



DNA sequence variation and methylation in an arsenic tolerant earthworm population

Peter Kille^{a,1}, Jane Andre^{a,b,1}, Craig Anderson^{a,c}, Hui Na Ang^{b,c}, Michael W. Bruford^a, Jacob G. Bundy^d, Robert Donnelly^a, Mark E. Hodson^{b,2}, Gabriela Juma^a, Elma Lahive^c, A. John Morgan^a, Stephen R. Stürzenbaum^e, David J. Spurgeon^{c,*}

^a Cardiff School of Biosciences, BIOSI 1, University of Cardiff, P.O. Box 915, Cardiff CF10 3TL, UK

^b Soil Research Centre, School of Human and Environmental Sciences, University of Reading, Reading RG6 6DW, Berkshire, UK

^c Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Wallingford, Oxfordshire OX10 8BB, UK

^d Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, South Kensington, London, UK

^e King's College London, Analytical and Environmental Sciences Division, 150 Stamford Street, London SE1 9NH, UK

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ABSTRACT

Evidence is emerging that earthworms can evolve tolerance to trace element enriched soils. However, few studies have sought to establish whether such tolerance is determined through adaptation or plasticity. Here we report results from a combined analysis of mitochondrial (cytochrome oxidase II, COII), nuclear (amplified fragment length polymorphism, AFLP) variation and DNA methylation in populations of the earthworm *Lumbricus rubellus* from sites across an abandoned arsenic and copper mine. Earthworms from the mine site population demonstrated clear arsenic tolerance in comparison to a naïve strain. COII and AFLP results suggest that *L. rubellus* from the unexposed and the adapted populations comprises two cryptic lineages (Lineages A and B) each of which was present across all of the sites. AFLP analysis by lineage highlighted variations associated with soil metal/metalloid concentrations (most clearly for Lineage A) suggesting a genetic component to the observed tolerance. The methylation sensitive AFLP (Me-AFLP) identified a high genome methylation content (average 13.5%) in both lineages. For Lineage A, Me-AFLP analysis did not identify a strong association with soil arsenic levels. For Lineage B, however, a clear association of methylation patterns with soil arsenic concentrations was found. This suggests that Lineage B earthworms utilise epigenetic mechanisms to adapt to the presence of contamination. These fundamentally different genetic adjustments in the two clades indicate that the two lineages employ distinct adaptive strategies (genetic or epigenetic) in response to arsenic exposure. Mechanisms driving this variation may be founded within the colonisation histories of the lineages.

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1. Introduction

Many invertebrate species are able to maintain viable populations in polluted soils where total and potentially bioavailable metal/metalloid concentrations greatly exceed toxicity values (e.g. LC₅₀s) for known naïve (and thus sensitive) populations (laboratory strains). This suggests that under trace element exposure, some invertebrate populations develop metal tolerance through behaviour or physiological adaptive traits (Posthuma and Van Straalen,

1993; Van Straalen and Roelofs, 2007). Mechanisms underpinning this tolerance have in some cases been shown to involve heritable changes in coding or promoter regions of metal efflux pumps (Callaghan and Denny, 2002) and thiol-rich peptides involved in sequestration (glutathione-S-transferases, phytochelatin, and metallothioneins) (Janssens et al., 2007; Vatamaniuk et al., 2005). In other cases, however, the mechanisms underlying tolerance remain unknown and/or unstudied.

For earthworms, one of the most functionally important of soil taxa (Lavelle et al., 1997), indirect evidence for metal tolerance is provided by the fact that earthworms can be collected from soils containing residue levels that significantly exceed toxic effect concentrations (Spurgeon and Hopkin, 1999a,b). However, difficulties in extrapolating toxicity data between the laboratory and field due to, for example, contaminant ageing and speciation (Arnold et al., 2003, 2007), mean that the mere presence of

* Corresponding author. Tel.: +44 1491 772 208; fax: +44 1491 692 424.
E-mail address: dasp@ceh.ac.uk (D.J. Spurgeon).

¹ PK and JA should be considered as joint first authors.

² Present address: Environment Department, University of York, Heslington, York YO10 5DD, UK.

earthworms in these polluted soils is not alone confirmation that adaptation has occurred (Davies et al., 2003; Donner et al., 2010). More directly in relation to tolerance, studies with successive generations of *Eisenia fetida* selected for tolerance to Zn over two generations found changes in the shape of concentration response relationships for survival that were indicative of tolerance development (Spurgeon and Hopkin, 2000). In the field, Langdon et al. (1999) noted that *Lumbricus rubellus* living in arsenic and copper polluted soil at two abandoned arsenic mines (Devon Great Consols, Carrock Fell) could survive in arsenic-spiked soil that was acutely toxic to earthworms from a clean site. This tolerance was preserved when the mine populations were reared on clean soil over two generations, suggesting a genetic basis for this phenotype (Langdon et al., 2009).

Despite indications of trace metal and metalloid tolerance in earthworms, the extent to which there is a genetic and/or physiological basis of this trait has not been fully investigated. A study of isozyme specific polymorphisms within *L. rubellus* populations known to be adapted to combined metal and flooding stress failed to identify adaptive variation (Simonsen and Klok, 2010), although the results of this study should be treated with some caution as enzymes known to be related to metal tolerance were not targeted. The only study that has, to date, identified a potential genetic basis for tolerance to adverse soil conditions in earthworms is that for *L. rubellus* living at a lead/zinc mine located at Cwmystwyth, Mid Wales. For this population, Andre et al. (2010) used mitochondrial (COII) and amplified fragment length polymorphism (AFLP) genotyping to demonstrate that a mine spoil associated population showed little genetic overlap (in AFLP profile) with individuals within populations at two less polluted sites.

While the assumption often is that individual/population survival is based on selection for increased tolerance, there is evidence emerging that the plastic responses driven by chemical influences on the epigenome may also be an important mechanism of adaptation (Mirouze and Paszkowski, 2011; Ren et al., 2011; Seong et al., 2011). Among the many epigenetic mechanisms, DNA methylation represents a key response. Given that earthworms have been recorded to possess a 13% methylated cytosine content in DNA (Regev et al., 1998), the potential for mediation of adaptive tolerance through

epigenetic DNA methylation should be considered. Here, we report a combined toxicological and genetic study, using mitochondrial (COII) and nuclear (AFLP) and DNA methylome analysis, for the earthworm *L. rubellus* sampled at sites of different metal pollution status within an As-contaminated mine complex – the Devon Great Consols (DGC) site in the UK. For the study, we sampled earthworms from a number of sites within DGC including one (Site 2 in this study) from which tolerant populations previously studied by Langdon et al. (2009, 1999) were collected. Adjacent and distant reference sites were also sampled. That arsenic, a major contaminant at the site, has been reported to induce epigenetic changes including hypo- and hyper-methylation of DNA (Ren et al., 2011; Zhong and Mass, 2001) makes the site particularly suited for the analysis of DNA methylation responses in earthworms. Initially the collected populations were screened to confirm that the tolerance previously reported for populations at study Site 2 was applicable to earthworms inhabiting this and other collection sites located in the mining area. Genetic analyses were then undertaken using these populations. The hypothesis tested was that *L. rubellus* populations from polluted sites that show evidence of tolerance, would include individuals with mitochondrial or nuclear genotypes and/or DNA methylation patterns that were distinct from those of intolerant earthworms from (adjacent) unpolluted locations.

2. Materials and methods

2.1. Site description, sampling and soil characterisation

This study was conducted at the abandoned Devon Great Consols mine complex located in the Tamar Valley, Devon, South-West UK (UK Ordnance Survey; Map coordinates for mine centre: SX426733 – N50:32:52 W4:13:25). This mine was worked for copper and arsenic from 1844 to 1900 and from 1915 to 1930. Across the site, the spoil from various extraction processes remain. The soils established on these wastes contain highly elevated concentrations of trace elements, including arsenic and copper. Earthworms (*L. rubellus*) were sampled from six locations in the region of the Devon Great Consols mine. Four locations (Sites 1–4) were situated on the mine and waste handling area (see Fig. 1). This

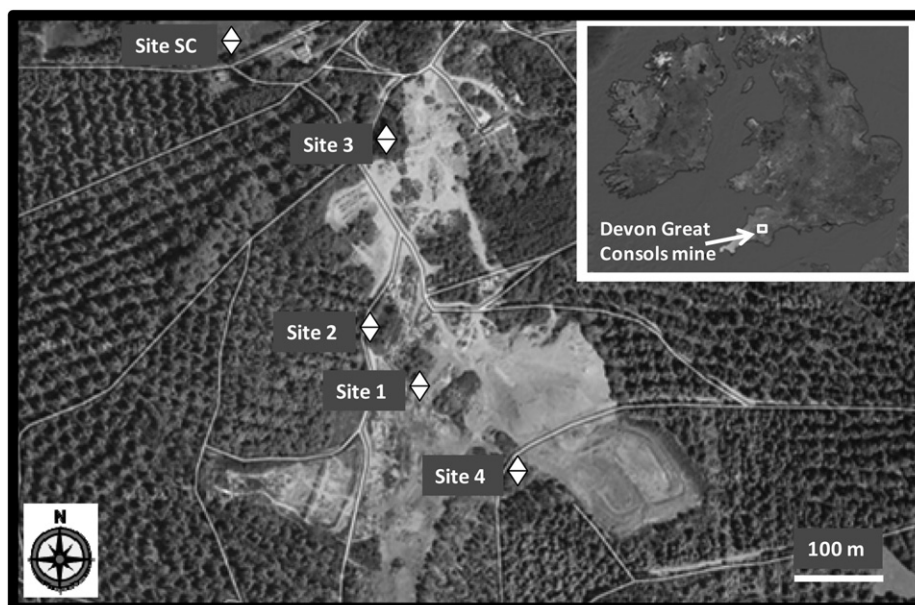


Fig. 1. Aerial images showing the location of the Devon Great Consols mine site in the South West UK (top right insert panel) and locations of the 5 sampling locations (Sites 1–4 and Site SC) situated in the area on, and immediately adjacent to, the Devon Great Consols mine workings.

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