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DNA isolation from single pieces of beetle frass: a resource for conservation genetic studies of *Gnorimus nobilis*

Max Blake, Niall J McKeown, Paul W Shaw

Population Genetics and Genomics Laboratory, Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK

Corresponding author: Max Blake [mab82@aber.ac.uk]

The Noble Chafer (*Gnorimus nobilis* (L. 1758), Coleoptera: Scarabaeidae: Cetoniinae; Figure 1) is a widespread Eurasian heartwood-rot specialist, feeding as a larva in veteran oak, willow and beech (Smith 2003). However, in the UK it is largely restricted to traditionally-managed orchards (Whitehead 2003). Post-war changes in orchard management practices have resulted in a loss of 87% of the UK's traditional orchards (PTES unpublished data). This reduction, with subsequent fragmentation of habitat represents a serious threat to *G. nobilis* in the UK (Mannerkoski 2010). At present, conservation practises are hindered by the complete absence of data on population demography. Genetic (DNA) markers represent powerful tools to study population demographics and connectivity that may be beyond the resolution of standard ecological approaches such as mark recapture.

The larvae of *G. nobilis* (third instar in particular) produce copious quantities of frass (hard pelleted faeces), which is used as an indicator of *G. nobilis* presence in a rot-hole by surveyors (Shenke 2010). The isolation of DNA from single frass pieces suitable for genetic analysis would represent an invaluable resource for future conservation genetic studies of Noble Chafer or other species where sampling live individuals is not desirable and/or practical. A few studies have used non-lethal DNA extraction techniques (e.g. Feinstein 2004; Donald *et al.* 2012; Lefort *et al.* 2012; Scriven *et al.* 2013), but only Strangi *et al.* (2013) have extracted DNA from frass *in-situ*, working with 100mg of frass from trees infested with *Anoplophora chinensis*. In the present study, a number of DNA extraction techniques were employed, with successful DNA isolation tested by subsequent PCR amplification of mitochondrial DNA (two Cytochrome Oxidase 1 gene regions) and nuclear (microsatellite) loci.

Three DNA extraction methods were assessed: a Chelex based method following McKeown & Shaw (2008); DNeasy Blood and Tissue Kit (Qiagen); CTAB-chloroform/isoamylalcohol method (Winnepenninckx *et al.* 1993). Each method was applied to 8 individual pieces of

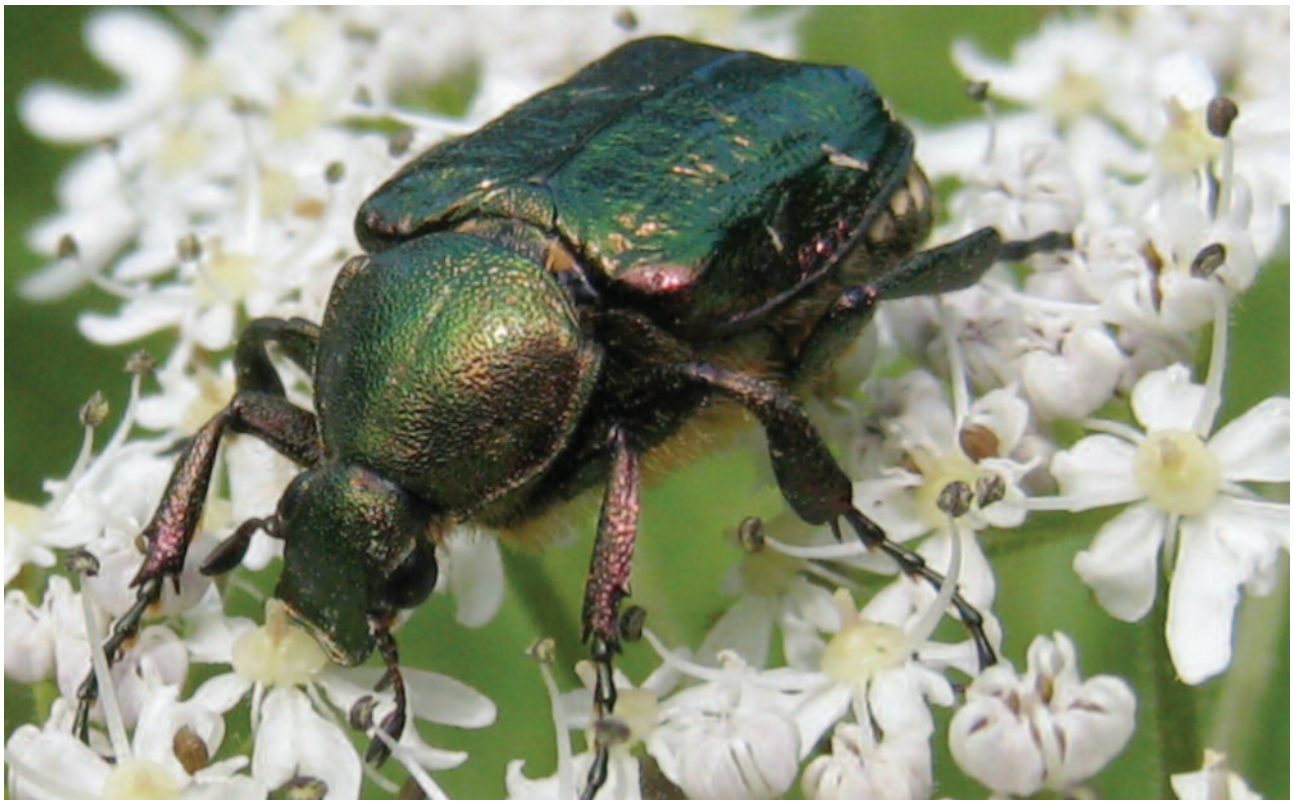

Figure 1: An adult male *Gnorimus nobilis* (Noble Chafer) in a typical feeding position on Hogweed (*Heracleum* sp.)
(photograph credit: Adam Bates)

	DNA region tested		
Extraction source and dilution	1st CO1 (219bp)	2nd CO1 (333bp)	Microsatellite locus (≈130bp)
Chelex Stock	0	0	-
Chelex 1/10	0	0	-
Chelex 1/100	1	0	-
Chelex 1/200	1	1	1
DNeasy Stock	6	0	-
DNeasy 1/10	24	13	-
DNeasy 1/100	23	23	-
DNeasy 1/200	24	19	23
Phenol-Chloroform Stock	0	0	-
Phenol-Chloroform 1/10	9	0	-
Phenol-Chloroform 1/100	22	13	-
Phenol-Chloroform 1/200	24	23	23

Table 1: PCR amplification success (number of reactions out of 24 tests displaying DNA products) from frass-derived DNA recovered using different extraction techniques. A dash indicates no test.

frass from three different sites in Worcestershire all collected between May and August 2013. Frass from third-instar *G. nobilis* larvae weighs 2.198mg (standard deviation 0.507) and a range of 1.1-3.2mg (n=30, dry weights from a single locality (Site 2)). All frass was crushed before digestion.

Extracted DNA was diluted with distilled water to four different treatments: no dilution, 1/10, 1/100, and 1/200. PCR mixtures contained 5 l of Biomix, 0.5 l of each primer, 3 l of the DNA, and 1 l of distilled water. PCR cycles for both CO1 primer combinations (1st region=219bp, 2nd region=333bp) were: an initial denaturation step for 3 minutes at 94°C, followed by 35 cycles of 30s at 94°C, 1 minute at 52°C and 1 minute at 72°C, with a final extension of 3 minutes at 72°C. The PCR cycle for the microsatellite locus (≈130bp) was an initial denaturation step for 3 minutes at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 55°C and 30s at 72°C, with a final extension of 3 minutes at 72°C. All 4 dilutions were used in the CO1 amplifications, whilst only 1/200 was used for the microsatellite loci. PCR success was scored by visualising the amplified DNA on a 2% agarose gel in TBE buffer. COI amplicons were cleaned using SureClean and sequenced using the respective PCR primers on an AB sequencing platform using BigDye technology. BLAST was used to ensure that the amplicons were derived from *G. nobilis*.

Success rates are reported in Table 1. Both DNeasy and CTAB-chloroform/isoamylalcohol extraction methods showed high PCR amplification success rates (90%+) at higher dilutions for all three DNA regions. All sequenced amplifications were aligned to reference material and confirmed as *G. nobilis*. BLAST results confirmed the sequences as the sister group to *G. variabilis.* The results show the potential to reconstruct individual genotypes from single frass pellets and thus non-invasive genetic monitoring. Information from individual genotypes can then be applied to diverse areas of molecular ecology; in particular conservation genetic studies focusing on threatened, cryptic, rot-hole associated scarabs such as *Osmoderma* and *Propomacrus*. The technique will be used to investigate patterns of neutral and adaptive genetic structuring in Noble Chafer to disentangle the roles of historical and recurrent processes on various spatial scales.

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