)	Outbreeding Schwerdtfeger (1929)
Parent adults in brood systems	One fertilized female	One male and 1 to 3 females Wermelinger (2004)	One male and 2 to 7 females Chararas (1962)
Voltinism	Semivoltine to univoltine (Grégoire 1988)	Uni- to multivoltine (1 to 3 generations/year) Wermelinger (2004)	Uni- to multivoltine Schwerdtfeger (1929)
Fecundity	100-150 eggs per female (Grégoire 1988)	Up to 80 eggs per female Wermelinger (2004)	10-16 eggs/female Schwerdtfeger (1929)
Dispersal capacity	Up to 40 km in flight mills (Rigaux, pers. comm.); approximately 5–6 km/year (O'neill & Evans 1999)	18 km in one hour, windborne Forsse & Solbreck (1985); found infesting logs up to 43 km away from spruce forests Nilssen (1984)	Found infesting logs up to 86 km away from spruce forests Nilssen (1984)
Aggregation pheromones	No (Grégoire 1988)	Yes Wermelinger (2004)	Yes Francke (1977)
Primarity (attack living trees)	Yes (Grégoire 1988)	Yes, when at high population densities Wermelinger (2004)	Yes Hedgren (2004)
Tree killers	Usually no (Grégoire 1988)	Yes when at high population density Wermelinger (2004)	Yes when at high population density Hedgren (2004)
Outbreaks	At the edge of its range; otherwise controlled by natural enemies (Grégoire 1988)	Yes, usually after climatic disturbance Wermelinger (2004)	Yes, usually after climatic disturbance Hedgren (2004)

*See discussion.

host death due to its association with pathogenic fungi, in particular *Ceratocystis polonica*, which can directly kill trees as demonstrated by inoculation experiments (Krokene & Solheim 1998) and reduce their defences either directly (Hammerbacher *et al.* 2013) or indirectly by depleting their phloem nonstructural carbohydrates and sapwood lipids (Lahr and Krokene 2013). The virulence of the species is mainly driven by life history traits typical of an opportunistic strategist (MacArthur 1960) well adapted to unstable and unpredictable resources, such as a short life cycle with 1–3 generation(s) per year (Botterweg 1982; Nageleisen 2004), abundant offspring with nearly 35 000 pre-emergent adults in each cubic metre of attacked tree (Gonzalez *et al.* 1996) and an efficient aggregation pheromone system allowing rapid and massive colonization (Andersson *et al.* 2009). The opportunistic strategy of *I. typographus* relies on a highly efficient dispersal capacity that facilitates the location of suitable hosts; movements from 19 to 50 km within a single generation have been recorded by flight-mill studies (Forsse & Solbreck 1985) and mark–release–recapture experiments (Botterweg 1982; Nilssen 1984; Franklin & Grégoire 1999).

In contrast, *D. micans* is usually associated with a specific predator, *Rhizophagus grandis* Gyll. (Coleoptera, Monotomidae), which keeps its populations under control and makes it a rather harmless parasite that usually does not kill its hosts, except in newly colonized areas that are devoid of this predator, and where outbreaks can occur. Due to a high level of resistance to the spruce defensive monoterpenes (Everaerts *et al.* 1988), single *D. micans* females are able to establish in the phloem of healthy standing trees. Although occasional high dispersal capacities are recorded under laboratory conditions (Forsse 1989; J.-C.Grégoire pers. com.), the abundance of suitable hosts in endemic conditions results in low actual migration rates and strong spatially structured attack densities (Gilbert & Grégoire 2003). Incidentally, this species has been able to invade new areas due to transport with spruce logs (Kobakhidze 1967; Bevan & King 1983; Pauly & Meurisse 2007).

Despite previous phylogeographic studies on *I. typographus* and the availability of historical data on *D. micans*, their evolutionary histories remain largely unknown. It was initially claimed that *I. typographus* shares the same history as its host, because a similar modern disjunction between the north-eastern and southern parts of Europe was detected in the mitochondrial structure (Stauffer *et al.* 1999). Later, studies based on microsatellites and ITS sequences (Sallé *et al.* 2007; Bertheau *et al.* 2013) failed to identify any genetic structure, and a reinvestigation of mitochondrial sequence data reached a similar conclusion, interpreted as a recent radiation of *I. typographus* populations from a unique refugium (Bertheau *et al.* 2013; Mayer *et al.* 2014). The incongruence between the two mitochondrial DNA data sets was explained by the presence of cryptic nuclear copies (Numts) amplified in some individuals of *I. typographus* (Bertheau *et al.* 2011, 2013).

Conversely, the current knowledge on *D. micans* history is rather scarce and mainly based on monitoring records and on comparisons with other congeneric species. The genus *Dendroctonus* is particularly abundant in North America, and *D. micans* is the only European representative, presumably originating from eastern Siberia (Schedl 1932). Its relatedness to the North American species *D. punctatus* has suggested that a founder population of that species crossed the Bering Strait from America to Eurasia in a period of low sea level during the last glacial maximum (LGM), and colonized Eurasia (Furniss 1996; Kelley & Farrell 1998). Recent palynologic (Brubaker *et al.* 2005) and phylogeographic studies (Anderson *et al.* 2006) attest the existence of spruces in eastern Beringia at that time.

Because dispersal strategies and mode of reproduction affect gene flow and effective population size (Pollak 1987; Nordborg 2001), we hypothesized that the variation in life history traits will have a differential impact on the phylogeographic pattern in the two species. More specifically, we tested the expectation that *D. micans* exhibits high levels of population structure across its range, but low genetic diversity within local populations, being a poor disperser relative to *I. typographus* and being characterized by small population sizes and sib mating. Conversely, we expected *I. typographus* to exhibit low population structure across its range and high genetic diversity within local populations, due to its much more efficient dispersion. Moreover, strong migration is likely to have erased, at least partially, the phylogeographic signal in this species, and phylogeographic structure is likely to be weaker. On the other hand, if the contrasting life history traits had little impact on genetic variation, the two species should exhibit comparable patterns. Indeed, because they are codistributed and associated with the same host plant, they would most likely be exposed to the same environmental disturbances and may have responded to them in a similar fashion.

To test these hypotheses, we have conducted an extensive sampling effort over the entire range of both species and have sequenced a mitochondrial DNA fragment for 265 *I. typographus* and 228 *D. micans* individuals and sequenced four nuclear DNA fragments for a fraction of them (85-132 *I. typographus* and 42-74 *D. micans*, depending on the locus considered). Although phylogeographic data have been collected in the past for *I. typographus*, our sampling covers a larger portion of its range and includes a larger number of loci, therefore offering the potential for new historical

inferences. Finally, because the geographic range of Norway spruce was much more restricted in the past (Tollefsrud *et al.* 2008) and was extended only recently by human activity for economic reasons, we compared the genetic variation of the two bark beetles between their native and recently colonized ranges, to investigate whether it is possible to infer the colonization routes into non-native regions.

Materials and methods

Samples collection

We collected 296 individuals of *I. typographus* in 121 localities and 228 individuals of *D. micans* in 110 localities in Europe, Siberia, Caucasus and the Russian Far East, using pheromone traps or by direct sampling of attacked trees. Additionally, we obtained four samples of the sister species *D. punctatus* Leconte from Canada (British Columbia) and Alaska (Noatak River). On each tree, individuals were gathered in a separate maternal gallery to avoid collecting siblings. Most of the samples were collected on native spruces but also occasionally on introduced spruces or native pines (Tables S1 and S2, Supporting information).

Insects were preserved in absolute ethyl alcohol until extraction, except for a few dry specimens from entomological collections (D-RU-Ka, D-HR and I-GE-4-1) or samples sent in other solvent blends (D-UK-2, I-Cz-1 and I-CH-4).

DNA extraction, sequencing and preliminary data analyses

Total genomic DNA was extracted from the entire beetle with a DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's protocol. All specimens were incubated overnight with ATL buffer and proteinase K at 56 °C. Dry specimens were additionally quick-frozen in liquid nitrogen preliminary to cutting into small pieces.

As increasing the amplicon size is one method proposed to reduce Numt co-amplification (Nardi *et al.* 2003; Miraldo *et al.* 2012), a fragment of the COI gene two times longer than previously analysed was amplified using primers TL2-N-3014 (Simon *et al.* 1994) and a modified version of C1-J-1751 (Mardulyn & Milinkovitch 2005), generating a 1250-bp long fragment to sequence. Despite the long fragment used, the electropherograms for 15 COI *I. typographus* sequences presented double peaks and were considered potential nuclear copies (Numts). By further comparing our entire COI data set with *I. typographus* sequences previously identified by Bertheau *et al.* (2011) as Numts, 31 additional sequences (of 296) that matched with ItNumt1-3 (JN133882-4) were deleted from our data set prior to analysis. None of the remaining sequences exhibited a stop codon.

In addition to the mitochondrial fragment, four nuclear coding gene fragments were amplified: arginine kinase (AK, amplicon size: ± 650 bp), elongation factor-1 α (EF-1α, amplicon size: ± 850 bp in *I. typographus* and 1150 in *D. micans*), glucose-6-phosphate dehydrogenase (G-6-PD, amplicon size: ± 850 bp) and wingless (Wg, amplicon size: ± 500 bp). With the exception of the primer pairs used to amplify the *D. micans* fragments of Wg (5′WG1 and 3′WG2, Ober 2002) and EF-1α (HaF2For1 and Cho10, Praz *et al.* 2008), all primer pairs were specifically designed for this study (see Material S1, Supporting information for further details) and are presented in Table S1 (Supporting information). All collected individuals of both species were sequenced for the COI marker, while only a subset of these was sequenced for each nuclear marker (Tables S2 and S3, Supporting information).

All PCRs were conducted with the Truestart Taq Polymerase (Fermentas) in the PCR mix provided by the manufacturer, under the following cycling conditions: one cycle of 120 s at 94 °C; 40 cycles of 45 s at 94 °C, 60 s at 47–55 °C (see Table S1, Supporting information for specific annealing temperatures) and 90 s at 72 °C, followed by a final elongation cycle of 120 s at 72 °C. Prior to sequencing, unincorporated dNTPs and primers were removed using NucleoFast Membranes (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. PCR products were sequenced on both strands using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems), and the same primers used for PCR amplification (except for the Wg fragments of *I. typographus*, for which we used WgR3, 5′-CGTGCTGGATGAAACTATC-3′, as reverse primer). Electrophoresis of sequencing products was conducted on a 3730 Applied Biosystems capillary sequencer. All electropherograms were checked manually in CodonCode Aligner to assess quality and detect the possible presence of double peaks.

We first attempted to analyse heterozygote PCR products with PHASE v2.1 (Stephens *et al.* 2001; Stephens & Donnelly 2003), after formatting the aligned sequences with the online version of SEQPHASE (Flot 2010). In PHASE, each analysis consisted of a burn-in of 1000, followed by a run of 1000 steps. The thinning interval was equal to one, and the analysis was repeated three times with a different seed number to ensure convergence of results. Haplotypes determined with a probability inferior to 90% were rejected. Those heterozygote PCR products for which the phase could not be inferred with sufficient reliability were cloned. They were purified

and ligated into a pGEM-3Z vector (Promega), then transferred to *Escherichia coli* JM109 competent cells. Between 10 and 15 clones per PCR product were sequenced, and these sequences were compared to the one initially obtained by direct sequencing.

Sequences were aligned with the Muscle algorithm (Edgar 2004) implemented in ALIGNER v3.5.7. (CodonCode Corporation).

Genetic diversity

To compare genetic diversity among populations and between species, standard diversity indices were calculated for each locus using ARLEQUIN 3.5 (Excoffier & Lischer 2010): the number of polymorphic sites (S), the number of haplotypes (h), haplotype diversity (HD) and nucleotide diversity (π) according to Nei (1987). Allelic richness (r) was computed for both species using the rarefaction method proposed by Petit *et al.* (1998) with Contrib (https://www6.bordeaux-aquitaine.inra.fr/biogeco/Production-scientifique/Logiciels/Contrib-Permut/Contrib). For each markers and geographic region, Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1997) summary statistics were calculated using ARLEQUIN. In the absence of direct selection, negative values of these statistics reflect an excessive number of alleles compared to expectations, which may indicate population size expansion, if we are willing to assume sequences were sampled from a panmictic (nonstructured) population.

Population structure

To compare population structure between the two species, genetic differentiation among geographic regions (as defined in the previous paragraph) was quantified in Arlequin by conducting an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992), using the Kimura-2-parameter pairwise differences among haplotypes (statistical test based on 10 000 permutations). Regions were defined by pooling sampling locations based on the presence of natural geographic barriers and the fragmented distribution of the host plant (18 geographic regions, each identified by a different colour in Fig. 2a).

We further investigated genetic structure of the European populations of *I. typographus* by applying the Bayesian procedure implemented in STRUCTURE v2.3.3 (Pritchard *et al.* 2000) after combining data from the five loci into a single input file. For each locus, we coded haplotypes as unique alleles by assigning them a single integer value. For the mitochondrial locus, the second allele of each individual was coded as missing data. We performed 20 independent runs with a burn-in of 10 000, a run length of 100 000 and predefined values of *K* varying from 1 to 10. We assumed a model of population admixture and used sampling location as prior information to improve the detection of structure. The optimal value for *K* was assessed by plotting delta *K* versus *K* (Evanno *et al.* 2005) as implemented in STRUCTURE HARVESTER (Dent & vonHoldt 2012). The *K*-value satisfying the three following criteria was considered optimal: a high delta *K*, an absence of group with a single individual and a stable assignment of individuals over replications. Replicate runs with the optimal delta *K* were aligned with CLUMPP 1.1.2b and the *FullSearch* algorithm (Jakobsson and Rosenberg 2007), and graphs were displayed in DISTRUCT 1.1 (Rosenberg 2004). We also calculated the mean probability of assignment over the 20 runs for each individual and plotted results on a map of Europe.

We conducted an AMOVA using the population structure suggested by STRUCTURE outputs to define subgroups. A similar analysis was not possible for *D. micans* due to the absence of sequence polymorphism in all nuclear genes. However, we performed locus-by-locus structure analyses with the spatial analysis of molecular variance (SAMOVA; Dupanloup *et al.* 2002) and the discriminant analysis of principal component (DAPC; Jombart 2008) on both species. Both SAMOVA and DAPC define groups of populations by maximizing the proportion of the total genetic variance due to differences between groups of populations (F_{ct}), but differ in their search algorithm and also in the geographic scale of analysis; SAMOVA investigates the data at the locality level, while DAPC works at the individual level, giving both a complementary view of intrapopulation variation. For SAMOVA, we used the multicluster version of this algorithm available in SPADS 1.0 (Dellicour & Mardulyn submitted, http://ebe.ulb.ac.be/ebe/SPADS_1.0.html) and let the number of *K* vary from 2 to 10. For each locus, the retained *K*-value maximized the F_{ct} statistic and did not include any single population group. The groups used for DAPC were determined a priori with the *K*-mean clustering algorithm. The analyses were run through the *ape* (Paradis *et al*. 2004) and *adegenet* (Jombart 2008) packages implemented in the R software (v 2.14., R Development Core Team 2011) on a standardized allele frequency table, obtained by scaleGen, with the binomial method. The optimal number of groups was selected with criteria based on the minimum Bayesian information criterion (BIC) and on a configuration without group of only one individual. Variations of F_{ct} and F_{sc} statistics with K (number of groups) were compared for both species (Fig. S3, Supporting information).

Networks and phylogeographic structure

Median-joining haplotype networks were inferred with Network version 4.6.1.1 (Bandelt *et al.* 1999) (Figs. 2b and 3). Gaps were considered as missing data and

recoded at the end of the sequence alignment as binary characters, irrespective of their length (Simmons & Ochoterena 2000).

To check whether strong migration within each species could have erased the historical signal in the DNA sequence data, we verified the presence of a significant phylogeographic structure within the European populations through the comparison of two statistics measuring population structure, N_{ST} and G_{ST}. While G_{ST} is only calculated from haplotype frequency data, N_{ST} also takes genetic distance between haplotypes into account. A significantly higher N_{ST} value is indicative of a correlation between genetic lineage and geographic distribution, thus of the presence of phylogeographic structure (Pons & Petit 1996). Computation of the indices and their statistical significance was performed with 1000 permutations in the program PERMUTCPSSR 2.0 (http://www.pierroton.inra.fr/genetics/labo/Software/Permut/).

Divergence time

In both species, we used coalescent-based approaches to estimate the time of divergence between East Asia and other populations, which would indicate a minimum time of presence of these species on the European continent (as they are thought to have colonized Eurasia from North America), assuming the observed differentiation has started to occur in Eurasia. For both species, we assessed divergence times by calibrating the COI molecular clock rate with the widely used substitution rate in insect of 0.0115 substitutions/site/my/lineage (Brower 1994) because of its proximity with the arithmetic mean retrieved from the literature on Coleoptera (0.0114) (0.0075, Farrell 2001; 0.008, Sota & Hayashi 2007; 0.0125, Caccone & Sbordoni 2001 and 0.0177, Papadopoulou *et al.* 2010). Divergence time estimates were obtained with BEAST for COI data in both species. A multilocus analysis was conducted with the *BEAST model (Heled & Drummond 2010) for *I. typographus*, assigning individual sequences to one of the three subspecies suggested by our network analyses (Eurasia, Russian Far-Eastern and Japanese subspecies) based on their geographic origin. The five genes were considered as different partitions, and the COI specified as mitochondrial to account for the assumed fourfold difference in effective population size. We fixed the rate of the COI marker at 0.0115 substitutions/year/lineage and let the algorithm estimate the rates for the four nuclear markers (lognormal tree prior). A relaxed lognormal molecular clock model was selected. Models of sequence substitution were initially selected with JMODELTEST v.0.1.1 (Posada 2008) under the BIC criterion. Results from two independent runs were pooled together after removing a 10% burn-in using LogCombiner and summarized using Tracer. The mean and the standard deviation priors of the nucleotide substitution rate (*ucld.mean* and *ucld.stdev*) for COI were set to 0.0115 and 0.33, respectively (which correspond to a 95% confidence interval of the lognormal distribution rates of 0.0075-0.0155 substitutions/site/my). The time of divergence estimated with BEAST was compared to an estimate obtained under the isolation-with-migration model (IMa2 v8.27.12) (Hey 2010, http://genfaculty.rutgers.edu/hey/software). Unlike BEAST, the Bayesian approach implemented in IMa2 implements a more realistic model because it considers the possibility of gene flow between sister species after they split from an ancestral species (Hey & Nielsen 2004). We also performed an IMa2 analysis at European scale for *I. typographus* between two population sets defined by STRUCTURE analysis. As IMa2 needs a rooted phylogenetic tree when more than two populations are defined, we used the trees generated by *BEAST. IMa2 analyses of *D. micans* were conducted on the mtDNA sequences alone (for lack of sufficient variation in the other loci) and considered only two populations (EurA and RuFE). IMa2 analyses of *I. typographus* were conducted with all loci combined and defining three groups; EurA, Jap and RuFE. For each locus, we opted for the HKY substitution model and ran the program three times (to check convergence) with different seed numbers under the Metropolis-Hastings Markov chain Monte Carlo algorithm (MCMC). After running exploratory analyses, we set the upper prior bounds to 0.5 for the migration rate (m), 50 for the population size (q) and 100 for the divergence time (t). For each run, we used a geometric heating model with 80 chains (*ha* = 0.975, *hb* = 0.75) of two million steps following a burn-in of 100 000 steps. After each run, we inspected parameter trend line plots for the absence of discernible pattern and verified that the effective sample sizes (ESS) values were superior to 50.

Demographic changes

We estimated changes in effective size over time separately for the northern and southern portions of the range of *I. typographus* (portions within which no significant population structure was detected), using a multilocus extended Bayesian skyline plot (EBSP; Heled & Drummond 2008) analysis in *BEAST (Heled & Drummond 2010; BEAST 1.7.4). MCMC chains were run for 50 million steps and sampled every 5000 steps. Ten per cent of the sampled trees were discarded as burn-in prior to inferring the estimates. We used an EBSP linear model, allowing population size to change continuously over time. We used a strict clock model, a HKY model

of nucleotide substitution for all loci, and we specified a substitution rate range of 0.0115–0.04 substitutions/site/my/lineage (flat prior) for the mitochondrial COI, to get a calibrated timescale for the skyline plots. For the nuclear genes, we defined large priors ranging from 0 to 1. A 'mitochondrial' ploidy type was assigned to the COI locus and an 'autosomal nuclear' ploidy type to all nuclear loci. A similar analysis could not be conducted for the other species, *D. micans*, as strong population structure was highlighted across its range, contradicting the EBSP assumption of a panmictic population.

Colonization of D. micans in regions where P. abies was recently introduced

We classified regions into native or non-native areas based on the published natural range of *P. abies* (Schmidt-Vogt 1977; Tollefsrud *et al.* 2008) and historical records of the invasion process (e.g. documented recent introduction in an area by human-mediated assistance) (Figs 2b and 3). The native range of the Norway spruce was thus considered to include the mountain ranges of the Alps, the Apennines, the Vosges, the Bohemian Massif, the Carpathians and the Bulgarian Mountains in the south and to run from north-eastern Poland to the Ural range in the North. We reasoned that because non-native regions were colonized in recent times only (during the 19th century at the latest), mutations did not have the time to differentiate introduced populations from their associated source populations. We therefore assumed that a haplotype found both in the native and non-native portions of the range was indicative of a recent colonization event and that the source population of this colonization was located in the portion of the native range in which it is found today. Only *D. micans* displayed sufficient population differentiation (COI locus) to allow such a comparison.

Results

The alignments of the nuclear sequences generated in this study exhibited, after the introduction of gaps and removing ambiguous sequence ends, fragment lengths of 985, 591, 712, 735 and 417 base pairs for *I. typographus* and 937, 556, 742, 505 and 702 bp for *D. micans* for the COI, AK, EF-1α, G-6-PD and Wg loci, respectively.

For *I. typographus*, the alignment of 265 COI sequences included 69 polymorphic sites, while those of the nuclear markers displayed 11 segregating sites for AK, 15 segregating sites for EF-1α, 28 segregating sites and 2 gaps for G-6-PD, 13 segregating sites and 1 gap for Wg. For *D. micans*, the alignments displayed 35 segregating sites for COI, only 1 segregating site for AK and E-F-1α and a complete absence of polymorphism for G-6-PD and Wg. All identified haplotypes were deposited in GenBank (accession references KF846083–KF846301).

Genetic diversity

Global haplotype and nucleotide diversity indices estimated per locus and per region are available as supplementary information (Tables S4–S9, Supporting information). While the total number of COI alleles found for *I. typographus* is more than twice as large as the number found for *D. micans*, estimated indices of genetic diversity are similar for the two species or even higher for *D. micans* (case of nucleotide diversity). The same indices calculated per region do not provide any obvious and systematic difference between the two species either. For the nuclear loci, on the other hand, genetic diversity was obviously much higher for *I. typographus*, as they were monomorphic (or only slightly polymorphic) for *D. micans*. These indices were therefore not calculated for this last species.

Although Tajima's D or Fu's F_S calculated per region was occasionaly significantly different from 0, we could not find any particular locus showing the same pattern in most regions (which would have suggested this locus to be under selection) or a region where most or all loci were affected (which would have suggested a past demographic change in the region).

Population structure

The STRUCTURE analyses on European populations of *I. typographus* returned the highest value of delta *K* for $K = 2$, and superior values of *K* lead only to additional groups that included a single individual. When plotted on a map, the geographic distribution of the two clusters suggested a geographic separation between the north and the south of the species range, delimited by the western Baltic and mid-Poland Plain (Fig. 1) The groups detected in the COI SAMOVA and DAPC are also consistent with a north–south subdivision (Figs S4–S5, Supporting information), but the northern group was less extensively distributed than in the STRUCTURE analyses with all markers combined. In the southern range, DAPC analyses identified an additional shallow level of substructure with the mitochondrial and nuclear Wg loci. The additional groups identified were difficult to correlate with the geographic distribution; however, cluster 3 and cluster 4 in Wg DAPC analysis (Fig. S6, Supporting information) seem restricted to central and south-west Europe, respectively, a pattern similar to that observed for haplogroups 1 and 6 in the COI

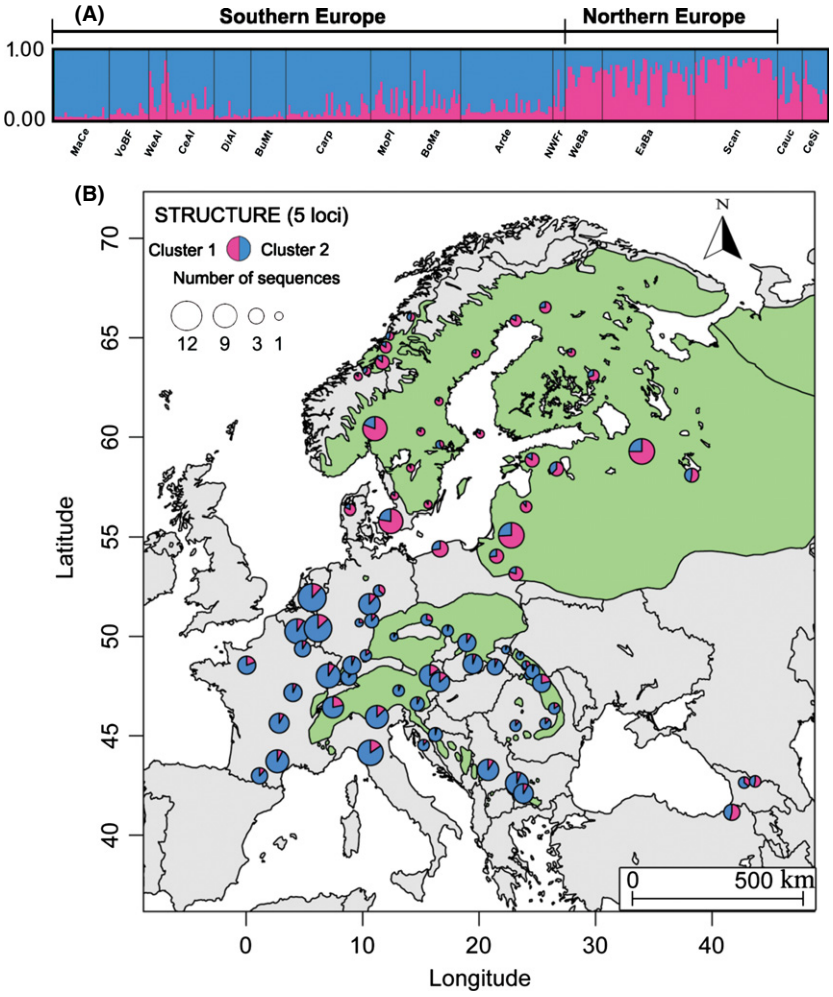

Fig. 1 Result from STRUCTURE analysis on all sequence data available for *Ips typographus* (a) Bar plot of individual clustering corresponding to the most probable subdivision of K = 2, each individual is represented by a vertical bar and populations correspond to the geographic groups of Ardennes (Arde), Bohemian Massif (BoMa), Bulgarian Mountains (BuMt), Carpathians (Carp), Caucasus (Cauc), Central Alps and Apennines (CeAl), Central Siberia (CeSi), Dinaric Alps (DiAl), eastern Baltic (EaBa), Massif Central (MaCe), Moldanubian Plain (MoPl), north-western France (NwFr), Scandinavia (Scan), Vosges & Black Forest (VoBF), Western Alps (WeAl), West Baltic (WeBa). (b) Geographic location of the two clusters; size of circles is proportional to the number of individuals sequenced. Cluster proportion in pie charts represents the overall assignment probability of individuals present at corresponding locations, averaged over 20 independent runs. The green area on the map delimits the native distribution of the host tree *Picea abies* after Schmidt-Vogt (1977) (downloaded from EUFORGEN 2009, www.euforgen.org). The map was generated with the mapplots package in R 2.15.1.

haplotype network (Figs 2 and 5). The AMOVA shows that the largest fraction of the variation is distributed within populations (from 73 to 87%, depending on the marker; Tables 2 and 3). When northern and southern populations were considered belonging to two distinct groups, the variation among groups was higher, with a significant value for COI (Φ_{CT}=0.20; $P < 0.001$) and Wg (Φ_{CT}=0.05; $P < 0.001$).

For *D. micans*, the continuous increase in F_{CT} values of the SAMOVA (Fig. S3, Supporting information) and the slight decrease in DAPC BIC criterion only for values above K = 15 (Fig. S 2, Supporting information) suggest that the analyses failed to detect any obvious geographic subdivision of the species range. In the AMOVA, the largest part of the variation was observed among populations within regions (Φsc= 0.67 $P < 0.001$), followed by the variation among regions (Φct= 0.29; $P < 0.001$; Table 2).

Demographic changes

Because the STRUCTURE analyses suggested that the range of *I. typographus* was mainly subdivided in a north and south region, with little structure within them, we estimated past demographic changes using ESBP, separately for these northern and southern regions. The generated plots are shown in Figs S7 and S8 (Supporting information). They suggest a relatively stable population size over time in both cases, possibly except for a very recent increase (not more than 100 years old) that could reflect a recent expansion following the human-mediated extension of the range of *Picea abies* in central and southern Europe.

Phylogeographic structure

Haplotype networks are shown in Figures 2–5. For *I. typographus*, N_{ST} was significantly higher than G_{ST} for COI (G_{ST} = 0.21, N_{ST} = 0.31; $P < 0.01$) and Wg markers (G_{ST} = 0.009, N_{ST} = 0.079; $P < 0.05$), revealing some level of phylogeographic structure for two markers of five. For *D. micans*, a strong population structure was detected by G_{ST} (0.46), but the level of structure did not significantly increase when including genetic distances among haplotypes (N_{ST} = 0.38).

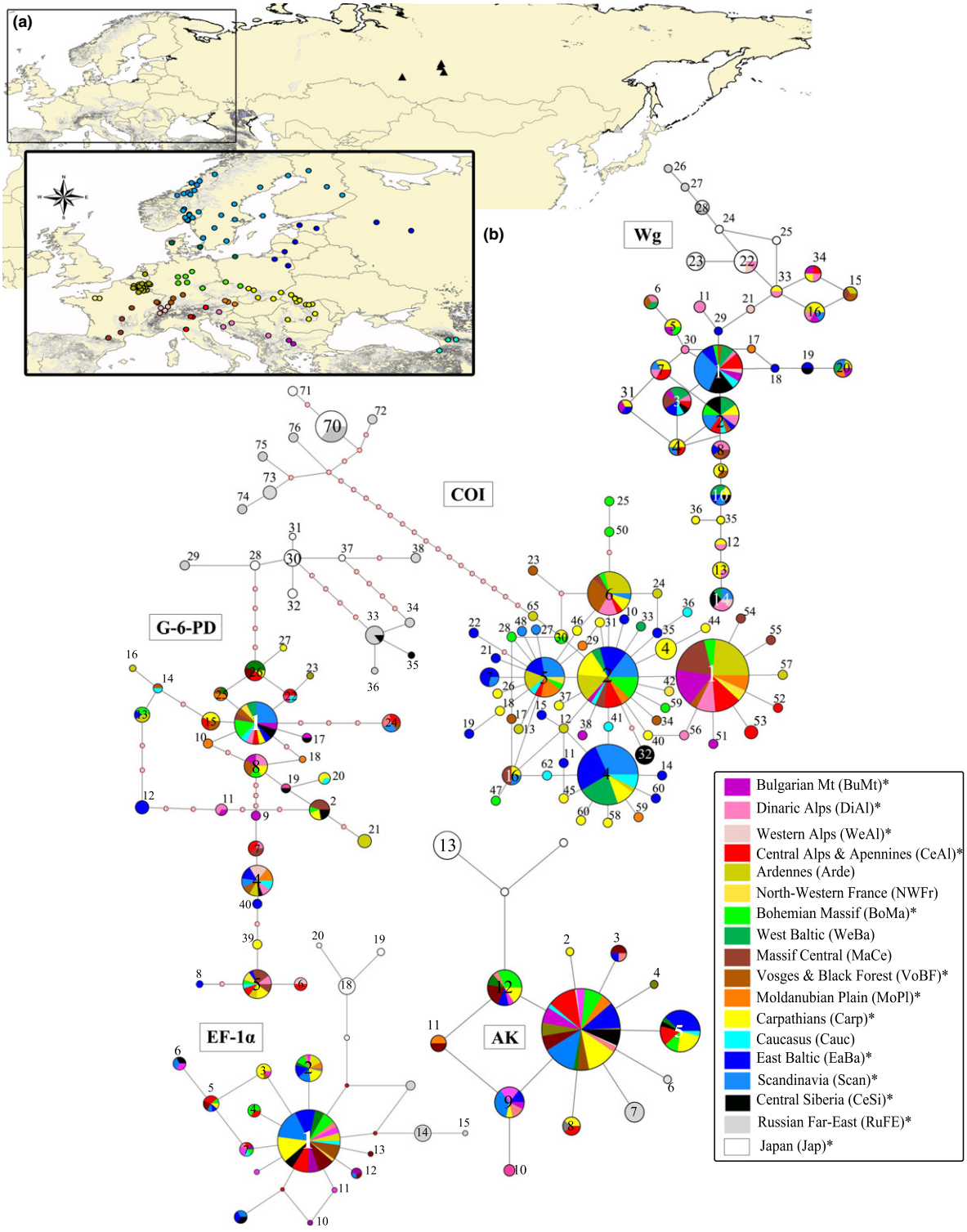

Fig. 2 Median-joining networks for the five molecular markers (Cytochrome oxydase I, COI; Arginine kinase, AK; Elongation factor-1 α, EF-1α; Glucose-6-phosphate dehydrogenase, G-6-PD; Wingless, Wg) sequenced for *Ips typographus*. Each haplotype is represented by a pie chart whose size is proportional to its frequency, and is identified by a unique number. Each line in the network represents a single mutational step. Small red circles indicate intermediate haplotypes that are not present in the sample. Sampling sites are shown on a map of Eurasia (a) and are merged into geographic entities corresponding to isolated regions, such as mountain ranges, identified by a specific colour. These colours are used in the haplotype networks to display the geographic distribution of haplotypes among regions. Asterisks indicate native areas.

Table 2 Analysis of molecular variance (AMOVA) among populations of *I. typographus* (a) and *D. micans* (b) on the mitochondrial locus (COI): d.f., degree of freedom; % var, percentage of total variance explained by each source of variation; *Φ*-stat, fixation indices; ***significant at probability $P < 0.001$; *significant at probability $P < 0.05$

		COI					
		(a) *I. typographus*			(b) *D. micans*		
Groups defined	Source of variation	d.f.	% var	*Φ*-stat	d.f.	% var	*Φ*-stat
(1) Grouping by geographic regions	Among groups (Φct)	12	19.52	0.20***	10	29.2	0.29***
	Among populations within groups (Φsc)	94	−0.35	0	88	47.46	0.67***
	Within populations (Φst)	128	80.84	0.19***	97	23.33	0.77***
(2) Two groups (northern[a]/southern[b] regions)	Among groups (Φct)	1	23.08	0.23***			
	Among populations within groups (Φsc)	105	5.24	0.07*			
	Within populations (Φst)	128	71.66	0.28***			

[a]Northern regions include eastern Baltic (EaBa), western Baltic (WeBa) and Scandinavia (Scan). [b]Southern regions include Vosges and Black Forest (VoBF), Central Alps (CeAl), Dinaric Alps (DiAl), Bulgarian Mountains (BuMt), Carpathians (Carp), Moldanubian Plain (MoPl), Bohemian Massif (BoMa), Ardennes (Arde) and north-west France (NWFr).

Table 3 Analysis of molecular variance (AMOVA) among populations of *I. typographus* on the nuclear loci: d.f., degree of freedom; % var, percentage of total variance explained by each source of variation; *Φ*-stat, fixation indices; ***significant at probability $P < 0.001$

		AK			EF-1α			G-6-PD			Wg		
Groups defined	Source of variation	d.f.	% var	*Φ*-stat	d.f.	% var	*Φ*-stat	d.f.	% var	*Φ*-stat	d.f.	% var	*Φ*-stat
(1) Grouping by geographic regions	Among groups (Φct)	13	4.48	0.04	13	−2.68	−0.03	13	−2.45	−0.03	12	2.62	0.03
	Among populations within groups (Φsc)	93	13.18	0.14***	56	29.42	0.29***	39	29.47	0.29***	43	10.83	0.11***
	Within populations (Φst)	121	82.34	0.18***	130	73.26	0.27***	111	72.98	0.27***	77	86.55	0.13***
(2) Two groups (northern[a]/southern[b] regions)	Among groups (Φct)	1	0.02	0.00	1	−0.87	0.00	1	0.33	0.00	1	4.66	0.05***
	Among populations within groups (Φsc)	105	17.38	0.17***	68	29.84	0.27***	51	26.94	0.27***	54	10.99	0.12***
	Within populations (Φst)	121	82.6	0.17***	130	71.02	0.27***	111	72.73	0.27***	77	84.35	0.16***

[a]Northern regions include eastern Baltic (EaBa), western Baltic (WeBa) and Scandinavia (Scan). [b]Southern regions include Vosges and Black Forest (VoBF), Western Alps (WeAl), Central Alps (CeAl),Dinaric Alps (DiAl), Bulgarian Mountains (BuMt), Carpathians (Carp), Moldanubian Plain (MoPl), Bohemian Massif (BoMa), Ardennes (Arde) and north-west France NWFr (excluded from Wg).

Divergence time

Overall, BEAST and *BEAST analyses of *I. typographus* produced convergent results with a mean estimate of divergence between Asian and European populations occurring around 200 000 years before present (BP). The IMa2 analysis approximately concurs, with a mean divergence time estimated at 167 729 years BP (Table 3).

D. micans estimations from the two programs were less congruent (based on a single locus), but agree on a more recent divergence between Asia and Europe. The mean divergence time was estimated at 17 000 years BP using IMa2 (highest posterior density confidence interval of 0–187 926 years) but at 86 300 years BP with BEAST (with a 95% confidence interval of 39 260–141 000 years) (Table 3).

The estimation of divergence time for the north–south separation of *I. typographus* within Europe (Fig. 1) provided a time range of 0–57 530 years.

Colonization history of D. micans

Figure 4 displays the geographic distribution of *D. micans* COI haplotypes, while highlighting the native and non-native range of the species. Identifying the haplotypes observed in a specific newly colonized (non-native) region allows highlighting native portions of the range from which these haplotypes may have originated. Although other unsampled areas of the native range may have actually served as source of the colonization (our sampling did obviously not cover the entire

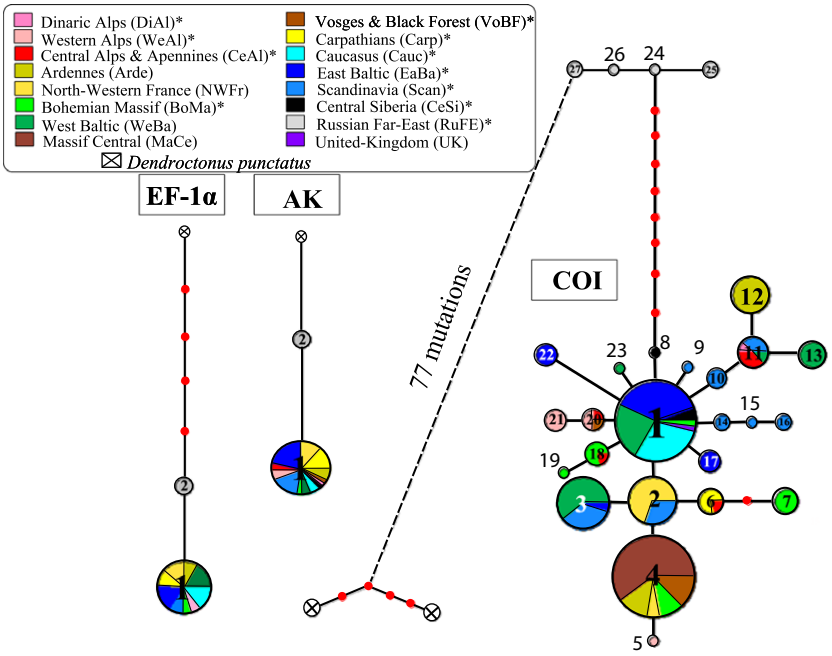

Fig. 3 Median-joining networks for the three polymorphic molecular markers (Cytochrome oxydase I, COI; Arginine kinase, AK; Elongation factor-1 α, EF-1α) sequenced for *Dendroctonus micans*. Each sequenced haplotype is represented by a pie chart whose size is proportional to its frequency, and is identified by a unique number. Each line in the network represents a single mutational step. Small red circles indicate intermediate haplotypes that are not present in the sample. Asterisks indicate native areas.

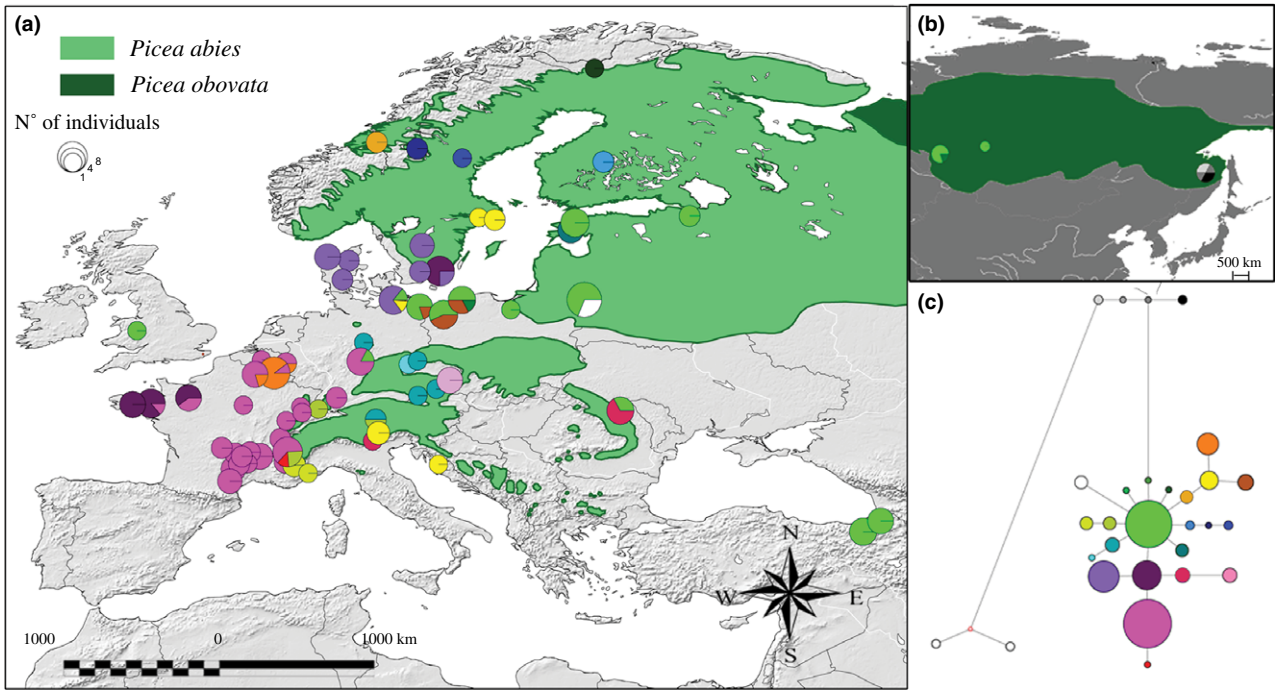

Fig. 4 Geographic distribution of 27 COI haplotypes of *Dendroctonus micans* across 110 collection sites in Eurasia. Each colour identifies a different haplotype in the network (panel c). The total surface of each circle is proportional to sample size, and colour proportions in each pie chart (panels a and b) represent haplotype frequencies. The light and dark shaded green areas indicate the native range of dominant host tree species: *Picea abies* and *P. obovata* after Schmidt-Vogt (1977), respectively (downloaded from EUFORGEN 2009, www.euforgen.org). The map was generated using the ARCGIS software. Panel a): European range; panel b): East Asian portion of the range.

native range), we can at least identify sampled native regions that could not have served as source (because the haplotypes from the source and sink populations are different).

Discussion

Overall, the observed results over population structure and genetic diversity largely favour our hypothesis that

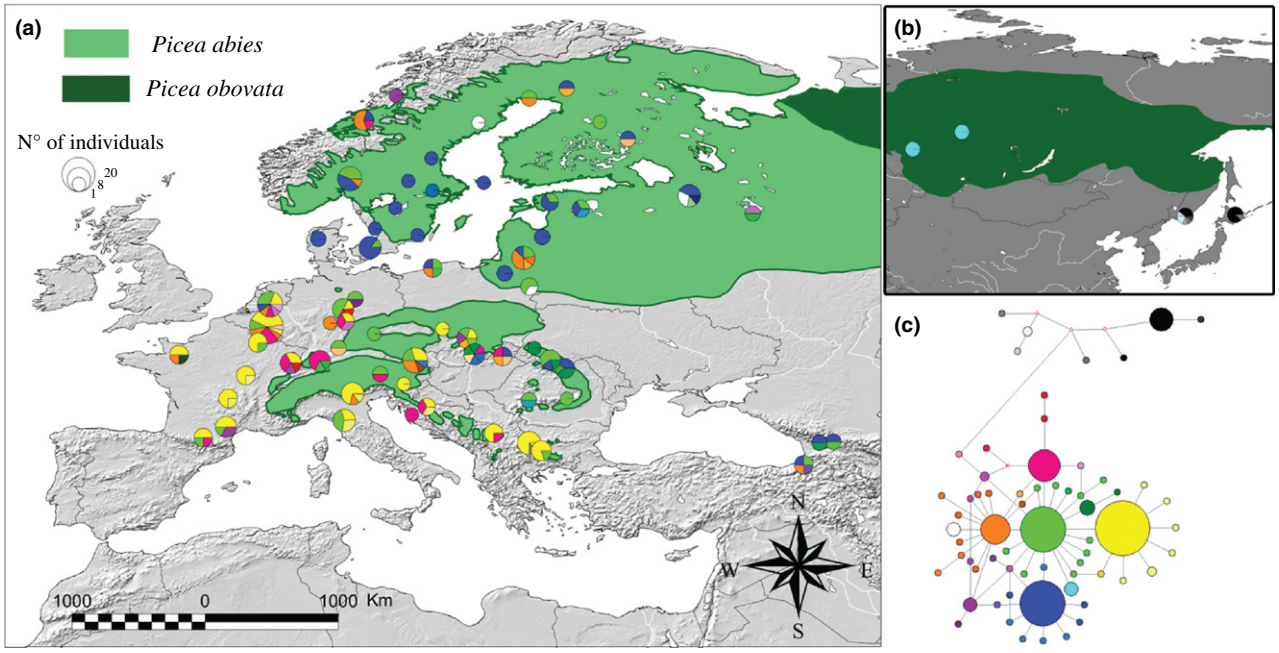

Fig. 5 Geographic distribution of 65 COI haplotypes of *Ips typographus* across 108 collection sites in Eurasia. Each colour identifies a different group of related haplotypes in the network (panel c). The total surface of each circle is proportional to sample size, and colour proportions in each pie chart (panels a and b) represent haplotype frequencies. The light and dark shaded green areas indicate the native range of dominant host tree species: *Picea abies* and *P. obovata* after Schmidt-Vogt (1977), respectively (downloaded from EUFORGEN 2009, www.euforgen.org). The map was generated using the ARCGIS software. Panel a): European range; panel b): East Asian portion of the range.

current patterns of genetic variation are strongly influenced by life history traits of the organism, rather than solely by the environmental disturbances and characteristics of the geographic areas that their distributions span. While the main body of the phylogeographic literature focuses on explaining phylogeographic patterns by past climatic events and geographic barriers, it is important to take other factors into account as well, that is ecological features or life history traits of the organisms. Indeed, although comparisons of phylogeographic patterns among various European species have suggested the existence of common glacial refuges for many temperate species (Hewitt 2004), other reviews have emphasized the variety of patterns highlighted for different codistributed organisms (Taberlet *et al.* 1998). The current phylogeographic pattern of an organism may therefore be better interpreted as generated by a combination of past environmental disturbances and barriers characterizing the studied region (assuming the species has not colonized this region too recently) and species-specific features. In fact, a species characterized by strong dispersal, such as *I. typographus*, may be less useful to study the impact of historical climate changes on its geographic distribution, because the phylogeographic signal contained within DNA sequences may be overridden by strong migration.

The patterns of genetic variation for the two species of bark beetles harbour major differences. First, we observed a considerably lower genetic diversity in *D. micans* than in *I. typographus*, both at the local scale (i.e. within sampling locations) and at the regional scale (as defined above), as well as at the level of the entire species range. This pattern is notably extreme for the four nuclear markers, with two loci displaying a complete lack of genetic diversity (single allele) across the entire range of *D. micans* and two others exhibiting just one additional allele for the Russian Far East samples. At the same time, we found much stronger population structure within *D. micans* than within *I. typographus* when focusing on COI (the only polymorphic locus available to study population structure in *D.* micans; see Φ_{ST}, Φ_{SC} and Φ_{CT} in Table 2). This combined pattern of stronger structure and lower diversity within sampling locations for *D. micans* relative to *I. typographus* is easily visualized in Figures 4 and 5 where sampling sites often display a single allele in *D. micans*, and more than one allele in *I. typographus*, and where the geographic spread of alleles is generally higher for *I. typographus*. It is further confirmed by the AMOVA (Table 2), where 81% of the source of variation in *I. typographus* was found within and 0% among populations, while 29% was assigned to variation

within populations and as much as 47% among populations for *D. micans*.

Influence of species-specific features

We suggest, as hypothesized, that these differences are related to the contrasting dispersal strategies and modes of reproduction in the two species. Indeed, each *D. micans* population gathers small colonies of highly related individuals, and sib mating seems to be the rule (Vouland *et al.* 1984; Grégoire 1988). The negative influence of inbreeding on genetic diversity was already reported for a few invertebrates, including insects (e.g. *Hypothenemus hampei*; Andreev *et al.* 1998), spiders (*Anelosimus eximi*; Agnarsson *et al.* 2010) and nematodes (*Caenorhabditis elegans*; Graustein *et al.* 2002). In addition, the slow dispersal of this species is achieved by a few related individuals moving over short distances. This probably results in successive founder events that reduce the effective size of populations and, as a consequence, their level of genetic variation (Graustein *et al.* 2002). A low effective recombination rate, which is expected in highly inbred species, can also reinforce this pattern (Nordborg 2000). If a selective sweep occurs at any locus of the genome, all other loci will be affected simultaneously as they are tightly linked to each other (Charlesworth 2003).

The higher population structure highlighted in *D. micans* for the mitochondrial DNA fragment is also probably related to the difference in dispersal distances between the females of the two species (Botterweg 1982; Nilssen 1984; Gilbert & Grégoire 2003). *D. micans* COI alleles are characterized by different geographic distributions, and copies of each allele tend to aggregate within a restricted area (see Fig. 4), which point towards low dispersal.

Influence of past environmental disturbances and geographic barriers. Although the population structure across the species entire range was much weaker for *I. typographus* than for *D. micans*, it was still possible to identify an interesting structure across its range, which divides it into a northern and southern (spanning central and south Europe) portion (Figs 1 and 5). This geographic subdivision was not detected in former studies that investigated genetic variation in this species at a large geographic scale (Stauffer *et al.* 1999; Sallé *et al.* 2007; Bertheau *et al.* 2013) and was highlighted here thanks to a more thorough sampling in northern Europe and/or a larger number of molecular markers (Fig. 1). This pattern can be interpreted as evidence of a past allopatric differentiation. This is further supported by the similar population structure observed in its host plant *P. abies* that also defines two main genetic groups located on either side of the mid-Polish lowlands (see green area on Fig. 1; Lagercrantz & Ryman 1990; Sperisen *et al.*1998; Vendramin *et al.* 2000; Heuertz *et al.* 2006; Tollefsrud *et al.* 2008). It can also be related to the quantitative assessment of a phylogeographic structure provided by the G_{ST}/N_{ST} comparison that resulted in a significant phylogeographic signal only for *I. typographus*. The absence of a phylogeographic structure in *D. micans* suggests that population structure is independent from genealogical relationships in this species: including the information on genetic distances among alleles does not significantly increase the level of population structure within that species (in other words, genetically related alleles are not, on average, geographically closer than less related alleles). It seems further confirmed by the distribution of COI alleles in both species: while these are present in a restricted portion of the range of *D. micans*, they are sometimes present in distant locations (revealing long-distance migration possibly mediated by human activities), like the yellow or blue alleles (Fig. 4) that are both present on the northern and southern part of their range. By contrast, *I. typographus* COI alleles are generally more widespread, but often restricted either to the north or to the south of the species range (Fig. 5). The stronger phylogeographic structure in the species associated with higher dispersal capacity is actually in contradiction with our initial expectation. We suggest that this may be explained by different colonization histories leading to the current range displayed by both species: from two separate refuges (possibly, but not necessarily, located somewhere in the north and south portions of their current distribution) for *I. typographus*, but from a single refuge for *D. micans*. In that case, the ancient geographic separation could still be reflected in the phylogeographic pattern, despite strong dispersion. Because we have estimated the divergence time between the north and the south regions to be in the range of 0–58 000 years ago (Table 4), this allopatric differentiation is then likely to have originated around the last glaciation or later.

The strong population structure found across the entire range of *D. micans* is a testimony to its long-time presence in Eurasia and contradicts a previously suggested hypothesis that it colonized Europe from eastern Siberia very recently, in the mid-19th century (Grégoire 1988). At the same time, the deep genealogic divergence between Eurasian and Far East Asian populations, found in both *D. micans* and *I. typographus*, reveals a long history of separation between the two regions. As the mean divergence time estimate separating the populations located in these two regions for *D. micans* is at least half the one estimated for I. *typographus*, we cannot fully exclude the possibility that two different evolutionary histories have shaped the observed deep

Table 4 Divergence time (T) A. between Far East and Eurasian populations estimated for both species and B. between northern and southern populations of Europe for *I. typographus*: (a) BEAST and *BEAST mean estimates and corresponding 95% confidence interval (CI) and (b) IMa2 mean estimates and corresponding 95% highest posterior density (HPD)

		a) BEAST (mit) and *BEAST (mit+nucl)			b) IMa2	
Species	Marker	Clock model	Mean T (year ago)	95% CI	Mean T (year ago)	95% HPD
A. Asia–Europe						
D. micans	mit	Relaxed	85 880	39 500–141 500	17 261	0–187 926
I. typographus	mit	Relaxed	216 000	106 000–345 900		
	mit+nucl	Fixed	220 000	192 700–318 900	167 792	0–295 740
B. south–north Europe	mit+nucl				3528	0–57 530

divergences in the two species. However, the discrepancy in the estimation between the two species could also be attributed to the fact that a single locus was available for *D. micans*, while five loci were used for *I. typographus*.

In any case, the time estimates in both species place the divergence at least during the last glacial maximum and probably well before that period at least for *I. typographus*. It indicates that both species have been present in Europe for a longer period of time than previously thought (Grégoire 1988; Bertheau *et al.* 2013), which could be established thanks to the inclusion of Far East samples that were not available in previous phylogeographic studies of these species.

Comparison with other European beetles associated with spruce

For the purpose of testing our hypothesis that current patterns of genetic variation are strongly influenced by life history traits, another useful comparison can be performed between the two studied species and another bark beetle with a similar distribution, *Pityogenes chalcographus* (Table 1). A comparative phylogeographic study between this species and *I. typographus* has indeed been previously conducted by Bertheau *et al.* (2013). *P. chalcographus* has a mating system and a migration behaviour much more similar to those of *I. typographus* (Bertheau *et al.* 2012), and it feeds not only on *P. abies* but on other Pinaceae species as well (Bertheau *et al.* 2009). This larger diet breadth probably translates into a globally less fragmented geographic distribution (Bertheau *et al.* 2012, 2013). Although the comparison between the two studies is not straightforward, because Bertheau *et al.* (2013) used different markers to compare *P. chalographus* to *I. typographus* and focused on a smaller portion of their range, an interesting comparison can be performed with COI sequences. Even though Bertheau *et al.* (2013) sequenced a smaller fragment, only partially overlapping with ours, all genetic diversity indices calculated across the entire species range highlighted a much stronger COI diversity for *P. chalographus* than for *I. typographus* and the overall population structure (as measured by G_{ST}) was much higher for *I. typographus*. Thus, when combining the COI results from both studies, it suggests a gradient of increasing genetic diversity and decreasing population structure across the three species, from *D. micans* to *P. chalographus*. While the latter species appears to have dispersal capabilities similar to those of *I. typographus*, a less fragmented range caused by a larger diet breadth could explain the lowest level of population structure and highest genetic diversity. Data from Bertheau *et al.* (2013) therefore seem compatible with, and even seem to reinforce, our initial hypothesis. Another phylogeographic study of a spruce beetle that seems compatible with our hypothesis is that of the large pine weevil, *Hylobius abieti* (Coleoptera: Curculionidae), which colonizes Scots pine (*Pinus sylvestris L.*) and Norway spruce (*Picea abies*) and exhibits low genetic differentiation among populations that can be related to a high dispersal capacity, similar to that of *I. typographus* (Conord *et al.* 2006).

Recent colonization history of D. micans

Finally, because the range of the host plant *P. abies* has been largely extended by human activity in recent times for economic reasons and because estimates of its native and current ranges are available (Schmidt-Vogt 1977; Köble & Seufert 2001; Tollefsrud *et al.* 2008), the strong association found between geography and genetic lineages in *D. micans* could be used to trace the recent colonization history of the beetle (Fig. 4). For example, Sitka stands of the north-western Baltic and Norway spruce stands of the Massif Central exhibit each a single maternal lineage across their entire range, and both regions are reported to have been colonized after the mid-19th century (Løvendal 1898; Grégoire 1988). This could suggest specific potential donor areas from the

native range of this species, because the geographic distribution of each allele is somewhat restricted in the native range, and the short time since colonization could not allow for mutations to differentiate the source from the recently colonized region. If these assumptions are correct, our data suggest the western Baltic (Denmark and North Germany) has been invaded by south-Scandinavian individuals through the crossing of the Øresund. This is in agreement with the first reports of *D. micans* in this region being in eastern Denmark (Sjaeland) (Løvendal 1898). Further, the Massif Central region (France), the north of France, the Ardennes and Burgundy (Morvan) appear to have been colonized either from Western Alps or from the Vosges and Black forest regions, which has been suggested previously (Chararas 1961; Pauly & Meurisse 2007). Another interesting observation is the presence of the same haplotype in the south-eastern part of Sweden and in the recently colonized region of Brittany (western France). This could be interpreted as an example of the colonization of *D. micans* into new areas through human-mediated transport (Gilbert *et al.* 2003). Although we cannot reject the possibility that it is the result of a parallel/reversal mutation that has occurred independently in the two distant regions, we find this less likely. Similarly, the Caucasian region exhibits a unique haplotype, commonly found in Siberia and eastern Baltic, which appears in agreement with historical data suggesting log trade from the former USSR as the source of invasion (Shavliashvili & Zharkov 1985). Note, however, that these inferences assume that we can estimate the native geographic distribution of the haplotypes from our current sampling (shown in Fig. 4) of the native range of *D. micans*. A more thorough sampling of the range is needed to test these inferences further, as the geographic distribution of a given haplotype may actually be larger than is suggested by our data.

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F.M., J.-C.G. and P.M. designed the project. F.M., F.P., J.-C.G., C.B., N.K., A.L. and B. Ø. collected the samples. F.M. performed most of the laboratory work and computer analyses. F.P. and L.G. contributed some to the laboratory work, and F.P. to data analysis. All authors contributed to data interpretation and to writing the manuscript. This work is part of the PhD thesis of F.M., performed under the supervision of P.M. and J.-C.G.

Data accessibility

Sampling information: Table S2 (Supporting information) for *Ips typographus* and Table S3 (Supporting information) for *Dendroctonus micans*. DNA sequence alignments, haplotype composition of each individual, GenBank Accession no. associated with each haplotype, and structure input file for *Ips typographus*: Dryad entry doi:10.5061/dryad.43cp5. DNA Sequences accessible through GenBank (KF846086–KF846301); haplotype definition (Tables S10–S17, Supporting information), GenBank Accession no. associated with each haplotype (Table S18, Supporting information), and haplotype composition of each individual (Tables S19–S20), available as supporting information.

Supporting information

Additional supporting information may be found in the online version of this article.

Material S1. Additional details on Material and Methods.

Fig. S1. Plot of Δ*K* for each K in STRUCTURE analysis on *I. typographus*.

Fig. S2. Plot of Bayesian information criteria in the discriminant analyses of principal component on COI sequence data sets for both species.

Fig. S3. Plot of the values of the fixation indices obtained from the SAMOVA on the mitochondrial COI locus for both species.

Fig. S4. SAMOVA results for *I. typographus* COI sequences ($K = 2$).

Fig. S5. DAPC results for *I. typographus* COI sequences ($K = 3$).

Fig. S6. DAPC results for *I. typographus* Wg sequences ($K = 4$).

Fig. S7. Extended Bayesian skyline plots showing changes in effective population size in southern population group of *I. typographus* over evolutionary time.

Fig. S8. Extended Bayesian skyline plots showing changes in effective population size in southern population group of *D. micans* over evolutionary time.

Table S1 Primers used for DNA amplification.

Table S2 Sampling sites and distribution of *I. typographus* haplotypes for 5 gene fragments. An asterisk identifies samples collected by pheromone traps.

Table S3 Sampling sites and distribution of *D. micans* haplotypes for 5 gene fragments. An asterisk identifies samples collected by pheromone traps.

Table S4 Intrapopulation variability in *I. typographus* based on COI sequences.

Table S5 Intrapopulation variability in *D. micans* based on COI sequences.

Table S6 Intrapopulation variability in *I. typographus* based on ArgK sequences.

Table S7 Intrapopulation variability in *I. typographus* based on EF-1α sequences.

Table S8 Intrapopulation variability in *I. typographus* based on G6PD sequences.

Table S9 Intrapopulation variability in *I. typographus* based on Wg sequences.

Tables S10–S17 Definition of haplotypes for all loci.

Table S18 GenBank accession for each haplotype.

Table S19 haplotype composition of each *I. typographus* individual.

Table S20 haplotype composition of each *D. micans* individual.