Stevenson-Holt, Claire D. ORCID: https://orcid.org/0000-0003-3635-5404 , Ramsey, Andrew D. ORCID: https://orcid.org/0000-0002-5550-9977 , Nevin, Owen ORCID: https://orcid.org/0000-0003-3513-8053 and Sinclair, Billy (2012) Assessing grey squirrel dispersal patterns within the landscape using sequence variation. Landscape Research, 38 (4). pp. 553-559.

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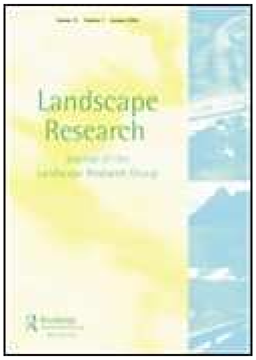

Assessing grey squirrel dispersal patterns within the landscape using DNA sequence variation.

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Assessing grey squirrel dispersal patterns within the landscape using sequence variation.

ABSTRACT

The grey squirrel *Sciurus carolinensis* is thought to have contributed to the decline of red squirrel *S. vulgaris* populations in the UK through resource competition and disease spread. This study used mtDNA sequencing to assess patterns of grey squirrel dispersal in the UK. Patterns of genetic variation within the dloop sequence were characterised for seven grey squirrel populations. Infiltration directions and potential barriers to dispersal are identified and discussed, with a focus on Cumbria, a county at the forefront of grey squirrel expansion. Understanding the dynamics of grey squirrel dispersal will aid their management at a landscape scale and enhance the conservation of red squirrels.

INTRODUCTION

The Eastern grey squirrel *Sciurus carolinensis* was first introduced to Britain between 1876 and 1929 (Middleton 1931; Shorten 1954; Lloyd 1983). Subsequent successful introductions and translocations occurred within the UK and by the 1930's populations were established in southeast England and rapidly spreading through the country (Shorten 1954). This expansion occurred simultaneously with the decline in native red squirrel *Sciurus vulgaris* populations (Lloyd 1962; Lloyd 1983). Studies suggest that interspecific resource competition occurs (Wauters et al. 2000; Gurnell et al. 2004) and that this, along with the effects of a particularly virulent squirrelpox virus (SQPV), carried by the grey squirrel, has caused the decline and extirpation of many red squirrel populations (Tompkins et al. 2003; Gurnell et al. 2004; Carroll et al. 2009).

Cumbria in North West England has been found to hold genetically unique populations of red squirrel which contain high levels of genetic diversity (Hale et al. 2004).

Concerns have been raised over the impact of expanding grey squirrel populations are having on red squirrel populations. Interspecific competition and SQPV transmission are thought to be highest at times of grey squirrel dispersal (Sainsbury et al. 2008). An understanding of the dispersal ecology and directional movements of the grey squirrel will aid grey squirrel management decisions and red squirrel conservation.

Recent work (Stevenson et al., in review) has indicated that the Cumbrian Mountain range is acting as a barrier to dispersal. Grey squirrel populations within Cumbria have been derived from the infiltration of individuals from two directions; to south Cumbria from Lancashire and to north Cumbria from Northumberland/ Scottish Borders. Hale et al. (2001) and Trizio et al. (2005) both suggest genetic analysis has enabled the identification of land cover types which either facilitate dispersal or provide barriers to red squirrels over large geographic scales. In this paper we report on variation within Cytochrome b (Cytb) and Dloop DNA sequences found within grey squirrel mtDNA, the first such report to date.

METHODOLOGY

Two grey squirrel accessions were collected from each of four known introduction points (see Middleton, 1931); Balloch near Loch Long; Dalkeith in Edinburgh; Henbury in Cheshire, and from Alice Holt Forest in Surrey. In addition, samples were collected from three established/ emerging populations within the UK; Doune, in Stirlingshire; Millom, in south Cumbria and Brampton, in north Cumbria (Figure 1).

DNA was extracted from the leg muscle tissue of 14 individuals using the QIAGEN DNeasy blood and tissue extraction kit and following the manufactures instructions (QIAGEN Ltd). Fragments of the two mtDNA sequence encoding Cytb and Dloop were amplified by polymerase chain reaction (PCR). Primers for *Sciurus carolinensis* Cytb were available from Meece et al. (2005), BM1 (5'-CCCCTCAGAATGATATTTGTCCTCA) and

BM2 (5'-CCATCCAACATCTCAGCATGATGAAA). Each PCR reaction had a reaction volume of 25 µl and containing 1 µl 1:10 mtDNA, 12.5 µl AmpliTaq Gold PCR master mix (Roche, USA), 1 µl 1:10 BM1 primer to distilled water, 1 µl 1:10 BM2, 9.5 µl distilled water. PCR amplification followed the protocol of Meece et al. (2005): denaturation of 3.5 min at 95ºC followed by 36 cycles of 30s at 95ºC, annealing 50s at 60 ºC, extension 40s at 72ºC, final extension of 5min at 72 ºC.

Primers for *Sciurus carolinensis* Dloop were taken from the published sequence of the Dloop (GenBank Accession no. AF111027; Barrett et al. 1999), Dloop forward primer 5'-GCCACCCCCAAGTTAAATGG-3' and Dloop reverse primer 5'-ATTCGTGCATTAATGCACTATCC-3'. Each PCR reaction for Dloop contained the same relative quantities of components as outlined above for cytb amplification apart from 5 µl of each primer was used. PCR amplification for Dloop sequence followed the protocol of Trizio et al. (2005), denaturation of 1 min at 94ºC followed by 40 cycles of 30s at 94ºC, annealing 30s at 52 ºC, extension 1min at 72ºC, final extension of 5min at 72 ºC.

Electrophoresis on 1% agarose gels were used to check the success of the PCR reactions for each sample. All PCR products were then cleaned following the methodology of the QIAquick PCR purification kit (QIAGEN Ltd). Sequencing of PCR products was carried out (DBS Genomics, Durham, UK).

Sequences for cytb and Dloop from each individual were aligned manually using BioEdit_R v7.0.4.1 Freeware (Ibis Therapeutics, California,USA) and Sequence Analysis 5.2.0 (Applied Biosystems) using the default settings within the programmes. A sequence from *S. vulgaris* (GenBank Accession no. AJ238588) was also included as an outgroup accession in the analysis. The sequences were entered into Paup 4.0 Beta 10 (Swofford, Illinois Natural History Survey, Illinois, USA) for phylogeny reconstruction and a 50%

majority rule consensus tree was created with 1000 bootstrap and jacknife replicates (Harrison and Langdale 2006).

RESULTS

MtDNA was successfully extracted from 14 grey squirrel individuals from selected locations around the UK. Cytb sequence data was analysed for all 14 accessions with no sequence variation observed. The 325bp sequence generated, demonstrated a 99% similarity match for grey squirrel cytochrome b gene in the Genbank BLAST search tool (Accession no. AY509680). Similarly, the 329bp Dloop sequence generated showed a 98% similarity match to that of grey squirrel (Accession no. AF111027). Dloop sequences were aligned in BioEdit and discrete points of variation were detected at 16 unique sites, representing 4.9 % of the overall sequence.

Parsimony analysis generated a 50% majority rule consensus tree from the grey squirrel dloop sequence data which was rooted against red squirrel (GenBank Accession no. AJ238588). The consensus tree (Figure 2) indicated that the grey squirrel samples are separated into four distinct clades; Clade I; Henbury, Balloch and accession S8 from Alice Holt; Clade II; Doune and accession S7 from Alice Holt; Clade III; Dalkeith and Brampton (north Cumbria); Clade IV; Millom (south Cumbria). The branching of samples from Dalkeith and Brampton is strongly supported with bootstrap values of 73% and jacknife values of 56%.

DISCUSSION

This study examines DNA sequence variation and its utility in assessing patterns of grey squirrel dispersal in the UK. Cytb sequences generated in this study demonstrated no detectable differences across the range of populations sampled. This may be due to the low mutation rate within the cytb coding sequence and the relatively short time frame of introduction and dispersal within the UK. Conversely, sequence variation was detected within the dloop sequence for these same accessions. Dloop sequence analysis showed significant statistical support for the distinct separation of north and south Cumbria grey squirrel populations.

Accessions from north Cumbria grouped with accessions from Dalkeith in Edinburgh. Individual accessions within north Cumbria may not necessarily have been derived from individuals dispersing south from Edinburgh. However, as they are both within the same clade they may have been derived from the same progenitor individuals from another location such as northeast England (Stevenson et al., in review). Similar patterns of dispersal have been seen in red squirrels, mediated by the afforestation of woodland between Cumbria and the Northeast (Hale & Lurz 2003).

The two samples taken from each population were generally grouped together within the phylogenetic tree, however, samples from Alice Holt Forest did not follow this pattern and were grouped separately with samples from elsewhere. David-Gray et al. (1998) study found high levels of genetic diversity within grey squirrel populations from Alice Holt Forest and attributed this to numerous introduction sites and translocations from different source populations, which could explain the results demonstrated in Figure 2 in this study.

The initial findings presented here demonstrate support for the hypotheses that the Cumbrian Mountain range is acting as a barrier to dispersal and that invasion into the area is coming from multiple directions. If grey squirrels colonised Cumbria with a northerly advance as suggested (Lowe 1993; Skelcher 1997) accessions from north and south Cumbria should be grouped together within a phylogenetic reconstruction. However, this is not the case, and the two populations separate out in the tree. This points towards separate populations and the effectiveness of the Cumbrian Mountain range as a barrier. Whilst it is acknowledged that a small number of individuals may try to disperse over this mountain barrier, the majority of individuals will choose a lower cost dispersal route. Further validation of prospective incursion directions will require an increase in the sample size for each of the seven locations and additional accessions from within Cumbria and adjoining areas.

This study has provided the first evidence of Dloop sequence variation within UK grey squirrel populations. Despite the small sample size, it supports the suggestion that the Cumbrian Mountains provide a barrier to grey squirrel dispersal and infers both northerly and southerly grey squirrel incursion routes into the county. Ultimately, further knowledge of dispersal and colonisation of grey squirrels will inform conservation policy and can be used to target resources for grey squirrel control and enable better protection for vulnerable red squirrel populations. We have shown here, that landscape genetics can provide evidence of population origin and genetic differences. Although this study has focused on grey squirrels in Cumbria, the techniques are equally applicable to other landscapes, validating and highlighting dispersal routes of invasive species and species of conservation concern.

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Figure 1. Geographical location of grey squirrel sample sites within the UK.

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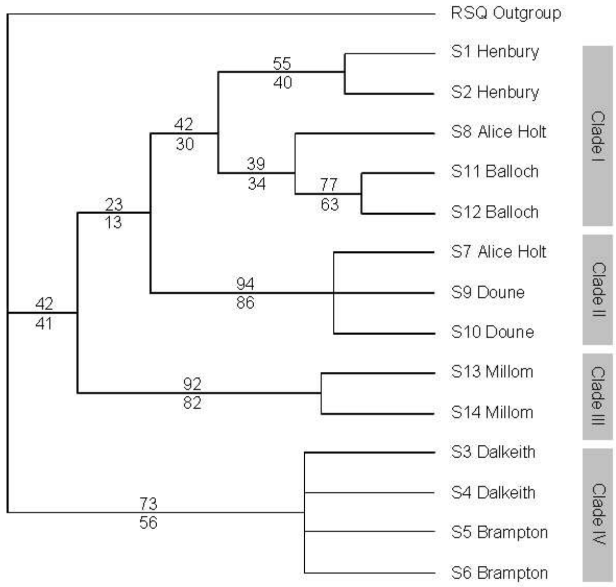

Figure 2. Phylogenetic tree showing Bootstrap and Jacknife analysis results above and below each group separation.
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