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

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Manuscript ID:	Draft
Manuscript Type:	Short Communication
Keywords:	least cost, mtDNA, grey squirrel, Sciurus, dispersal

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Manuscripts

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3 1 Assessing grey squirrel dispersal patterns within the landscape using sequence variation.  
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8 3 ABSTRACT  
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10 4 The grey squirrel *Sciurus carolinensis* is thought to have contributed to the decline of  
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12 5 red squirrel *S. vulgaris* populations in the UK through resource competition and disease  
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14 6 spread. This study used mtDNA sequencing to assess patterns of grey squirrel dispersal in the  
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16 7 UK. Patterns of genetic variation within the dloop sequence were characterised for seven grey  
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18 8 squirrel populations. Infiltration directions and potential barriers to dispersal are identified  
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20 9 and discussed, with a focus on Cumbria, a county at the forefront of grey squirrel expansion.  
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22 10 Understanding the dynamics of grey squirrel dispersal will aid their management at a  
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24 11 landscape scale and enhance the conservation of red squirrels.  
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34 13 INTRODUCTION  
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36 14 The Eastern grey squirrel *Sciurus carolinensis* was first introduced to Britain between  
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38 15 1876 and 1929 (Middleton 1931; Shorten 1954; [Lloyd 1983](#)). Subsequent successful  
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40 16 introductions and translocations occurred within the UK and by the 1930's populations were  
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42 17 established in southeast England and rapidly spreading through the country (Shorten 1954).  
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44 18 This expansion occurred simultaneously with the decline in native red squirrel *Sciurus*  
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46 19 *vulgaris* populations ([Lloyd 1962](#); [Lloyd 1983](#)). Studies suggest that interspecific resource  
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48 20 competition occurs ([Wauters et al. 2000](#); [Gurnell et al. 2004](#)) and that this, along with the  
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50 21 effects of a particularly virulent squirrelpox virus (SQPV), carried by the grey squirrel, has  
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52 22 caused the decline and extirpation of many red squirrel populations ([Tompkins et al. 2003](#);  
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54 23 [Gurnell et al. 2004](#); [Carroll et al. 2009](#)).

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57 24 Cumbria in North West England has been found to hold genetically unique  
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59 25 populations of red squirrel which contain high levels of genetic diversity ([Hale et al. 2004](#)).

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3 26 Concerns have been raised over the impact of expanding grey squirrel populations are having  
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5 27 on red squirrel populations. Interspecific competition and SQPV transmission are thought to  
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8 28 be highest at times of grey squirrel dispersal (Sainsbury et al. 2008). An understanding of the  
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10 29 dispersal ecology and directional movements of the grey squirrel will aid grey squirrel  
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12 30 management decisions and red squirrel conservation.

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15 31 Recent work (Stevenson et al., in review) has indicated that the Cumbrian Mountain  
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17 32 range is acting as a barrier to dispersal. Grey squirrel populations within Cumbria have been  
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19 33 derived from the infiltration of individuals from two directions; to south Cumbria from  
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22 34 Lancashire and to north Cumbria from Northumberland/ Scottish Borders. Hale et al. (2001)  
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24 35 and Trizio et al. (2005) both suggest genetic analysis has enabled the identification of land  
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26 36 cover types which either facilitate dispersal or provide barriers to red squirrels over large  
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29 37 geographic scales. In this paper we report on variation within Cytochrome b (Cytb) and Dloop  
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31 38 DNA sequences found within grey squirrel mtDNA, the first such report to date.

#### 32 33 34 39 35 36 40 METHODOLOGY

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38 41 Two grey squirrel accessions were collected from each of four known introduction  
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40 42 points (see Middleton, 1931); Balloch near Loch Long; Dalkeith in Edinburgh; Henbury in  
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42 43 Cheshire, and from Alice Holt Forest in Surrey. In addition, samples were collected from  
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44 44 three established/ emerging populations within the UK; Doune, in Stirlingshire; Millom, in  
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46 45 south Cumbria and Brampton, in north Cumbria (Figure 1).

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48 46 DNA was extracted from the leg muscle tissue of 14 individuals using the QIAGEN  
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50 47 DNeasy blood and tissue extraction kit and following the manufactures instructions  
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52 48 (QIAGEN Ltd). Fragments of the two mtDNA sequence encoding Cytb and Dloop were  
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54 49 amplified by polymerase chain reaction (PCR). Primers for *Sciurus carolinensis* Cytb were  
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56 50 available from Meece et al. (2005), BM1 (5'-CCCCTCAGAATGATATTTGTCCTCA) and

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3 51 BM2 (5'-CCATCCAACATCTCAGCATGATGAAA). Each PCR reaction had a reaction  
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5 52 volume of 25 µl and containing 1 µl 1:10 mtDNA, 12.5 µl AmpliTaq Gold PCR master mix  
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8 53 (Roche, USA), 1 µl 1:10 BM1 primer to distilled water, 1 µl 1:10 BM2, 9.5 µl distilled water.  
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10 54 PCR amplification followed the protocol of Meece et al. (2005): denaturation of 3.5 min at  
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12 55 95°C followed by 36 cycles of 30s at 95°C, annealing 50s at 60 °C, extension 40s at 72°C,  
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14 56 final extension of 5min at 72 °C.

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17 57 Primers for *Sciurus carolinensis* Dloop were taken from the published sequence of the  
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19 58 Dloop (GenBank Accession no. AF111027; Barrett et al. 1999), Dloop forward primer 5'-  
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21 59 GCCACCCCAAGTTAAATGG-3' and Dloop reverse primer 5'-  
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23 60 ATTCGTGCATTAATGCACTATCC-3'. Each PCR reaction for Dloop contained the same  
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25 61 relative quantities of components as outlined above for cytb amplification apart from 5 µl of  
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27 62 each primer was used. PCR amplification for Dloop sequence followed the protocol of Trizio  
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29 63 et al. (2005), denaturation of 1 min at 94°C followed by 40 cycles of 30s at 94°C, annealing  
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31 64 30s at 52 °C, extension 1min at 72°C, final extension of 5min at 72 °C.

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34 65 Electrophoresis on 1% agarose gels were used to check the success of the PCR  
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36 66 reactions for each sample. All PCR products were then cleaned following the methodology of  
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38 67 the QIAquick PCR purification kit (QIAGEN Ltd). Sequencing of PCR products was carried  
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40 68 out (DBS Genomics, Durham, UK).

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43 69 Sequences for cytb and Dloop from each individual were aligned manually using  
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45 70 BioEdit\_R v7.0.4.1 Freeware (Ibis Therapeutics, California,USA) and Sequence Analysis  
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47 71 5.2.0 (Applied Biosystems) using the default settings within the programmes. A sequence  
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49 72 from *S. vulgaris* (GenBank Accession no. AJ238588) was also included as an outgroup  
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51 73 accession in the analysis. The sequences were entered into Paup 4.0 Beta 10 (Swofford,  
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53 74 Illinois Natural History Survey, Illinois, USA) for phylogeny reconstruction and a 50%

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3 75 majority rule consensus tree was created with 1000 bootstrap and jackknife replicates (Harrison  
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5 76 and Langdale 2006).  
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## 10 78 RESULTS

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12 79 MtDNA was successfully extracted from 14 grey squirrel individuals from selected  
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14 80 locations around the UK. Cytb sequence data was analysed for all 14 accessions with no  
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16 81 sequence variation observed. The 325bp sequence generated, demonstrated a 99% similarity  
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18 82 match for grey squirrel cytochrome b gene in the Genbank BLAST search tool (Accession no.  
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20 83 AY509680). Similarly, the 329bp Dloop sequence generated showed a 98% similarity match  
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22 84 to that of grey squirrel (Accession no. AF111027). Dloop sequences were aligned in BioEdit  
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24 85 and discrete points of variation were detected at 16 unique sites, representing 4.9 % of the  
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26 86 overall sequence.  
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31 87 Parsimony analysis generated a 50% majority rule consensus tree from the grey  
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33 88 squirrel dloop sequence data which was rooted against red squirrel (GenBank Accession no.  
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35 89 AJ238588). The consensus tree (Figure 2) indicated that the grey squirrel samples are  
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37 90 separated into four distinct clades; Clade I; Henbury, Balloch and accession S8 from Alice  
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39 91 Holt; Clade II; Doune and accession S7 from Alice Holt; Clade III; Dalkeith and Brampton  
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41 92 (north Cumbria); Clade IV; Millom (south Cumbria). The branching of samples from  
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43 93 Dalkeith and Brampton is strongly supported with bootstrap values of 73% and jackknife  
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45 94 values of 56%.  
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## 52 96 DISCUSSION

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55 97 This study examines DNA sequence variation and its utility in assessing patterns of  
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57 98 grey squirrel dispersal in the UK. Cytb sequences generated in this study demonstrated no  
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59 99 detectable differences across the range of populations sampled. This may be due to the low  
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3 100 mutation rate within the cytb coding sequence and the relatively short time frame of  
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5 101 introduction and dispersal within the UK. Conversely, sequence variation was detected  
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8 102 within the dloop sequence for these same accessions. Dloop sequence analysis showed  
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10 103 significant statistical support for the distinct separation of north and south Cumbria grey  
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12 104 squirrel populations.

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15 105       Accessions from north Cumbria grouped with accessions from Dalkeith in Edinburgh.  
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17 106 Individual accessions within north Cumbria may not necessarily have been derived from  
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19 107 individuals dispersing south from Edinburgh. However, as they are both within the same  
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21 108 clade they may have been derived from the same progenitor individuals from another location  
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23 109 such as northeast England (Stevenson et al., in review). Similar patterns of dispersal have  
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25 110 been seen in red squirrels, mediated by the afforestation of woodland between Cumbria and  
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27 111 the Northeast (Hale & Lurz 2003).

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31 112       The two samples taken from each population were generally grouped together within  
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33 113 the phylogenetic tree, however, samples from Alice Holt Forest did not follow this pattern and  
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35 114 were grouped separately with samples from elsewhere. David-Gray et al. (1998) study found  
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37 115 high levels of genetic diversity within grey squirrel populations from Alice Holt Forest and  
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39 116 attributed this to numerous introduction sites and translocations from different source  
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41 117 populations, which could explain the results demonstrated in Figure 2 in this study.

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45 118       The initial findings presented here demonstrate support for the hypotheses that the  
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47 119 Cumbrian Mountain range is acting as a barrier to dispersal and that invasion into the area is  
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49 120 coming from multiple directions. If grey squirrels colonised Cumbria with a northerly  
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51 121 advance as suggested (Lowe 1993; Skelcher 1997) accessions from north and south Cumbria  
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53 122 should be grouped together within a phylogenetic reconstruction. However, this is not the  
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55 123 case, and the two populations separate out in the tree. This points towards separate  
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57 124 populations and the effectiveness of the Cumbrian Mountain range as a barrier. Whilst it is

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3 125 acknowledged that a small number of individuals may try to disperse over this mountain  
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5 126 barrier, the majority of individuals will choose a lower cost dispersal route. Further validation  
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7 127 of prospective incursion directions will require an increase in the sample size for each of the  
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9 128 seven locations and additional accessions from within Cumbria and adjoining areas.

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12 129 This study has provided the first evidence of Dloop sequence variation within UK  
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14 130 grey squirrel populations. Despite the small sample size, it supports the suggestion that the  
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16 131 Cumbrian Mountains provide a barrier to grey squirrel dispersal and infers both northerly and  
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18 132 southerly grey squirrel incursion routes into the county. Ultimately, further knowledge of  
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20 133 dispersal and colonisation of grey squirrels will inform conservation policy and can be used to  
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22 134 target resources for grey squirrel control and enable better protection for vulnerable red  
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24 135 squirrel populations. We have shown here, that landscape genetics can provide evidence of  
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26 136 population origin and genetic differences. Although this study has focused on grey squirrels in  
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28 137 Cumbria, the techniques are equally applicable to other landscapes, validating and  
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30 138 highlighting dispersal routes of invasive species and species of conservation concern.

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#### 37 38 140 REFERENCES

- 39  
40  
41 141  
42 142 [Carroll, B., P. Russell, J. Gurnell, P. Nettleton, and A. W. Sainsbury. 2009. Epidemics of](#)  
43  
44 143 [squirrelpox virus disease in red squirrels \(\*Sciurus vulgaris\*\): Temporal and serological](#)  
45  
46 144 [findings. \*Epidemiology and Infection\* \*\*137\*\*:257-265.](#)
- 47  
48  
49 145 David-Gray, Z. K., J. Gurnell, and D. M. Hunt. 1998. DNA fingerprinting reveals high levels  
50  
51 146 of genetic diversity within British populations of the introduced non-native grey squirrel  
52  
53 147 (*Sciurus carolinensis*). *Journal of zoology* **246**(4):443-445.
- 54  
55  
56 148 Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap.  
57  
58 149 *Evolution* **39**(4):783-791.
- 59  
60



- 1  
2  
3 150 Gurnell, J., L. A. Wauters, P. W. W. Lurz, and G. Tosi. 2004. Alien species and interspecific  
4  
5 151 competition: Effects of introduced eastern grey squirrels on red squirrel population dynamics.  
6  
7 152 *Journal of Animal Ecology* **73**(1):26-35.  
8  
9  
10 153 Hale, M. L., P. W. W. Lurz, and K. Wolff. 2004. Patterns of genetic diversity in the red  
11  
12 154 squirrel (*Sciurus vulgaris* L.): Footprints of biogeographic history and artificial introductions.  
13  
14 155 *Conservation Genetics* **5**:167-179.  
15  
16  
17 156 Hale, M. L., and P. W. W. Lurz. 2003. Morphological changes in a British mammal as a result  
18  
19 157 of introductions and changes in landscape management: The red squirrel (*Sciurus vulgaris*).  
20  
21 158 *Journal of zoology* **260**(2):159-167.  
22  
23  
24 159 Hale, M. L., P. W. W. Lurz, M. D. F. Shirley, S. Rushton, R. Fuller, and K. Wolff. 2001.  
25  
26 160 Impact of landscape management on the genetic structure of red squirrel populations.  
27  
28  
29 161 Harrison, C. J., and J. A. Langdale. 2006. A step by step guide to phylogeny reconstruction.  
30  
31 162 *The Plant Journal* **45**:561-572.  
32  
33  
34 163 Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for  
35  
36 164 assessing confidence in phylogenetic analysis. *Systematic Biology* **42**(2):182.  
37  
38  
39 165 Lloyd, H. G. 1962. The distribution of squirrels in England and Wales, 1959. *Journal of*  
40  
41 166 *Animal Ecology* **31**:157-166.  
42  
43  
44 167 Lloyd, H. G. 1983. Past and present distribution of red and grey squirrels. *Mammal Review*  
45  
46 168 **13**(2-4):69-80.  
47  
48  
49 169 Lowe, V. P. W. 1993. The spread of the grey squirrel (*Sciurus carolinensis*) into Cumbria  
50  
51 170 since 1960 and its present distribution. *Journal of Zoology* **231**:663-667.  
52  
53 171 Meece, J. K., C. E. Reynolds, P. J. Stockwell, T. A. Jenson, J. E. Christensen, and K. D. Reed.  
54  
55 172 2005. Identification of mosquito bloodmeal source by terminal restriction fragment length  
56  
57 173 polymorphism profile analysis of the cytochrome B gene. *Journal of Medical Entomology*  
58  
59 174 **42**(4):657-667.  
60

- 1  
2  
3 175 Middleton, A. D. 1931. The grey squirrel. The introduction and spread of the American grey  
4  
5 176 squirrel in the British Isles, its habits, food, and relations with the native fauna of the country.  
6  
7  
8 177 Sidgwick and Jackson, London.  
9  
10 178 Sainsbury, A. W., R. Deaville, B. Lawson, W. A. Cooley, S. S. J. Farelly, M. J. Stack, P.  
11  
12 179 Duff, C. J. McInnes, J. Gurnell, P. H. Russell, S. P. Rushton, D. U. Pfeiffer, P. Nettleton, and  
13  
14 180 P. W. W. Lurz. 2008. Poxviral disease in red squirrels *Sciurus vulgaris* in the UK: Spatial and  
15  
16 181 temporal trends of an emerging threat. *EcoHealth* 5(3):305-316.  
17  
18 182 Shorten, M. 1954. Squirrels. Collins, London.  
19  
20 183 Skelcher, G. 1997. The ecological replacement of red by grey squirrels. Pages 67-78 in J.  
21  
22 184 Gurnell and P. Lurz, editors. The conservation of red squirrels, *Sciurus vulgaris* L. People's  
23  
24 185 Trust for Endangered Species, London.  
25  
26 186 Stevenson, C. D., Watts, K., Nevin, O.T., Ramsey, A. D. and S. Bailey. In review. Validation  
27  
28 187 and creation of a 'best fit' resistance set within a spatially explicit behaviourally-informed  
29  
30 188 landscape model using historical observations of species invasion and information theory.  
31  
32 189 Tompkins, D. M., A. R. White, and M. Boots. 2003. Ecological replacement of native red  
33  
34 190 squirrels by invasive greys driven by disease. *Ecology Letters* 6(3):189-196.  
35  
36 191 Trizio, I., B. Crestanello, P. Galbusera, L. A. Wauters, G. Tosi, E. Matthysen, N., and H. C.  
37  
38 192 Hauffe. 2005. Geographical distance and physical barriers shape the genetic structure of  
39  
40 193 Eurasian red squirrels (*Sciurus vulgaris*) in the Italian Alps. *Molecular ecology* 14(2):469-  
41  
42 194 481.  
43  
44 195 Wauters, L. A., P. W. W. Lurz, and J. Gurnell. 2000. Interspecific effects of grey squirrels  
45  
46 196 (*Sciurus carolinensis*) on the space use and population demography of red squirrels (*Sciurus*  
47  
48 197 *vulgaris*) in conifer plantations. *Ecological Research* 15(3):271-284.  
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Figure 1. Geographical location of grey squirrel sample sites within the UK.

186x273mm (300 x 300 DPI)

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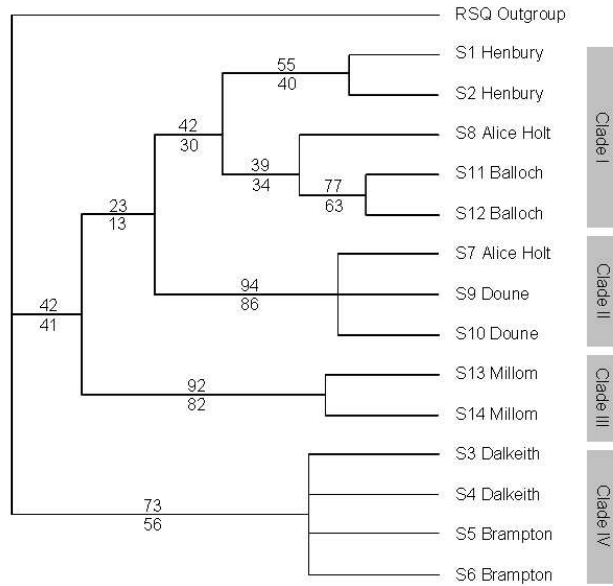


Figure 2. Phylogenetic tree showing Bootstrap and Jackknife analysis results above and below each group separation.

254x190mm (96 x 96 DPI)