RESEARCH ARTICLE

Successful reintroduction of an endangered veteran tree specialist: conservation and genetics of the Great Capricorn beetle (*Cerambyx cerdo*)

L. Drag · L. Cizek

Abstract Habitat fragmentation is one of the main threats to biodiversity. Reintroductions or translocations may mitigate its effects by allowing species with limited dispersal ability to exploit otherwise inaccessible habitat patches. Despite the fact that reintroductions are among the most effective conservation measures, they are rarely used for invertebrates. In this study we investigate the potential of reintroductions as a conservation measure for beetles, and present the first genetic results for an endangered veteran tree specialist. After translocation of 10 adults in 1987, a population of the Great Capricorn beetle reappeared in Hluboká nad Vltavou (Czech Republic) in 1990s. Using population genetic analyses of 79 individuals based on nine microsatellite loci and 82 individuals based on the mitochondrial COI gene we assessed the origin of this population, and compared its genetic variation, population structure and demography to the alleged source population (southern Moravia) and to the closest autochthonous population (Třeboňsko). Although the reintroduced and the closest autochthonous populations are geographically close (24 km), their mutual genetic distance was much higher than that between each of them and the geographically distant (>150 km) potential source population in southern Moravia. The genetic diversity of the reintroduced population was the lowest from the three studied populations and represented a subset of the alleged source population suggesting its establishment due to a translocation from southern Moravia. Despite the lower genetic variation at the reintroduced site, our results suggest that reintroductions could serve as a highly effective measure in biodiversity conservation and in some cases it may be the only chance to prevent extirpation of many endangered populations.

Keywords Xylophagous insect · Threatened · Saproxylic · Doudlebia · Oaks

Introduction

Habitat fragmentation is one of the main threats to the survival of organisms with limited dispersal abilities (Hanski and Gilpin 1997; Hanski 2005). By allowing such organisms to exploit otherwise inaccessible habitat patches, reintroductions or translocations may thus reduce the effect of fragmentation, boost biodiversity conservation by direct spread of an endangered species (Hayward 2011), or improve ecosystem functioning through reintroduction of keystone species (Gibbs et al. 2008). Reintroduction is an effective and increasingly common conservation measure (Seddon et al. 2007). It is, however, also highly taxonomically biased, with only 3 % of reintroduction studies dealing with invertebrates (Bajomi et al. 2010), and very few of them focusing on the most diverse group on the Earth: the beetles. Except for the campaign to reintroduce the American burrowing beetle *Nicrophorus americanus* (e.g. Amaral et al. 1997), and several, mostly unsuccessful attempts to reintroduce or translocate endangered beetles in Northern America and New Zealand (Nothnagle 2002;

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Knisley et al. 2005; Sherley et al. 2010), beetle translocations are rarely performed, or are under-reported in scientific literature. This is surprising given that in comparison to for example some large mammals the reintroduction of beetles is an inexpensive and logistically simple task (Perrotti et al. 2001).

Beetles depending on old trees and dead-wood (=saproxylic beetles) constitute a diverse, functionally important, but numerically decreasing portion of global biodiversity (Berg et al. 1994; Jonsson et al. 2005; Müller et al. 2008; Nieto and Alexander 2010; Lindenmayer et al. 2012). The notoriously poor dispersal abilities of some species make them especially sensitive to habitat fragmentation (Ranius 2006). Moreover, numerous populations of endangered veteran tree specialists inhabit isolated sites where their long-term survival is compromised by the absence of trees that would become the next generation of veteran trees (Alexander 1998; Miklín and Čížek 2014). On the other hand, suitable management measures allow for surprisingly rapid restoration of habitats for at least some veteran tree specialists (Sebek et al. 2013). Reintroduction to sites that have regained suitable conditions might therefore be the key to preventing local extirpation of many species due to fragmentation. It is, however, unclear, to what extent such attempts are likely to succeed, as no cases of successful reintroduction of an endangered saproxylic beetle have been reported (but see Karlsson 2012).

Small and isolated populations are prone to bottlenecks and founder events and thus susceptible to genetic degradation caused by increased rates of inbreeding or loss of genetic diversity due to genetic drift, both reducing long-term fitness (Frankham et al. 2002). Since the number of translocated individuals is usually small, the newly established population is susceptible to the above-mentioned problems. Evaluation of the genetic variation in reintroduced populations should therefore be one of the main concerns following a reintroduction attempt. Despite this fact, there are no studies on the genetic structure of a reintroduced beetle population available, and there are surprisingly few studies available on population genetics of non-pest saproxylic beetles (e.g. Jonsson et al. 2003; Audisio et al. 2009; Oleksa et al. 2013; Ahrens et al. 2013; Solano et al. 2013).

The Great Capricorn beetle (Cerambyx cerdo, Linnaeus, 1758) is an important umbrella species and considered an ecosystem engineer (Buse et al. 2008). It is a globally threatened and internationally protected (Council of the European Communities 1992; Nieto and Alexander 2010; IUCN 2013) inhabitant of old, sun exposed oaks (Quercus spp.). Mainly in northern parts of its distribution range it is rare and rapidly declining (e.g. Sláma 1998; Ehnström and Axelsson 2002; Starzyk 2004; Ellwanger 2009). In the Czech Republic, the beetle inhabited most of the lowlands and major river valleys till the last century (Sláma 1998).

Today, its continuous range has shrunk to south-east of the country and only six isolated populations remain in the rest of the republic. Following a reintroduction attempt in 1987, however, the beetle re-appeared at a site where it was considered extinct.

In order to investigate the potential of reintroductions of saproxylic invertebrates as a tool for their conservation, we apply population genetic analyses based on ten microsatellite loci and a fragment of the mitochondrial COI gene, to (i) assess the origin of the reintroduced population by comparing it to the alleged source population and to the closest autochthonous population and (ii) compare genetic variation, population structure and demography of the reintroduced population, the large autochthonous population and the isolated autochthonous population. Finally, (iii) we present data on the development of the reintroduced population over time.

Materials and methods

Sites

The reintroduction site, Hluboká nad Vltavou (Hluboka) is located in the south-western region of Czech Republic, 115 km south of Prague (49.045°N, 14.441°E, 380 m a.s.l.) in the Budejovicka Basin, near the river Vltava. Large open-grown oaks of up to 200 years old (with maximum diameter at breast height ∼160 cm) inhabited by the beetle are found in alleys, on fishpond dams and on wooded meadows. The locality is protected as a Site of Community Importance (total area: 67.2 ha), with C. cerdo as one of its target species (see Albert et al. 2012 for details). At the locality and in its wider environs, C. cerdo was present till mid 1950s, with the last record in 1953 (Kletečka and Klečka 2003). Despite intensive activity of amateur and professional entomologists in the area during the second half of 20th century, only old larval galleries indicating past occurrence were found before the reintroduction event.

The nearest site inhabited by C. cerdo is Třeboňsko (Trebon; 48.995°N, 14.769°E, 450 m a.s.l., 24 km from Hluboka), a protected landscape area found in the Třeboň basin, where an isolated autochthonous population inhabits old oaks on several fishpond dams (Kletečka and Klečka 2003).

The source population, located in southern Moravia (Moravia; 48.774°N, 16.797°E, 190 m a.s.l., 174 km from Hluboka), inhabits mostly oak woodlands along the lower Dyje (Thaya) river (see Miklín and Čížek 2014 for details). It is a large population connected to other populations in western Slovakia and north-eastern Austria and most likely also to the rest of Slovakia and Hungary.

Fig. 1 Establishment of the Great Capricorn beetle, *Cerambyx cerdo*, population at the site Hluboká nad Vltavou (Hluboka) since its reintroduction in 1987
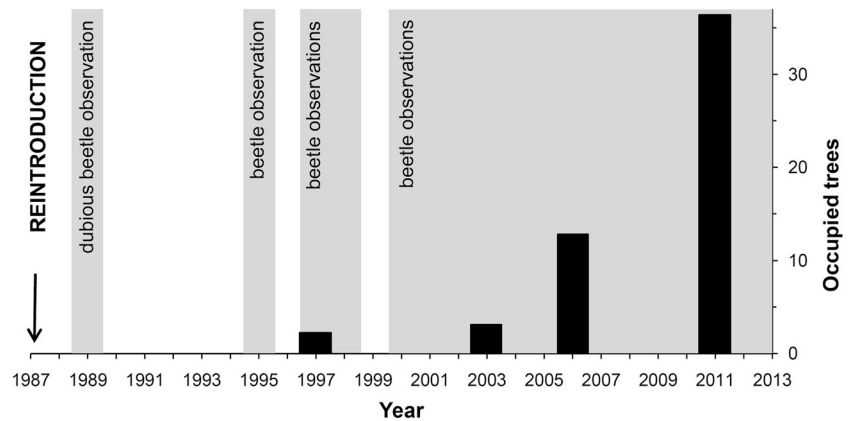
Reintroduction and establishment of the population

Seven females and three males of *C. cerdo* were collected in Lednice Chateau Park (48.806°N, 16.817°E) on the 11 and 12 of June 1987. One day later, they were released on five oaks on Podskalska meadow (49.044°N, 14.439°E) near Hluboka (Anonymous, personal communication). The reintroduction was carried out without any institutional or legal backing.

After several decades of no sightings in the area, a single dead and damaged adult beetle was found ca 0.5 km from the reintroduction site in 1989 (Kletečka and Klečka 2003). Given the circumstances of the finding, this record may concern the remains of one of the translocated individuals. The first observation of a live individual comes from 1995. Beetles were then observed in 1997 and 1998. Since 2000, beetles and fresh exit holes have been observed by numerous entomologists on a yearly basis and became more abundant. In 1997, fresh exit holes were observed on two trees (one was cut down in 1997, the other a few years later). In 2003, Kletečka and Klečka (2003) found three oaks with fresh exit-holes. In 2006, 14 inhabited trees were found (Hauck and Cizek 2006). Today, there are >30 inhabited trees at the site (Fig. 1). The reintroduction site, Podskalska meadow, remains the centre of the population. Most inhabited trees are found <300 m from this site, despite the fact that the most distant inhabited tree was found 3.8 km away (personal observation).

DNA extraction and analyses

In 2011, we sampled 84 individuals from three sites in the Czech Republic. A middle leg from each individual was taken and stored in a vial containing ethanol for molecular analyses. Genomic DNA was extracted using the Genomic DNA Mini Kit Tissue (Geneaid) following the manufacturer's protocol. In total, 79 individuals (29 from Hluboka,

29 from Moravia and 21 from Trebon; Table 1) were genotyped for 10 polymorphic microsatellite loci (Drag et al. 2013). Since locus CC_06 exhibited deviation from HWE (Hardy–Weinberg equilibrium) as well as high estimated frequencies of null alleles for the Trebon and Moravia populations, we decided to exclude it from further analyses and all results presented here are based on 9 loci. PCR products were run on an ABI 3730XL sequencer (Applied Biosystems) by Macrogen (Korea) and allelic patterns were scored using the software GeneMapper 3.7 (Applied Biosystems). For 82 individuals (33 from Hluboka, 27 from Moravia and 22 from Trebon; Table 1) a partial fragment (814 bp long) of the mitochondrial gene cytochrome c oxidase subunit I (COI) was amplified and sequenced. We used universal forward and reverse primers; C1-J-2183 (alias Jerry) (5′ CAA CAT TTA TTT TGA TTT TTT GG) and TL2-N-3014 (alias Pat) (5′ TTC AAT GCA CTT ATT CTG CCA TAT TA) (Simon et al. 1994). PCR cycling parameters included a denaturation step at 95 °C for 5 min, followed by 40 cycles of 92 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min 30 s, with a final elongation step at 72 °C for 10 min. Sequencing was performed on both strands by the commercial company Macrogen (Korea).

Microsatellite data analyses

Loci characteristics and genetic diversity

For each population we calculated the number of alleles and the observed and expected heterozygosity using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Frequencies of null alleles were estimated by FreeNA (Chapuis and Estoup 2007) and the linkage disequilibrium between all pairs of loci as well as the HWE was tested with Genepop 4.1.3 (Raymond and Rousset 1995; Rousset 2008) using the default parameters. We calculated the allelic richness (AR) in FSTAT v2.9.3.2 (Goudet 2002).

Table 1 Genetic diversities of the three sampled populations of the Great Capricorn beetle (*Cerambyx cerdo*) derived from nine microsatellite loci and cytochrome c oxidase I (COI)

Population	Locality	Microsatellites					MtDNA (COI)				
		N	N_A	AR	H_o	H_e	N	H	h	π	P
Reintroduced	Hluboka	29	3.7	3.55	0.482	0.491	33	2	0.061	0.00007	1
Source	Moravia	29	5.0	4.59	0.592	0.636	27	8	0.510	0.00194	13
Nearest native	Trebon	21	3.9	3.87	0.522	0.576	22	3	0.537	0.00179	7

N number of individuals analyzed; N_A average number of alleles across the nine loci; *AR* average allelic richness per locus, based on a minimum of 19 individuals; H_o observed heterozygosities; H_e expected heterozygosities; *H* number of haplotypes; *h* haplotype diversity; π nucleotide diversity; *P* number of polymorphic sites

Population structure

Genetic differentiation was evaluated by computing pairwise F_{ST} values for all pairs of populations in Arlequin 3.5.1.3 (Excoffier and Lischer 2010). The significance of the derived genetic distances was tested by 99,999 permutations. To avoid any potential bias by null alleles, we also calculated the pairwise F_{ST} values using a correction for null alleles with FreeNA (Chapuis and Estoup 2007).

The population structure of our dataset was assessed using Structure 2.3.4 (Pritchard et al. 2000), assuming an admixture model (individuals may have mixed ancestry) and correlated allele frequencies (closely related populations might have correlated allele frequencies). We ran Structure for values of K ranging from 1 to 5 with 100,000 burn-in and 1,000,000 MCMC steps for ten replicates for each K. The best K value was chosen according to Evanno et al. (2005) using the online software Structure Harvester (Earl and vonHoldt 2012). The obtained results for a given K were post-processed using CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) and visualized in Distruct 1.1 (Rosenberg 2004).

To assess the genetic structure of the studied populations, we also used factorial correspondence analysis (FCA) using the software Genetix 4.05 (Belkhir et al. 1996-2004). All individuals were grouped on multiple factorial axes based on shared alleles and formed clusters with similar genotypes in a 2D space graph.

Demography

Single-sample estimates of effective population size (N_e) were calculated using the linkage disequilibrium (LD) method (Waples and Do 2008) implemented in NeEstimator v2 (Do et al. 2014). The LD method assumes the selective neutrality of the marker and that the populations are isolated as well as assuming that the species has discrete generations; all of these assumptions were met where possible. Because of the small amount of missing data in our dataset, NeEstimator v2 which implements an improved method to account for missing data (Peel et al.
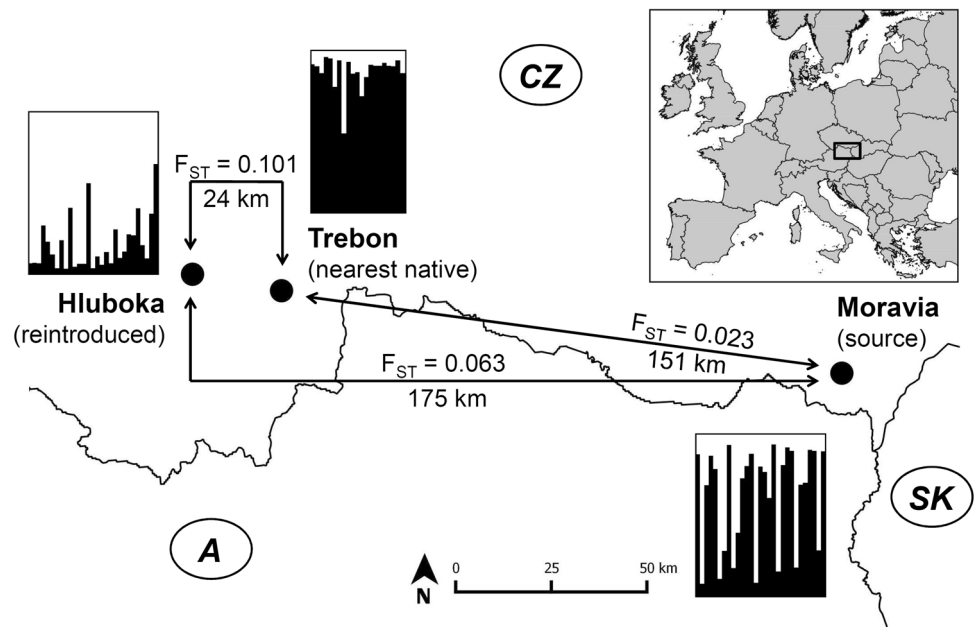
2013) represented a better choice compared to LDNe or other frequently used software. Also, this program allows for screening out rare alleles. The critical values in our analysis were set as a default to 0.05, 0.02 and 0.01. A jackknife method was used to construct 95 % confidence intervals (Waples and Do 2008).

We searched for a putative founder event by using the 'changes in allele frequencies test' and 'heterozygosity excess test', both implemented in Bottleneck 1.2.02 (Cornuet and Luikart 1996). The first of these tests expects that many rare alleles are lost following a population bottleneck: resulting in changes in the shape distribution of allele frequencies, the second assumes that the allele number is reduced faster than the heterozygosity during the bottleneck: resulting in higher observed heterozygosities than expected relative to the total number of alleles available in such a population (Cornuet and Luikart 1996). Based on Luikart and Cornuet (1998), we considered two proposed models; the stepwise-mutation model (SMM), and the two-phased model (TPM) (variance = 30 %, SMM = 70 %). The statistical significance of each model was evaluated by Wilcoxon's signed rank test.

COI data analyses

The sequences from each individual were edited and aligned (MUSCLE, default settings) in Geneious 6.1.6 (Biomatters) and translated into amino acid sequences to check for the presence of stop codons. All unique sequences were submitted to GenBank (KM516109–KM516119 accession number). Standard genetic indices (the number of polymorphic sites, the number of haplotypes and the nucleotide and haplotype diversities) were computed using the program DnaSP 5.10 (Librado and Rozas 2009). As most of the sequences showed low genetic variation (a low number of haplotypes), no phylogenetic trees were reconstructed. Instead, a haplotype network was produced using the statistical parsimony method (95 % connection limit; Templeton et al. 1992) implemented in the program TCS 1.21 (Clement et al. 2000).

Results

Microsatellite data

Loci characteristics and genetic diversity

All loci were polymorphic with the number of alleles per locus ranging from four to nine (Online Resource S1). The highest average number of alleles (5) was found in the Moravia population and the lowest (3.7) was found in the Hluboka population (Table 1). The same pattern was observed for the allelic richness. The average observed and expected heterozygosity over all loci ranged from 0.482 to 0.592, and from 0.491 to 0.576 respectively. After the Bonferroni correction for multiple tests, all loci were in HWE in all studied populations. No linkage disequilibrium was found between the pairs of loci except for the pair: CC_03 and CC_07 in Trebon ($p = 0.00254$). An estimated frequency of null alleles per locus >10 % was found in loci CC_08 in Trebon (19 %) and CC_03 in Moravia (13 %).

Population structure

F_{ST} values were significantly different in all pairwise comparisons and showed a rather low amount of genetic differentiation (Fig. 2). We found the highest F_{ST} (0.101)

between Hluboka and Trebon, i.e. the two geographically closest (24 km) populations. Both populations were equally genetically differentiated from the geographically most distant one. We gained similar results after the ENA (=excluding null alleles) correction (Chapuis and Estoup 2007) with the highest value of $F_{ST} = 0.119$ between Trebon and Hluboka.

The Bayesian clustering analysis in Structure identified the best number of clusters as two (K = 2). Almost all individuals from the Hluboka population were assigned to one cluster, whereas nearly all individuals from Trebon fell into the other cluster corroborating the higher genetic differentiation (measured by F_{ST}) between Hluboka and Trebon. The Moravia population consisted of individuals assigned to both clusters (Fig. 2).

In accordance with previous, the results of the factorial correspondence analysis (FCA) demonstrated practically non-overlapping ranges on the first axis for the populations of Hluboka and Trebon. The first axis explained 9.07 % of variation in the data, while the second axis explained 6.77 % of variation (Fig. 3).

Demographic factors

Depending on the minimal threshold of rare allele frequency, the N_e estimates ranged between 98–111 individuals for Hluboka and 29–34 individuals for Trebon. For Moravia a negative value indicated that N_e was unrecognizable from infinity, i.e. N_e was large or limited data were available (Waples and Do 2010). The confidence intervals were, however, extremely broad for all populations (Table 2).

Fig. 3 Factorial correspondence analysis (FCA) scatter diagram of the reintroduced (*empty circles*), source (*grey circles*), and nearest native (*black*) populations of the Great Capricorn beetle, *Cerambyx cerdo,* based on analysis of nine microsatellite loci. The number of analysed individuals was 29 for the reintroduced, 28 for the source and 21 for the nearest native populations
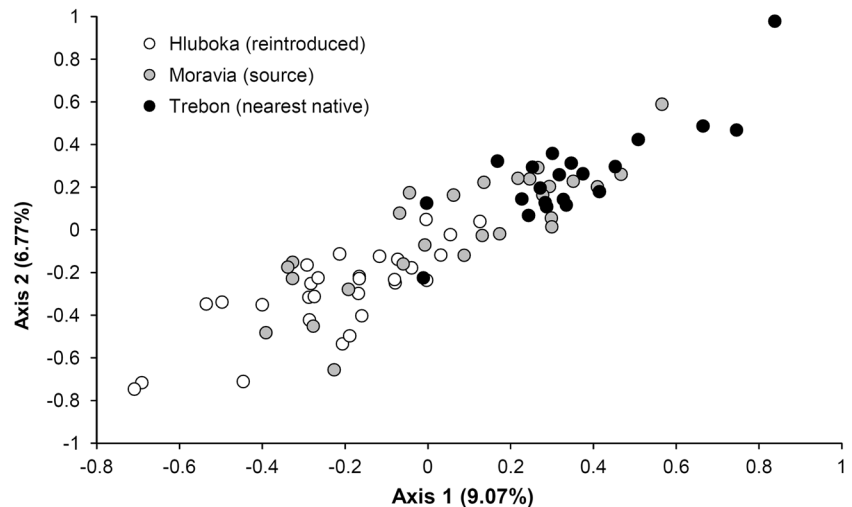

Table 2 Single sample estimates of effective population size (N_e) for the three sampled populations of the Great Capricorn beetle, *Cerambyx cerdo,* and the probability values of Wilcoxon's test for bottleneck effects using the stepwise mutation model (SMM) and two-phase model (TPM) based on nine microsatellite loci

Population	Locality	Estimates of N_e			Wilcoxon's test	
		0.05 (CI 95 %)	0.02 (CI 95 %)	0.01 (CI 95 %)	SMM	TPM
Reintroduced	Hluboka	111 (24-inf.)	101 (34-inf.)	98 (24-inf.)	0.8496	0.3672
Source	Moravia	inf. (59-inf.)	inf. (64-inf.)	inf. (99-inf.)	0.8984	0.0820
Nearest native	Trebon	29 (11-inf.)	34 (13-inf.)	34 (13-inf.)	0.4102	0.1504

For N_e estimates, alleles with a frequency <5, 2 and 1 % were excluded

The results of tests for heterozygosity excess in Bottleneck gave congruent results under the different mutation models. Both SMM and TPM models did not detect heterozygosity excess in all three studied populations (Table 2). Although according to allele frequency histograms many rare alleles were lost in the Hluboka population (Online Resource S1), all populations had a normal L-shaped distribution under both mutation models.

COI data

Based on the 814 bp long fragment of the mitochondrial gene COI, we identified 11 different haplotypes. The overall haplotype diversity (h) was 0.398 and the nucleotide diversity (π) was 0.0016 (Table 1). Markedly low diversity values were found in the Hluboka population (h = 0.061, π = 0.0001) compared to the other populations studied. Statistical parsimony analysis produced the haplotype network with one haplotype dominant in all three populations (79 % of all individuals) and ten other haplotypes separated by one or more mutations (Fig. 4). Except for the dominant haplotype, no haplotypes were shared among populations. Based on the 33 samples, the Hluboka population was entirely composed of individuals bearing the most common haplotype, with the exception of one individual where a unique mutation in one position was found.

F_{ST} values were significantly different in all pairwise comparisons and showed a similarly low amount of genetic differentiation as F_{ST} values based on microsatellites. Specifically, we found the highest F_{ST} (0.142) between Hluboka and Trebon, and almost equal distance between Hluboka and Moravia (0.04) and Trebon and Moravia (0.053).

Discussion

Our results present the first documented evidence supporting the success of an endangered saproxylic beetle reintroduction, the first population genetic analysis of a reintroduced beetle population, as well as the first population genetic study of the endangered Great Capricorn beetle, *Cerambyx cerdo*.

Origin of the reintroduced population

The Hluboka population was genetically closer to the geographically distant Moravia population than to the nearby Trebon population. The genetic makeup of the Hluboka
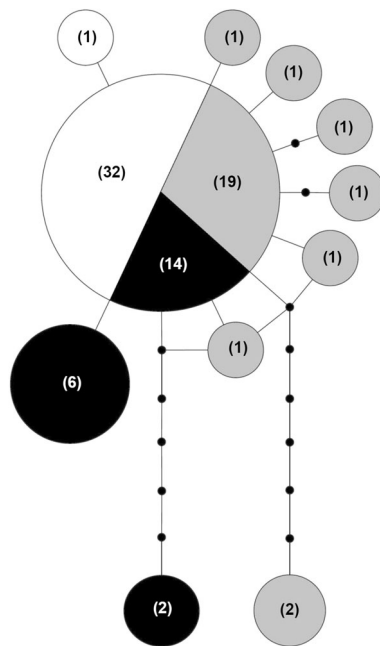
Fig. 4 Haplotype network of mtDNA (COI) of the Great Capricorn beetles, *Cerambyx cerdo* from the three populations, including the Hluboka (reintroduced), Moravia (source) and Trebon (closest native) population. The network was constructed using statistical parsimony method; each haplotype is represented by a circle; the circle color corresponds to one population (*white*: reintroduced; *grey*: source; *black*: nearest native). The circle size is proportional to the haplotype frequency. The number in brackets represents the number of individuals bearing the haplotype within a given population. Small black circles indicate missing haplotypes that are not present in the sample, but are necessary to link all observed haplotypes to the network

population represented a subset of the Moravia population (*but see below*). Natural colonisation, unnoticed survival of a local population, or translocation might have been the cause for the recent re-appearance of *C. cerdo* in Hluboka. Due to the geographical distances between Hluboka and the other neighbouring populations, natural colonisation other than from Trebon is unlikely. The high genetic differentiation between Hluboka and Trebon, however, rules out colonisation from Trebon as well. The unnoticed survival of a native population in Hluboka is also highly improbable since it would be expected to be genetically very close to that in Trebon given the formerly continuous distribution of *C. cerdo* in the region in the past (Kletecka and Klecka 2003), the rather high dispersal abilities observed in large longhorn beetles (e.g. Smith et al. 2001; Drag et al. 2011; Torres-Vila et al. 2013; David et al. 2013), and the low genetic differentiation over much larger distances. Reintroduction from the more distant Moravian source is, therefore, the most parsimonious explanation for the observed genetic structure.

Facts not favouring the translocation hypothesis include the existence of a unique COI haplotype, and the lack of

evidence for a bottleneck according to the calculations of Bottleneck software. Given the rarity of the unique haplotype in Hluboka (a single individual out of 33) and the relatively high haplotype diversity in the source population, it is certainly possible that the haplotype was not detected due to its rarity in the source population. We, nevertheless, can not rule out the possibility of further reintroductions from an unknown source. On the other hand, the low diversity of the maternally inherited COI sequences in the reintroduced population suggests that the number of females founding the population was indeed low. The lack of the evidence for bottleneck (results are based on microsatellites) in the reintroduced population can be explained by the beetlés biology. *C. cerdo* females usually mate with more than one male (personal observation). The seven females reintroduced in 1987 were collected as adults, making them most likely to be mated at the site of their origin. Hence, the translocation of 10 beetles might have resulted in translocation of genetic information of substantially more individuals. Also, the rapid growth of the population in the last 20 years (see above) probably allowed for the survival of even rare alleles due to the small effect of genetic drift (Nei et al. 1975). On the other hand, of the three studied populations the Hluboka population has the lowest allelic richness and haplotype diversity suggesting the presence of the founder effect in the past. We therefore conclude that the Hluboka population was most likely established due to the recent translocation from Moravia.

Autochthonous populations

The Moravia population exhibited the highest genetic diversity and it was genetically equidistant from the two other populations. Its estimated infinite N_e, together with high allelic and haplotype diversity indicates that the Moravia population is part of continuous (or very recently fragmented) distribution of *C. cerdo* in the region. In the past, the population probably reached further northwest along the Danube River and was connected with the Trebon population. The presence of two unique COI haplotypes in Trebon, however, suggests its longer isolation or connection to other populations, perhaps to those inhabiting the lower Vltava river valley.

Reliable estimates of N_e can be obtained with the LD method when populations are small ($N_e < 200$) and well sampled (~ 50 individuals, Waples and Do 2010). These requirements have not been met for any of the studied populations which might have resulted in the broad confidence intervals of the N_e estimates. Despite that, the estimates seem rather realistic for the Moravia and the Hluboka populations (see above). The N_e estimates may thus also be realistic for Trebon despite being surprisingly

low. Although such low N_e values are not rare even for beetles (e.g. Beebee 2007; Kajtoch et al. 2014), and although the number of analysed individuals would need to be higher to obtain more rigorous estimates, this is currently the only information on N_e available for Trebon population. Despite all the reservations, the low N_e is worrying and active management measures (Buse et al. 2007; Albert et al. 2012) should be considered to support this isolated, autochthonous population.

Translocation of saproxylic beetles

Nearly three decades after the reintroduction event the *C. cerdo* population is among the two largest of the several populations in Bohemia (the western part of the Czech Republic) where the beetles range collapsed in the past century (Sláma 1998). It is thus the first successful documented reintroduction of a threatened saproxylic beetle. Although the amount of habitat available at the reintroduction site is crucial, similar biotic and abiotic conditions at the source and reintroduction sites, as well as the rather similar genetic make-up of the source population and that of the population originally found in the area (*see above*) probably contributed to the success of the reintroduction attempt (Armstrong and Seddon 2008).

Reintroductions or translocations could serve as highly effective measures in beetle conservation. Moreover, our results suggest that as long as suitable conditions are present, just a few individuals (albeit mated; *see above*) are enough to found a viable population, thus minimising the potentially negative effect of removing individuals from the source population. On the other hand the translocation of few individuals resulted in the substantial loss of the mitochondrial and the partial loss of the nuclear diversity in the reintroduced population compared to the source. It is unclear whether such loss may compromise a long-term survival of the population.

Prior to a reintroduction several considerations need to be taken into account. First is the possibility of long-term survival of the reintroduced population. Second and perhaps more important in this case, it is the potential effect of the reintroduction on the exploited habitat and populations of other endangered organisms associated with it. The autochthonous population of *C. cerdo* in Hluboka probably died out due to temporal shortage of suitable trees in the mid 20th century. *C. cerdo* presence most likely increases the mortality of old oaks (Martín et al. 2005; Buse et al. 2008). If too few suitable oaks and their future successors are available at the site and/or in its vicinity, the reintroduced population might cause the locally available senescent oaks to die off, thus creating a temporal gap in their availability. Such a gap might be fatal to the population as well as to local populations of other organisms associated

with the habitat. On the other hand, *C. cerdo* is considered an ecosystem engineer whose presence on oaks allows for their colonisation by other threatened organisms (Buse et al. 2008). Its reintroduction may thus boost biodiversity conservation as long as the size and age structure of the local open-grown oak population enables gradual replacement of dead trees, or if suitable managements such as coppicing, pollarding, and wood pasture are applied to increase the amount of suitable habitat.

Careful consideration of reintroductions is particularly important for organisms with a potentially high impact on their habitat. Given the ever increasing habitat fragmentation combined with highly specific substrate requirements and low dispersal abilities of many organisms depending on old trees and/or dead wood, reintroductions and translocations may represent the only chance to prevent extirpation of many populations of endangered beetles, and are thus likely to become more common in the future.

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