

# Conflicting mitochondrial and nuclear data in the six toothed spruce bark beetle *Pityogenes chalcographus*



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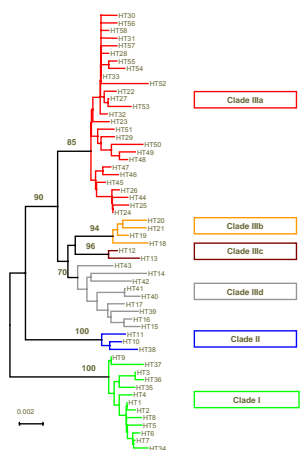


Figure 1. Phylogenetic tree of *P. chalcographus* mitochondrial DNA.

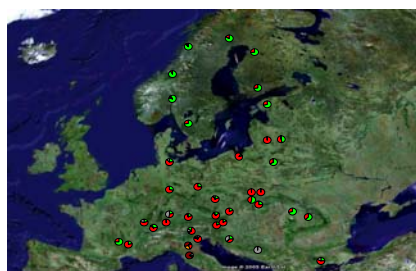


Figure 2. Sampling sites and proportion of individuals belong to distinct clades. Colors in the pie charts correspond with the colored branches in Figure 1.

Locus	Alleles sampled	Allelic richness	H <sub>e</sub>	H <sub>s</sub>	H <sub>i</sub>	F <sub>IS</sub> Italy	F <sub>IS</sub> N-Europe	F <sub>IS</sub> C-Europe	F <sub>IS</sub> SE-Eur.
44N	12	8.70	.492	.727	.732	.386	.303	.396	.186
46C	9	6.22	.133	.439	.438	.828	.884	.667	.591
41E	24	16.17	.481	.905	.913	.515	.426	.414	.516
44D	27	13.79	.237	.756	.759	.731	.852	.507	.643
All			.336	.707	.711	.593	.545	.470	.482

Table 1. Microsatellite data of 107 individuals which successfully amplified for all available loci. The individuals were grouped by geographical region.

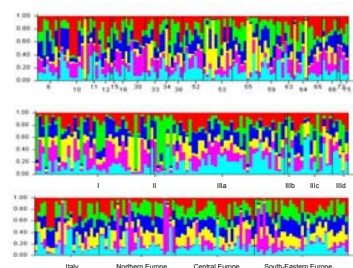


Figure 3. Output of *Structure* software [11] when microsatellite data are grouped by sampling location (top), mitochondrial haplotype (middle) or geographical region (bottom).

## References

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## Hypothesis

In the mid 1970ies, crossing barriers between Northern and Central European populations of *P. chalcographus* were observed for the first time and the possibility of race differentiation was hypothesized [1]. The restriction of the beetle's host tree to at least three refugial areas during Quaternary glaciations [2] was suggested as a potential cause of allopatric speciation with secondary contact after the Holocene temperature amelioration.

## The mitochondrial approach

The haplotypes of 653 individuals from 39 populations, covering the natural range of Norway spruce in Europe, were determined by a combination of sequencing and SSCP [3,4]. Phylogenetic reconstruction revealed a six-branched tree with the two major clades I and IIIa containing 93% of the individuals screened (Figure 1). These two clades exhibited a sympatric distribution on most of the European terrain, with clade I dominating in Northern and clade IIIa in Central Europe (Figure 2). Applying molecular clocks [5], a separation 1 to 1.5 myr ago was suggested. Thus, mitochondrial results support the hypothesis of an allopatric origin and secondary sympatry of the observed clades.

## The microsatellite approach

Despite the fact that microsatellite isolation and analysis in coleopteran species is a tedious process [6], four highly polymorphic loci were identified. We investigated 288 individuals with known mitochondrial haplotype. Rate of PCR failure ranged between 12 and 37% per locus. In 107 individuals all loci were successfully genotyped. For subsequent data analysis results were clustered by distinct populations, geographical regions and mitochondrial haplotypes. Contrary to the mitochondrial results, all statistics indicate a homogenous population structure and high gene flow all over Europe (Table 1, Figure 3).

## The scnDNA approach

Several single copy nuclear (scn) genes are established as potential markers in coleopterans. We sequenced 860 bp of the 28S ribosomal RNA [7] and 1 kbp of the elongation factor-1 $\alpha$  [8] gene of 56 individuals representing all mitochondrial haplotypes. Alignment of the sequences again revealed no mutations between European populations.

## Putative reasons for contrasting genealogies

- Due to haploid genotype and maternal inheritance, mitochondria have a 4-fold reduced effective population size relative to their nuclear counterparts. Thus, the **impact of genetic drift** is much higher than in nuclear genes. Historical barriers to gene flow may have lead to a complete sorting of mitochondrial lineages, while nuclear genes may have retained ancestral variation.
- Sex biased dispersal rates are good candidates for nuclear and mitochondrial incongruence. The **flight capacity**, which is higher in male beetles [9], may at least episodically lead to nuclear genetic exchange between populations, leaving the more stationary female mitochondrial genome unaffected.
- Maternally inherited **endosymbionts** shaping mitochondrial genomes are widespread among arthropods. The  $\alpha$ -proteobacterium *Wolbachia pipientis* was identified as causal agent of indirect mitochondrial selection [10]. High mitochondrial diversity between populations may reflect the presence of more than one symbiont strain maintaining a mitochondrial genetic structure in spite of ongoing nuclear gene flow.

## Further investigations

We intend to perform large scale assays for the presence of *Wolbachia* and the implementation of AFLP as an additional nuclear marker.