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MICROSATELLITE LETTERS

Isolation and characterization of ten microsatellite loci for the wood-living and threatened beetle *Cucujus cinnaberinus* (Coleoptera: Cucujidae)

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Abstract *Cucujus cinnaberinus* is an obligate saproxylic beetle distributed in Europe and considered as near threatened in its entire range (IUCN red list). Ten polymorphic microsatellites were characterized among 26 and 45 individual samples obtained from dead trees in the Czech Republic and Norway respectively. All loci were polymorphic in both samples with number of alleles per locus ranged from two to eleven in the Norwegian sample and from two to six in the Czech sample. The genetic difference between the Czech and the Norwegian material were highly significant ($P < 0.0001$). For several trees the sampled genotypes gave evidence that more than two parents were responsible for the breeding. These microsatellite loci should prove useful in the study of population structure including mate and dispersal patterns for this species.

Keywords SSR markers · Population genetics · Coleoptera · Red-listed beetle

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Introduction

The beetle *Cucujus cinnaberinus* (Scopoli 1763) lives primarily in recently dead, broad-leaved trees in eastern and northern parts of Europe and has status as near threatened at the IUCN redlist (Nieto et al. 2010). The major threat is degradation and loss of habitat, resulting in fragmentation and isolation of populations. In Scandinavia the species is primarily connected to aspen (*Populus tremula* L.), which occurs scattered in the managed boreal forest (Sverdrup-Thygeson 2008). Given the ephemeral and in many areas scattered nature of the habitat, resource competition may be strong. It is possible that vital resources might be monopolized by the first female to colonize a dead trunk, which would decrease the availability of suitable habitats further. Despite the species' need for conservation, nothing is known about its genetic structure.

Twenty-six larva or pupa from nine trees were sampled in Pardubice and Jihomoravsky Regions in Czech Republic and 45 larva or pupa from twelve trees were sampled in Telemark and Aust-Agder county in South Norway. Number of individuals from each tree varied from one to eleven. Genomic DNA was extracted using DNeasy™. Tissue Kit (Qiagen). The DNA from six individual samples from each country were pooled and sent to GenoScreen, Lille, France (www.genoscreen.fr). One µg DNA was used

Table 1 Characterization of microsatellites in *C. cinnaberius*

Locus	Repeat motif	Primer sequence (5′–3′)	Accession no.	Size	Norway			Czech Republic		
					Na	Ho	He	Na	Ho	He
Cuc-3	$(CA)_{11}$	F: CCAGGGGTTCTCCAATTCTT R: TGCGTTTGCTATTTTCCAAT	KJ156632	168–174	2	0.422	0.475	4	0.692	0.727
Cuc-4	$(GA)_9$	F: AGGACGCCGATGTGTAAGTC R:CCCTAACTGCAAATCCTCTCG	KJ156633	193–201	4	0.467	0.534	2	0.038	0.038
Cuc-5	$(CA)_{12}$	F: TTATTGGCCACACAGATCCA R: TGGATTGCCGGTAAGGTTTA	KJ156634	193–201	3	0.200	0.236	4	0.462	0.570
Cuc-6	$(CA)_{11}$	F: CCAGTCTTCTGGCACGAGTT R: GGTCGAACGGAAGAAACAAC	KJ156635	175–183	4	0.400	0.366	3	0.240	0.215
Cuc-7	$(CA)_{14}$	F: TCATTTGGCGCGATAAAGTC R: CATTTCGCCTGGGAGTAAGA	KJ156636	137–149	2	0.067	0.064	2	0.538	0.497
Cuc-8	$(GT)_{14}$	F: AAAACTTAGATTTGTTGAGAACGAG R: CGCCGCCAGATAACGTATT	KJ156637	160–251	11	0.795	0.723	6	0.577	0.686
Cuc-11	$(CA)_{11}$	F: TTAACGACGTGCCTGAACAC R: CGCACTTTTTGTATAGCAGCA	KJ156638	147–153	4	0.600	0.564	4	0.560	0.686
Cuc-12	$(GT)_{11}$	F: AACTTTTGTCAAACCGCCAC R: AGCAGCTACGTGACAGGAGA	KJ156639	128–148	6	0.636	0.565	3	0.400	0.447
Cuc-13	$(AC)_{10}$	F: ACCCCGAGTTGCATCACATA R: GCCTCTCCAAGGGTTTCTTT	KJ156640	180–200	7	0.733	0.717	6	0.692	0.655
Cuc-14	$(AC)_{12}$	F: CCGTTCGAGTTTTCCAAATA R: CCTCGTTCGTTTCCTTCTGT	KJ156641	101–111	5	0.548	0.576	3	0.227	0.208

Listed are microsatellite locus designation, the repeat structure, primer sequences, GeneBank accession number, size range of amplified alleles, number of alleles (N_a) and observed (H_{obs}) and expected heterozygosities (H_{exp})

for the development of microsatellites libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries as described in Malausa et al. (2011).

Primers were designed for thirteen of the microsatellite motives and tested for amplification and polymorphism using DNA from seven *C. cinnaberius* from each of the Czech Republic and Norway. Forward primers were labeled with fluorescent, and microsatellite loci were amplified on a GeneAmp PCR System 9700 (Applied Biosystems) in 10 µl reaction mixtures containing 20–40 ng DNA, 3 pmol of each primer, 1× Key buffer (VWR), 0.2 mM dNTP and 0.5 U Taq DNA polymerase (VWR). Thermocycling parameters after denaturation at 95 °C in 2 min were 30 cycles of 95 °C for 30 s, annealing temperature 55 °C for 30 s and 72 °C for 45 s followed by 10 min at 72 °C. The PCR products were electrophoresed using an ABI 3100 sequencer (Applied Biosystems).

Of the thirteen primer sets, ten gave satisfactory amplifications and showed polymorphism, while one did not amplify and two were monomorphic. Linkage disequilibrium and deviations from Hardy–Weinberg equilibrium within countries together with testing for genetic differentiation between countries were determined using Genepop version 4.0.7 (Rousset 2008). We used MICKROCHECKER version 2.2.3 (Van Oosterhout et al. 2004) to test for the presence of null alleles.

All loci were polymorphic in both the Norwegian and Czech samples with number of alleles per locus ranging from two to eleven (Table 1). No loci deviated from Hardy–Weinberg equilibrium after Bonferroni correction ($\alpha = 0.05$), neither was there any significant linkage disequilibrium among paired loci after correction for multiple tests. Null alleles were not detected. Exact test of genetic differentiation was highly significant between the two samples ($P < 0.0001$) with significant difference in nine of the ten markers used. In eight out of thirteen trees with more than two beetles sampled, the genotypes in minimum two loci gave evidence that they descended from more than one mating pair. The reason for this was presence of more than four alleles, or more than three alleles together with a homozygote, or more than two alleles together with two different homozygotes. Thus, there is no indication of one female monopolizing a resource with a large number of offspring. These ten microsatellite loci should prove useful in further studies of population structure including mating and dispersal pattern which are important for estimates of efficient population sizes and for making efficient protection plans for this threatened species.

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