Forensic Science International: Genetics 6 (2012) 798–809

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

A forensic DNA profiling system for Northern European brown bears (*Ursus arctos*)

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ARTICLE INFO

Article history:
Received 9 November 2011
Received in revised form 10 February 2012
Accepted 11 March 2012

Keywords:
Wildlife forensics
STRs
Brown bear
Non-invasive genetic sampling
Population genetics

ABSTRACT

A set of 13 dinucleotide STR loci (G1A, G10B, G1D, G10L, MU05, MU09, MU10, MU15, MU23, MU26, MU50, MU51, MU59) were selected as candidate markers for a DNA forensic profiling system for Northern European brown bear (*Ursus arctos*). We present results from validation of the markers with respect to their sensitivity, species specificity and performance (precision, heterozygote balance and stutter ratios). All STRs were amplified with 0.6 ng template input, and there were no false bear genotypes in the cross-species amplification tests. The validation experiments showed that stutter ratios and heterozygote balance was more pronounced than in the tetranucleotide loci used in human forensics. The elevated ratios of stutter and heterozygote balance at the loci validated indicate that these dinucleotide STRs are not well suited for interpretation of individual genotypes in mixtures. Based on the results from the experimental validations we discuss the challenges related to genotyping dinucleotide STRs in single source samples. Sequence studies of common alleles showed that, in general, the size variation of alleles corresponded with the variation in number of repeats. The samples characterized by sequence analysis may serve as standard DNA samples for inter laboratory calibration. A total of 479 individuals from eight Northern European brown bear populations were analyzed in the 13 candidate STRs. Locus MU26 was excluded as a putative forensic marker after revealing large deviations from expected heterozygosity likely to be caused by null-alleles at this locus. The remaining STRs did not reveal significant deviations from Hardy–Weinberg equilibrium expectations except for loci G10B and MU10 that showed significant deviations in one population each, respectively. There were 9 pairwise locus comparisons that showed significant deviation from linkage equilibrium in one or two out of the eight populations. Substantial genetic differentiation was detected in some of the pairwise population comparisons and the average estimate of population substructure (F_{ST}) was 0.09. The average estimate of inbreeding (F_{IS}) was 0.005. Accounting for population substructure and inbreeding the total average probability of identity in each of the eight populations was lower than 1.1×10^{-9} and the total average probability of sibling identity was lower than 1.3×10^{-4}. The magnitude of these measurements indicates that if applying these twelve STRs in a DNA profiling system this would provide individual specific evidence.

1. Introduction

DNA markers such as microsatellites have been extensively used in conservation genetics to study population diversity, impact of genetic drift and level of inbreeding in a variety of species [1]. One of the best studied mammalian species in conservation genetics is the European brown bear (*Ursus arctos*) [2]. Most data used in recent wildlife genetic studies of brown bear are from genotyping a collection of dinucleotide STRs that were isolated from brown bear and black bear (*Ursus americanus*) [3,4]. The development of non-invasive genetic sampling techniques has allowed sampling of living populations of large carnivores like the brown bear [3,5]. Non-invasive genetic sampling techniques open for long-term genetic monitoring of threatened carnivores that

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doi:10.1016/j.fsigen.2012.03.002

occur at low densities. Capture-mark-recapture (CMR) analysis allows important parameters such as abundance, survival and migration to be studied [6,7]. As part of the population management of Northern European brown bear in Norway their abundance have been monitored since 2006 by use of CMR analyses of data from non-invasive samples typed in a set of dinucleotide STRs (see e.g. [8]).

The brown bears of Northern Europe are listed as threatened, but are often involved in conflicts with humans, livestock depredations and illegal hunting. When investigating wildlife crime, genetic analyses of sample materials could provide species specific identification of bear. Furthermore, if using a set of bear specific STRs, forensic genetic analyses would have the potential to provide individual specific bear profiles from a variety of sample materials and provide a means for traceability of bear products (e.g. food, trophy objects and medicine). Experience from population management of brown bears in Norway indicates that approximately 65–70% of non-invasive sample materials may be successfully typed in more than 6 STR loci [8]. Similar success rates when analyzing non-invasive sample materials have been reported by others [9].

Genetic identification of individuals by use of a DNA profiling system would rely on relevant reference data. The lack of such reference data may be a limitation when developing DNA profiling systems for large carnivores. However, these existing dinucleotide markers commonly used in wildlife population management may be used in a forensic DNA profiling system if the necessary allele frequencies from living populations could be retrieved in co-operation with population management laboratories. Dinucleotide STRs are widely used in population monitoring and conservation genetic studies of brown bear [1]. Tetranucleotide STRs are expected to have less stutter and less difference in heterozygote balance than dinucleotide STRs, and they are thus, the preferred markers in human forensics. However, one reason why dinucleotide STRs are commonly used in conservation genetics is that these markers has proven to work well in sample materials like faeces and hair due to relatively short amplicon sizes [1,3,8–10]. European laboratories that monitor bear populations already use very similar sets of dinucleotide STRs [10–12] while tetranucleotide STRs, on the other hand, are not used at all for DNA analysis of brown bear. Thus, a selection of dinucleotide STRs seems to be the preferred forensic markers to include in a European bear DNA profiling system. The ISFG recommendations, although pointing out the benefits of using tetranucleotide STRs, support the use of dinucleotide STRs if they are already in widely use in a non-human species [15]. Several recommendations regarding validation and use of non-human DNA in forensic genetics have been suggested to justify their application as evidence in court [13–15]. Such validation studies, that demonstrate the performance of any new markers, may be particularly important if applying dinucleotide STRs instead of the tetranucleotide STRs [15].

One aim of this study were to perform the recommended validation tests on thirteen dinucleotide microsatellite markers (G1A, G10B, G1D, G10L, MU05, MU09, MU10, MU15, MU23, MU26, MU50, MU51, MU59) commonly used for bear population management and conservation genetics. The validations tests could aid in the selection of markers for a forensic DNA profiling system for the brown bear in Northern Europe. The validation tests included species specificity testing, measurements of sensitivity as well as measurements of precision, stutter and heterozygote balance. Selected common alleles from all STR loci were sequenced to explore the allelic size variation at the sequence level. Another aim of this study was to provide allele frequency distributions as well as relevant forensic genetic parameters for the selected markers from eight bear populations in Northern Europe.

2. Materials and methods

2.1. Population material from eight brown bear populations in Northern Europe

The individual profiles in the population material are from samples collected in specific areas in Northern Europe ($n = 479$). Fig. 1 shows a map of Northern Europe with the approximate location of each of the eight populations indicated (P1–P8). A total of 290 of the individuals were from Norway, of which 233 individual profiles were obtained by typing non-invasive samples (fecal scats and hair) collected in the field as part of the monitoring of bears in Norway from 2006–2009, and 57 were obtained from legally shot bears in the same period (tissue and/or blood). The individuals were from four geographically separated areas; North-eastern Norway (P1, $n = 74$), North-western Norway (P2, $n = 34$), Middle Norway (P3, $n = 81$) and South-eastern Norway (P4, $n = 101$). The individuals from Middle Sweden were collected in 2009 in the county of Västerbotten (P5, $n = 84$) and the individuals from Finland were from the Kainuu area (P7, $n = 44$) and collected during 2005–2008. The sample materials from Sweden and Finland were scats collected in the field. The individuals from Russia were from two areas; the Pinega Strict Nature Reserve in Archangelsk (P6, $n = 27$) and Karelia (P8, $n = 35$). The sample materials were scats collected in the field in Pinega during 2005–2008 and tissue samples from legally shot bears in Karelia in the period 2005–2007.

2.2. DNA extraction, PCR amplification and STR analysis

DNA was extracted from hair-roots and tissue using the Qiagen DNeasy Tissue kit (Qiagen) and from faeces using the Invitek Stool kit (Invitek). Faeces were stored in stool collection tubes with DNA stabiliser (Invitek) or in plastic bags and kept at −20 °C until DNA extraction. The hair samples were stored dry and dark in paper envelopes until DNA extraction. No quantification of DNA concentrations was performed on the extracted samples. Instead,

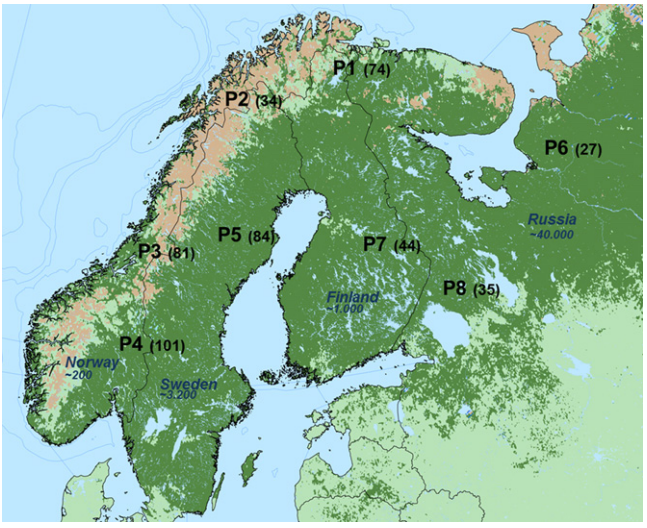

Fig. 1. The figure shows a map of northern Europe (including Norway, Sweden, Finland and Russia). The borders are shown as black lines, forest areas as green, scrub/bush and cropland as light green, tundra and mountains as brown and water as blue. The sample location of each of the eight bear populations, P1–P8 (with number of individuals in brackets) are indicated on the map. P1 (74); North eastern Norway $n = 74$, P2; North-western Norway $n = 34$, P3; Middle Norway $n = 81$, P4; South-Eastern Norway $n = 101$, P5; Middle Sweden $n = 84$, P6; Pinega in Russia $n = 27$, P7; Kainuu in Finland $n = 44$, P8; Karelia in Russia $n = 35$. Estimates of the total number of bears in each of the four countries are given in blue below the name of the country. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)