Morphological and functional variation between isolated populations of British red squirrels (Sciurus vulgaris)

P. G. Cox1,2, P. J. R. Morris3, J. J. Hennekam2 & A. C. Kitchener4,5

1Department of Archaeology, University of York, York, UK
2Hull York Medical School, University of York, York, UK
3School of Geosciences and Department of Integrative Biology, University of South Florida, Tampa, FL, USA
4Department of Natural Sciences, National Museums Scotland, Edinburgh, UK
5Institute of Geography, School of Geosciences, University of Edinburgh, Edinburgh, UK

Keywords
skull morphology; masticatory biomechanics; mechanical advantage; geometric morphometrics; Sciuridae; Sciurus vulgaris; isolated populations.

Abstract
Isolation due to habitat fragmentation can lead to morphological and functional variation between populations, with the effect being well documented in rodents. Here, we investigated whether such morphological variation could be identified between British populations of the Eurasian red squirrel (Sciurus vulgaris). This species was once widespread across Great Britain, but suffered a severe population decline across the 20th century, leaving a highly fragmented distribution. The aim was to test for morphological and biomechanical variation of the mandible between the remaining British red squirrel populations, and between British and continental European red squirrels. Linear and geometric morphometric methods were used to analyse shape in a sample of over 250 red squirrel hemi-mandibles from across Britain plus a sample from Germany representing the central European subspecies. Procrustes ANOVA identified significant shape variation between populations, with particularly distinct differences being noted between red squirrels from Germany and several British red squirrel populations, which may reflect their evolutionary history. Linear biomechanical measurements showed that the red squirrels from Formby and Jersey had a significantly lower mechanical advantage of the temporalis muscle than other British populations, suggesting they were less efficient at gnawing. This functional difference may be related to many factors, such as founder effect, potential inbreeding and/or past supplemental feeding with less mechanically resistant food items.

Introduction
It is well established that fragmentation of a population and the resulting isolation of the fragments can readily lead to morphological variation (e.g. Snorrason, et al., 1994; Losos, Warheit & Schoener, 1997; Grant, 1999; Sumner, Moritz & Shine, 1999; Renaud & Millien, 2001). Such phenotypic change in isolated populations has been shown by many studies to be very rapid, with morphological variation following environmental change or introduction to a new habitat being detectable in just a few generations (Losos et al., 1997; Hale & Lurz, 2003; Kristjansson, 2005; Renaud et al., 2013). Island populations are known to undergo especially fast morphological change (Lister, 1989; Millien, 2006, 2011; Evans et al., 2012), with the effect being well known in murid rodents (e.g. Pergams & Ashley, 1999, 2001; Yom-Tov, Yom-Tov & Moller, 1999: Renaud et al., 2013, 2015; Pergams et al., 2015). This phenomenon is also seen in mainland species subjected to habitat fragmentation (Schmidt & Jensen, 2003; Pergams & Lawler, 2009; Stumpp, Fuzessy & Paglia, 2018). The most frequently reported phenotypic changes are those of body size or mass (e.g. Schmidt & Jensen, 2003; Yom-Tov, Yom-Tov & Jarrell, 2008; Gardner et al., 2011). However, several studies have demonstrated that skeletal shape, i.e. the relative proportions and orientations of bony structures, can also undergo change in a short period of time (e.g. Pergams & Lacy, 2008; Nagorsen & Cardini, 2009; Franssen, 2011; Yazdi & Adriaens, 2011; Doudna & Danielson, 2015; Renaud et al., 2015). Such morphological changes resulting from evolution on islands or in habitat fragments may also have functional consequences. For example, changes to cranio-mandibular morphology can impact feeding biomechanics via changes to masticatory muscle lever arms and hence, potentially, bite force. This has been noted between isolated populations of the same species in finches (Herrel et al., 2005), lizards (Herrel et al., 2008), and shrews (Cornette et al., 2012).

An ideal case study for studying the impact of population fragmentation and isolation is the British population of
Rayfield, 2014). The first hypothesis will be tested using geometric morphometric methods (GMM). It is also hypothesized that morphological variation in mandibular shape between squirrel populations will directly reflect differences in feeding biomechanics, as variation in the position of masticatory muscle insertions relative to their origins on the skull will impact the ability of those muscles to produce bite force. This second hypothesis will be tested by estimating the mechanical advantage (MA) of three of the muscles of mastication (following Casanovas-Vilar & van Dam, 2013). MA is a measure of the efficiency with which muscle force can be converted to output force at the teeth, and is an important component of overall bite force.

Materials and methods

Sample

The sample comprised 258 red squirrel skeletons, with associated location data, from National Museums Scotland (Edinburgh, UK), most of which were collected between 1994 and 2006. The sample included individuals from most areas of Great Britain and its offshore islands where red squirrels have
been present over the last three decades, plus a number of specimens from the island of Jersey and from Germany, representing continental European populations. The specimens chosen for analysis were grouped into the following geographical regions (Fig. 1): North Scotland (n = 83); South Scotland (18); North England (48); Formby (29); Thetford (11); Isle of Wight (9); Jersey (22); and Germany (38). For the purposes of this study, ‘North Scotland’ and ‘South Scotland’ refer to areas of Scotland north and south of the Central Belt, respectively, and ‘North England’ includes the counties of Northumberland and Cumbria. From each specimen, one hemi-mandible was selected for analysis. Where both hemi-mandibles were present and undamaged, the right was used in preference to the left.

**Morphometrics**

Hemi-mandibles were laid flat with the external lateral surface facing upwards on paper marked with 0.5 mm squares (for scaling purposes). The specimens were photographed with a Panasonic Lumix DMC-TZ60 camera, secured on a tripod at a constant distance from the bench. A set of 12 two-dimensional landmarks, based on previous studies of rodent mandibles (e.g. Zelditch et al., 2008; 2015; Casanovas-Vilar & van Dam, 2013), was recorded from each photograph using the tpsDig2 software (Rohlf, 2018). The landmark set is illustrated and described in Fig. 2a, and the raw landmark co-ordinates for each specimen are given in Data File S1. Landmark co-ordinates from all 258 specimens were aligned via generalized Procrustes superimposition and then subjected to a principal components analysis (PCA). Size differences between populations were analysed with a permutational ANOVA with 1000 repeats. The effects of population (factor) and mandible size (log centroid size, covariate) on mandibular shape were assessed using Procrustes ANOVA. The statistical significance of differences between populations was assessed with a pairwise permutation test of 1000 repeats. All GMM analyses were carried out with the geosphere v3.0.6 package (Adams & Otárola-Castillo, 2013; Adams et al., 2018) in the R statistical environment (R Core Team, 2017).

**Biomechanics**

To examine the functional significance of any morphological variation between red squirrel populations, the MA of three of the major masticatory muscles – temporalis, superficial masseter and deep masseter – was estimated from linear measurements of the jaw (following Casanovas-Vilar & van Dam, 2013). MA was calculated as the ratio of the muscle in-lever to the biting out-lever. The muscle in-levers were measured as the linear distance from the condyle to the extreme points of the muscular insertions. The most dorsally inserting fibres of the temporalis (T_d) were at the tip of the coronoid process (landmark 4), and the most ventral temporalis fibres (T_v) inserted at the base of the coronoid (landmark 3). The most dorsal and ventral insertion points of the superficial masseter (SM_d, SM_v) were at the posterior tip and ventral-most point of the angular process (landmarks 8 and 9, respectively). Finally, the most anterior insertion of the deep masseter in-lever (DM_a) was taken to be the anterior-most point on the margin of the masseteric fossa (landmark 11). The posterior-most insertion of the deep masseter was coincident with that of the superficial masseter, that is landmark 8. All muscle in-levers were compared to the out-lever representing incisor biting. As many of the specimens had missing, damaged or dislocated incisors, the out-lever was measured from the condyle to landmark 1, the dorsal margin of the incisor alveolus process where it crosses molar alveolar margin; 4, tip of coronoid process; 5, anterior-most point of the deep masseter in-lever (DM_a); 6, posterior-most point of condyle articular surface; 7, anterior-most point on curve between condyle and angular process; 8, posterior tip on masseteric ridge; and 12, ventral-most point on incisor alveolar margin. Levers: DM_a, anterior deep masseter in-lever; l, incisor out-lever; SM_d, dorsal superficial masseter in-lever; SM_v, ventral superficial masseter in-lever; T_d, dorsal temporalis in-lever; and T_v, ventral temporalis in-lever.

A number of caveats to the biomechanical analysis should be noted to ensure that the results here are interpreted with appropriate levels of caution. Firstly, MA is correctly calculated using moment arms (not lever arms, as here), that is the perpendicular distance between the condyle and the vector.
running between the muscle origin and insertion. This was not possible as it would have required articulation of the skull and mandible in the photographs, and intact skulls were not available for many specimens, because they were road casualties. Lever arms have been used to provide a reasonable first approximation of MA in a number of previous studies (e.g. Casanovas-Vilar & van Dam, 2013; Renaud et al., 2015; Gomes Rodrigues et al., 2016; Jones & Law, 2018; West & King, 2018), but it should be noted that variations in mandibular morphology can rotate lever arms thus changing moment arms without altering the length of the lever arms. Secondly, the representation of a muscle insertion as a single point is a clear over-simplification as the temporalis, superficial masseter and deep masseter all have large attachment sites on the squirrel mandible (Cox & Jeffery, 2011, 2015). However, this was mitigated to some degree by taking the extreme points of the muscle insertions. Lastly, the analysis was conducted in 2D, which ignores any lateral component to the mandible. This was felt to be a justified approximation to the 3D morphology because squirrel hemi-mandibles are sciuromorphous and therefore largely planar (Hautier, Cox & Lebrun, 2015). Despite these simplifications, it was felt that the results generated were still meaningful, as the simplifications were consistent across all specimens.

The impact of population on masticatory muscle MA was assessed using permutational ANOVA with log centroid size of the mandible as the covariate. Post hoc pairwise permutation tests of 1000 repeats were used to assess the significance of differences in MA between pairs of populations. A false discovery rate controlling procedure was used to adjust the P values to account for multiple testing (Benjamini & Hochberg, 1995). All analyses were undertaken in R using the packages vegan v. 2.5-6 (Oksanen et al., 2019) and RVAideMemoire v. 0.9-77 (Hervé, 2020).

**Results**

**Size analysis**

Log centroid sizes of red squirrel mandibles are displayed in Fig. 3, and means are given in Table S1. A permutational ANOVA test revealed significant size differences between red squirrel populations \((F = 15.8, \ P < 0.001)\). Post hoc permutation tests found that the Formby, Germany and Jersey populations were significantly larger than most other populations, but that there was no difference between Formby and Jersey (Table S2).

**Shape analysis**

The distribution of individuals across the first two principal components, representing 24.7% and 17.8% of total shape variance respectively, is shown in Fig. 4a alongside the shape changes along those axes. Considerable overlap of all red squirrel populations is seen on this plot, with most populations being distributed widely across both axes. Only the squirrels from Germany diverge from this pattern, being found mostly on the negative half of the first component. Shape change along PC1 is characterized by hemi-mandibles with an elongate body, a wide angular process, a caudally positioned masseteric ridge and a posteriorly positioned coronoid at the negative end (i.e. the specimens from Germany), to hemi-mandibles with a foreshortened body, a more slender angle, a masseteric ridge that reaches more rostrally and an anteriorly located coronoid process at the positive extreme. PC2 represents a change from a short, deep mandibular body with an anteriorly situated masseteric ridge, a tall coronoid process and a broad angular process (negative PC2 scores), to a more elongate but shallower mandible, with a posteriorly located masseteric ridge, a less superiorly extended coronoid and a narrower angle that extends less posteriorly (positive PC2 scores).

Greater distinction between some of the squirrel populations is visible on the third principal component (11.5% of total variance). Despite considerable overlap, the populations from North Scotland, South Scotland, North England, Jersey and Formby are broadly positioned in that order along PC3 from negative to positive (Fig. 4b). Hemi-mandibles at the negative end of PC3 (i.e. specimens from North Scotland) have an anteriorly positioned coronoid process and a posteriorly extended angular process, whereas hemi-mandibles at the positive end of PC3 (i.e. specimens from Formby) have a posteriorly positioned coronoid and a much smaller angular process that does not extend posteriorly.

A Procrustes ANOVA revealed significant differences \((P = 0.001)\) in mandible shape between populations, with such differences representing about 15% of total shape variation (Table 1). Logged centroid size did not have a significant effect on shape, and nor did the interaction between size and population. Post hoc pairwise permutation tests (Table 2) revealed that the dataset is divided into two groups, one comprising the populations from Germany, Isle of Wight and Thetford, and the other formed by Formby, Jersey, North England, and both Scottish populations.

**Biomechanical analysis**

The distributions of the MA of the jaw-closing muscles within each of the red squirrel population are shown in the box plots in Fig. 5, and means and standard deviations for each population are given in Table S3. Differences between the populations are evident for the dorsally and ventrally inserting fibres of the temporalis, as well as for the deep masseter, but not for either part of the superficial masseter. This result was confirmed by the permutational ANOVA tests \((T4: F = 25.7, P = 0.0001; Tv: F = 15.8, P = 0.0001; DM: F = 25.7, P = 0.0006)\).

The largest difference in MA is seen between the Formby red squirrels and all other populations for the dorsally inserting temporalis fibres (Fig. 5a). This was shown to be highly significant by the pairwise permutation tests. These post hoc tests also noted a significantly lower MA in Jersey squirrels and a significantly higher MA in the North England population, but to a much lesser extent (Table S4). The MA of the ventrally inserting fibres of the temporalis seems to split the sample into...
two groups: Scotland and North England with a higher MA, and the remaining populations with a lower MA (Fig. 5b). Again, this is supported by the post hoc tests (Table S5). Finally, the deep masseter MA is slightly lower in the German red squirrels compared to most other populations (Fig. 5e). Significant differences between Germany and all other populations except Jersey and Thetford were found in the pairwise permutation tests (Table S6).

Discussion

The results of this study show significant differences in mandibular morphology and biomechanical estimates between British red squirrel populations, and between British and continental European red squirrels. The first hypothesis of this study, that there are differences in mandibular morphology between populations of red squirrels, is supported by the Procrustes ANOVA. This analysis indicates that inter-population differences have a significant, although low (15%), contribution to mandibular shape. Despite significant size differences between some populations (Fig. 3; Table S2), size did not appear to have a significant effect on mandibular shape (Table 1). The hypothesis is further supported by the PCA, which clearly separates German red squirrels from other populations on PC1, and partly separates a number of the other populations on PC3 (Fig. 4). The second hypothesis, which predicted significant differences in biomechanical performance between red squirrel populations, was also supported by the results here (Fig. 5). Clear differences between red squirrel populations were seen in the MA of the masticatory muscles (particularly the temporalis, but also the deep masseter), indicating that the identified morphological differences have potential functional consequences.

The clearest distinction is between the red squirrel population from Germany and most other populations in this study, both in shape and in size. This result was largely expected as the German population was included in the analysis as an out-group, supposedly a different subspecies (S. v. fuscoater) from the British red squirrels (S. v. leucourus). The German red squirrels have a more elongate mandible, with a more posteriorly positioned masseteric ridge and coronoid process. These characteristics have shortened the in-levers of the deep masseter and temporalis muscles, respectively, leading to a lower MA for both these muscles. The morphological variation is likely a reflection of the separate evolutionary history of the German squirrels from the British populations, but whether the differences are selectively driven, owing to differences in habitat and diet, or simply the result of genetic drift cannot be ascertained from the data here. Functionally, the reduced MA of the temporalis and masseter will tend to reduce the proportion of muscle force that is converted to bite force. However, as the German red squirrel mandibles are larger than those of other populations (Fig. 3), the corresponding increase in size of the masticatory muscles may offset the reduction in MA, leading to similar overall bite forces between German and British red squirrels.

The Isle of Wight and Thetford populations were also shown to be significantly different from all other British red squirrel populations by the Procrustes ANOVA. However, this is not obviously reflected in the PCA, where the Thetford
specimens are spread widely across the first component and the Isle of Wight squirrels are found in the centre of both PC1 and PC2. The significant difference in the morphology of the Isle of Wight squirrels may reflect a long-standing isolation of this population from other British red squirrels, but may also be an artefactual consequence of the low sample size of this population ($n = 9$). The difference in morphology of the now-extinct Thetford red squirrel population may also be an effect of low sampling ($n = 11$), but could also potentially be related to inbreeding in a small declining population at the time the samples were collected (no more than 40 living individuals in 1995; Gurnell, Sainsbury & Venning, 1997). This could have negatively affected the biomechanical capabilities of the mandible, as increased developmental instability and fluctuating asymmetry in the craniodental region have been suggested by some researchers to be associated with inbreeding and homozygosity (Leamy, Routman & Cheverud, 2002; Schaefer et al., 2006). In contrast, others have found no evidence for

Figure 4 Principal components analysis of red squirrel mandibular shape. (a) PC1 versus PC2; (b) PC1 versus PC3. Convex polygons drawn around individuals from each population. Wireframes illustrate shape changes along each principal component from −0.05 to 0.05. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com].

Morpho-functional variation in British red squirrels P. G. Cox et al.
Table 1 Procrustes ANOVA of mandibular shape between red squirrel populations with a covariate of log centroid size [Colour table can be viewed at zslpublications.onlinelibrary.wiley.com].

|                     | df | SS  | MS  | Rsq | F    | Z    | P   |
|---------------------|----|-----|-----|-----|------|------|-----|
| Log CS              | 1  | 0.009 | 0.009 | 0.001 | 0.403 | −0.333 | 0.614 |
| Population          | 7  | 0.979 | 0.140 | 0.147 | 6.017 | 3.843 | 0.001 |
| Size:Pop            | 7  | 0.051 | 0.007 | 0.008 | 0.311 | −1.446 | 0.921 |
| Residuals           | 242 | 5.625 | 0.023 | 0.844 |       |       |     |
| Total               | 257 | 6.664 |       |       |       |       |     |

Significant P values highlighted.

Table 2 Pairwise comparisons between squirrel populations: Z scores (below diagonal) and P values from permutation tests (1000 permutation rounds) (above diagonal) [Colour table can be viewed at zslpublications.onlinelibrary.wiley.com].

|                | North Scotland | South Scotland | North England | Formby | Thetford | Isle of Wight | Jersey | Germany |
|----------------|----------------|----------------|---------------|--------|----------|---------------|--------|---------|
| North Scotland | 0.99           | 0.957          | 0.956         | 0.002  | 0.04     | 0.989         | 0.001  |         |
| South Scotland | −1.351         | 0.994          | 0.963         | 0.002  | 0.072    | 0.998         | 0.005  |         |
| North England  | −1.263         | −1.304         | 0.981         | 0.002  | 0.038    | 0.987         | 0.001  |         |
| Formby         | −1.252         | −1.204         | −1.388        | 0.002  | 0.042    | 0.97          | 0.001  |         |
| Thetford       | 6.752          | 4.761          | 6.391         | 5.578  | 0.074    | 0.002         | 0.104  |         |
| Isle of Wight  | 2.27           | 1.468          | 2.086         | 1.894  | 1.612    | 0.047         | 0.138  |         |
| Jersey         | −1.358         | −1.214         | −1.323        | −1.265 | 5.243    | 1.68          | 0.001  |         |
| Germany        | 7.334          | 4.26           | 6.464         | 5.734  | 1.128    | 0.368         | 5.062  |         |

Significant comparisons highlighted.
muscles. Furthermore, permutational ANOVA tests showed population to have a significant effect on Td MA even after controlling for log centroid size.

Lastly, differences in diet could have a role in driving morpho-functional variation between red squirrel populations. In general, red squirrels have a diet largely composed of mechanically resistant items such as nuts and seeds. The diet of Scottish red squirrels has been shown to be very heavily dominated by Scots pine seeds (*Pinus sylvestris*), which have to be accessed from within the cone. Tittensor (1970) found pine seeds in 100% stomachs of the 52 Scottish red squirrels analysed, and Moller (1983) noted that 97% feeding red squirrels sighted in East Scotland were eating pine seeds. In Cumbria, in the North of England, red squirrels were recorded as feeding principally on hazel nuts (*Corylus avellana*) and yew seeds (*Taxus baccata*) (Tonkin, 1983). In contrast, although pine seeds are important in the diet of the Formby squirrels, until recently they also made use of a wide variety of supplemental foods including fruit, human snacks (biscuits, crisps, chips, ice cream) and, importantly, peanuts (Shuttleworth, 1996), which have in the past represented between 25% and 57% of the red squirrel diet at Formby (Shuttleworth, 1997, 2000). For several decades, the squirrel population at Formby was provided with peanuts year-round by the National Trust and by the public (Gurnell & Pepper, 1993; Rice-Oxley, 1993; Shuttleworth, 2000), although this practice is now much reduced and actively discouraged by the National Trust (A. Brockbank, pers. comm.). Peanut shells are much less mechanically resistant than pine seeds or hazel nuts. Thus, it is possible that the morphology of the mandible, and hence the efficiency of gnawing, has changed in response to this change in diet, as has been noted in previously in mice (Renaud & Auffray, 2010; Anderson et al., 2014; West & King, 2018). It is proposed that the less mechanically demanding peanut shells may have led to the reduction in temporalis MA, as this muscle is important for gnawing through nut shells, but that the forces required to process shelled peanuts, hazel nuts and pine seeds at the molars are fairly similar, so few differences are seen in the massteter MAs, as those muscles have greater importance in protraction of the mandible (Hiiemae, 1971) and molar chewing (Weijs & Dantuma, 1975; Offermans & Devree, 1993) respectively. The more posteriorly extended coronoid process in the Formby squirrels, revealed by GMM, is also consistent with previous work indicating that squirrels with more graniorous diets have less well-developed coronoid processes (Michaux et al., 2007).

**Figure 5** Box plots of masticatory muscle MA in red squirrel populations. (a) Most dorsally inserting fibres of the temporalis; (b) most ventrally inserting fibres of the temporalis; (c) most dorsally inserting fibres of the superficial masseter; (d) most ventrally inserting fibres of the superficial masseter; and (e) most anteriorly inserting fibres of the deep masseter. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.
A similar effect, albeit to a lesser extent, may also have occurred in the Jersey squirrels. Here, red squirrels are known to feed on the seeds of both conifers and deciduous trees, but in certain areas are also provided with supplemental food by local residents (Magris & Gurnell, 2002). Like the Formby squirrels, the Jersey specimens have a posteriorly positioned coronoid process, leading to a lower temporalis MA (although not as low as the Formby squirrels), which may be a result of a less mechanically demanding supplemental diet.

The morpho-functional changes seen in the Formby and Jersey red squirrels could either be an evolutionary response that has occurred over a number of generations (as seen in insular populations, e.g. Herrel et al., 2008; Cornette et al., 2012; Renaud et al., 2015), or a plastic response occurring across the lifetime of each individual exposed to supplemental feeding (as seen in laboratory animals raised on different diets, e.g. He & Kiliaridis, 2003; Enomoto et al., 2010; Anderson et al., 2014). To tease apart, these two possibilities would require a larger, well-dated sample of red squirrel specimens spanning a number of decades.

The interpretation of the results above could have important implications for conservation efforts related to British red squirrels. Although tentative, the conclusion sheds light on what more needs to be learned to enable valid decision-making in conservation strategies. Supplemental feeding of red squirrels is popular amongst members of the public and can provide important buffering against natural variations in food availability (Magris & Gurnell, 2002). However, it may have unsuspected impacts on skeletal morphology if the food provided is less mechanically demanding than that available in the wild. Many conservation strategies in the UK and beyond involve translocation of individuals from well-populated areas or captive-breeding facilities to bolster threatened populations or initiate new ones (e.g. Venning, Sainsbury & Gurnell, 1997; Ogden et al., 2005; Poole & Lawton, 2009). If supplemental feeding is to some degree implicated in changes in mandibular morphology that reduce the efficiency of gnawing, then diet must be taken into consideration in captive breeding and during translocations. The results here suggest red squirrels may not thrive if moved to a habitat with a more mechanically demanding food source, or if supplementary feeding is withdrawn suddenly. This is consistent with previous research demonstrating that translocated red squirrels tend to survive longer in release sites that have a similar habitat to the origin site (Kenward & Hodder, 1998) and that animals released in unfamiliar habitat will tend to disperse away from the release site (Morris, Meakin & Sharafi, 1993; Bright & Morris, 1994).

The conclusion that supplemental feeding has led to morphological change and the consequences this could have for conservation strategy is tantalizing, but at the moment still preliminary, and should be placed in the context of the caveats outlined above, in particular the uncertainty around the provenance and evolutionary history of the present British populations. Future studies are planned that combine genetic data with morphological data from the skull as well as the mandible, a longer time series of squirrels and that take advantage of more sophisticated biomechanical modelling techniques, in order to better understand the drivers of morpho-functional change in fragmentary populations such as British red squirrels.

Acknowledgements

Data collection was supported by the Department of Archaeology Research and Scholarship Fund, University of York. ACK thanks the late Hon. Vincent Weir for supporting the preparation of red squirrel specimens. The authors thank Kat Fingland, Mel Tonkin, Gill Whelan and Andrew Brockbank for helpful conversations about this research. The manuscript was improved by the insightful comments of Helder Gomes Rodrigues and an anonymous reviewer. PGC thanks Graham and Tis Buckley for accommodation during data collection.

Author contributions

PGC conceived the study, photographed the mandibles, undertook analyses and drafted the manuscript. PJRM landmarked the specimens. JJH carried out GMM analyses. ACK provided access to specimens and associated metadata. All authors interpreted results, contributed to the final manuscript and approved its contents.

References

Adams, D.C. & Otárola-Castillo, E. (2013). geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* 4, 393–399.

Adams, D.C., Collyer, M.L., Kaliontzopoulou, A. & Sherratt, E. (2018). geomorph: software for geometric morphometric analyses. *R package version 3. 6*, https://cran.r-project.org/web/packages/geomorph/index.html

Anderson, P.S.L., Renaud, S. & Rayfield, E.J. (2014). Adaptive plasticity in the mouse mandible. *BMC Evol. Biol.* 14, 85.

Ball, S.S. & Roth, V.L. (1995). Jaw muscles of New World squirrels. *J. Morphol.* 224, 265–291.

Barratt, E.M., Gurnell, J., Malarky, G., Deaville, R. & Bruford, M.W. (1999). Genetic structure of fragmented populations of red squirrel (*Sciurus vulgaris*) in the UK. *Mol. Ecol.* 8, S55–S63.

Benjamini, Y. & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* 57, 289–300.

Bright, P.W. & Morris, P.A. (1994). Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. *J. Appl. Ecol.* 31, 699–708.

Cartmel, S. (1997). A study of red and grey squirrels in Clocaenog Forest, North Wales: a preliminary study. In *The conservation of red squirrels, Sciurus vulgaris L.*, 89–95, Gurnell, J. & Luzr, P.W.W. (Eds). London: PTES.

Casanovas-Vilar, I. & van Dam, J. (2013). Conservatism and adaptability during squirrel radiation: what is mandible shape telling us? *PLoS One* 8, e61298.

Chantry, J., Dale, T.D., Read, J.M., White, S., Whitfield, F., Jones, D., McInnes, C.J. & Begon, M. (2014). European red
squirrel population dynamics driven by squirrelex and at a gray squirrel invasion interface. *Ecol. Evol.* **4**, 3788–3799.

Corrêa, R., Herrel, A., Cosson, J.-F., Poitevin, F. & Baylac, M. (2012). Rapid morpho-functional changes among insular populations of the greater white-toothed shrew. *Biol. J. Linn. Soc.* **107**, 322–331.

Cox, P.G. & Baverstock, H. (2016). Masticatory muscle anatomy and feeding efficiency of the American beaver, *Castor canadensis* (Rodentia, Castoridae). *J. Mammal. Evol.* **23**, 191–200.

Cox, P.G. & Jeffery, N. (2015). The muscles of mastication and the function of the medial pterygoid. In *Evolution of the rodents: advances in phylogeny, functional morphology and development* : 350–372. Cox, P.G. & Hautier, L. (Eds). Cambridge: Cambridge University Press.

Cox, P.G., Faulkes, C.G. & Bennett, N.C. (2020). Masticatory musculature of the African mole-rats (Rodentia: Bathyergidae). *PeerJ* **8**, e8847.

Doudna, J.W. & Danielson, B.J. (2015). Rapid morphological change in the masticatory structures of an important ecosystem service provider. *PLoS One* **10**, e0127218.

Druzinsky, R.E. (2010). Functional anatomy of incisal biting in *Aplodontia rufa* and sciurid rodents – Part 1: masticatory muscles, skull shape and digging. *Cells Tissues Organs* **191**, 510–522.

Enomoto, A., Watahiki, J., Yamaguchi, T., Irie, T., Tachikawa, T. & Maki, K. (2010). Effects of mastication on mandibular growth evaluated by microcomputed tomography. *Eur. J. Orthodont.* **32**, 66–70.

Evans, A.R., Jones, D., Boyer, A.G., Brown, J.H., Costa, D.P., Ernest, S.K.M., Fitzgerald, E.M.G., Fortelius, M., Gittleman, J.L., Hamilton, M.J., Harding, L.E., Lintulaakso, K., Lyons, S.K., Okie, J.G., Saarinen, S.R., Sibly, R.M., Smith, F.A., Stephens, P.R., Theodor, J.M. & Uhen, M.D. (2012). The maximum rate of mammal evolution. *Proc. Nat. Acad. Sci. USA* **109**, 4187–4190.

Franssen, N.R. (2011). Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish. *Evol. Appl.* **4**, 791–804.

Gardner, J.L., Peters, A., Kearney, M.R., Joseph, L. & Heinsohn, R. (2011). Declining body size: a third universal response to warming. *Trends Ecol. Evol.* **26**, 285–291.

Ginot, S., Agret, S. & Claude, J. (2018). Bite force performance, fluctuating asymmetry and antisymmetry in the mandible of inbred and outbred wild-derived strains of mice (*Mus musculus domesticus*). *Evol. Biol.* **45**, 287–302.

Gomes, Rodrigues H., Sumbera, R. & Hautier, L. (2016). Life in burrows channelled the morphological evolution of the skull in rodents: the case of African mole-rats (Bathyergidae, Rodentia). *J. Mammal. Evol.* **23**, 175–189.

Gorniak, G.C. (1977). Feeding in golden hamsters, *Mesocricetus auratus*. *J. Morphol.* **154**, 427–458.

Grant, P.R. (1999). *Ecology and evolution of Darwin’s finches*. Princeton, NJ: Princeton University Press.

Gurnell, J. (1987). *The natural history of squirrels*. London: Christopher Helm.

Gurnell, J. & Pepper, H. (1993). A critical look at conserving the British Red Squirrel *Sciurus vulgaris*. *Mammal. Rev.* **23**, 127–137.

Gurnell, J., Sainsbury, A.W. & Venning, T. (1997). Conserving the red squirrel *Sciurus vulgaris* in Thetford Forest. Peterborough: English Nature Research Report.

Gurnell, J., Clark, M.J., Lurz, P.W.W., Shirley, M.D.F. & Rushton, S.P. (2002). Conserving red squirrels (*Sciurus vulgaris*): mapping and forecasting habitat suitability using a Geographic Information Systems Approach. *Biol. Conserv.* **105**, 53–64.

Gurnell, J., Wauters, L.A., Lurz, P.W.W. & Tosi, G. (2004). Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. *J. Animal Ecol.* **73**, 26–35.

Gurnell, J., Lurz, P. & Bertoldi, W. (2014). The changing patterns in the distribution of red and grey squirrels in the North of England and Scotland between 1991 and 2010 based on volunteer surveys. *Hystra* **25**, 83–38.

Hale, M.L. & Lurz, P.W.W. (2003). Morphological changes in a British mammal as a result of introductions and changes in landscape management: the red squirrel (*Sciurus vulgaris*). *J. Zool.* **260**, 159–167.

Hale, M.L., Lurz, P.W.W. & Wolff, K. (2004). Patterns of genetic diversity in the red squirrel (*Sciurus vulgaris* L.): footprints of biogeographic history and artificial introductions. *Conserv. Genet.* **5**, 167–179.

Harris, S. & Yalden, D. (2008). *Mammals of the British Isles*. 4th edn. Southampton: The Mammal Society.

Harvie-Brown, J.A. (1880–1881). The history of the squirrel in Great Britain. *Proc. R. Phys. Soc. Edin.* **5**, 343–348; 6, 31–63; 6,115–183.

Hautier, L., Cox, P.G. & Lebrun, R. (2015). Grades and clades among rodents: the promise of geometric morphometrics. In *Evolution of the rodents: advances in phylogeny, functional morphology and development* : 277–299. Cox, P.G. & Hautier, L. (Eds). Cambridge: Cambridge University Press.

He, T. & Kiliaridis, S. (2003). Effects of masticatory muscle function on craniofacial morphology in growing ferrets (*Mustela putorius furo*). *Eur. J. Oral Sci.* **111**, 510–517.

Herrel, A., Podos, J., Huber, S.K. & Hendry, A.P. (2005). Bite performance and morphology in a population of Darwin’s finches: implications for the evolution of beak shape. *Funct. Ecol.* **19**, 43–48.

Herrel, A., Huyghe, K., Vanhooydonck, B., Backeljau, T., Breugelmans, J., Grbac, I., Van Damme, R. & Irschick, D. (2008). Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *Proc. Nat. Acad. Sci. USA* **105**, 4792–4795.
Hervé, M. (2020). RVAideMemoire: testing and plotting procedures for biostatistics. https://CRAN.R-project.org/package=RVAideMemoire

Hiiemae, K. (1971). The structure and function of the jaw muscles in the rat (Rattus norvegicus L.) III. The mechanics of the muscles. Zool. J. Linn. Soc. 50, 111–132.

Hobbs, A.M. (2005). Assessment of the status of the red squirrel in central Wales. Mid Wales Red Squirrel Project Report.

Jones, K. & Law, C.J. (2018). Differentiation of craniomandibular morphology in two sympatric Peromyscus mice (Cricetidae: Rodentia). Mammal Rev. 63, 277–283.

Kristjansson, B.K. (2005). Rapid morphological changes in African mole-rats (Rodentia: Bathyergidae). Zool. J. Linn. Soc. 117, 447–462.

Kenward, R.E. & Hodder, K.H. (1998). Red squirrels (Sciurus vulgaris) released in conifer woodland: the effects of source habitat, predation and interactions with grey squirrels (Sciurus carolinensis). J. Zool. 244, 23–32.

Kristjansson, B.K. (2005). Rapid morphological changes in threespine stickleback, Gasterosteus aculeatus, in freshwater. Environ. Biol. Fish 74, 357–363.

LaRose, J.P., Meredith, A.L., Everest, D.J., Fiegna, C., McInnes, C.J., Shaw, D.J. & Milne, E.M. (2010). Epidemiological and post-mortem findings in 262 red squirrels (Sciurus vulgaris) in Scotland, 2005 to 2009. Vet. Rec. 167, 297–302.

Leamy, L.J., Routman, E.J. & Cheverud, J.M. (2002). An epistatic genetic basis for fluctuating asymmetry of mandible size in mice. Evolution 56, 642–653.

Lister, A.M. (1989). Rapid dwarving of red deer on Jersey in the Last Interglacial. Nature 342, 539–542.

Lloyd, H.G. (1983). Past and present distribution of red and grey squirrels. Mammal Rev. 13, 69–80.

Losos, J.B., Warheit, K.I. & Schoener, T.W. (1997). Adaptive differentiation following experimental island colonization in Anolis lizards. Nature 387, 70–73.

Low, P.W. & Gardiner, A.S. (1983). Is the British squirrel (Sciurus vulgaris leucourus Kerr) British? Mammal Rev. 13, 57–67.

Magnis, L. & Gurnell, J. (2002). Population ecology of the red squirrel (Sciurus vulgaris) in a fragmented woodland ecosystem on the Island of Jersey, Channel Islands. J. Zool. 256, 99–112.

Markov, T.A. (1995). Evolutionary ecology and developmental instability. Ann. Rev. Entomol. 40, 105–120.

Marr, M.M. & MacLeod, N. (2019). Geographical variation in Eurasian red squirrel (Sciurus vulgaris L.), 1758 mandibles and the issue of subspecies-level organization: a failure of history? Biol. J. Linn. Soc. 128, 337–359.

Mathews, F., Kubasiewicz, L.M., Gurnell, J., Harrower, C.A., McDonald, R.A. & Shore, R.F. (2018). A review of the population and conservation status of British mammals. A report by the Mammal Society under contract to Natural England, Natural Resources Wales and Scottish Natural Heritage. Peterborough: Natural England.

McIntosh, A.F. & Cox, P.G. (2016). Functional implications of craniofacial morphology in African mole-rats (Rodentia: Bathyergidae). Biol. J. Linn. Soc. 117, 447–462.

Michaux, J., Chevret, P. & Renaud, S. (2007). Morphological diversity of Old World rats and mice (Rodentia, Muridae) mandible in relation with phylogeny and adaptation. J. Zool. Syst. Evol. Res. 45, 263–279.

Millien, V. (2006). Morphological evolution is accelerated among island mammals. PLoS Biol. 4, e321.

Millien, V. (2011). Mammals evolve faster on smaller islands. Evolution 65, 1935–1944.

Møller, H. (1983). Foods and foraging behaviour of red (Sciurus vulgaris) and grey (Sciurus carolinensis) squirrels. Mammal Rev. 13, 81–98.

Morris, P.A., Meakin, K. & Sharafi, S. (1993). The behaviour and survival of rehabilitated hedgehogs (Erinaceus europaeus). Animal Welf. 2, 53–66.

Nagorsen, D.W. & Cardini, A. (2009). Tempo and mode of evolutionary divergence in modern and Holocene Vancouver Island marmots (Marmota vancouverensis) (Mammalia, Rodentia). J. Zool. Syst. Evol. Res. 47, 258–267.

Offermans, M. & De Vree, F. (1993). Electromyography and mechanics of mastication in the springhare, Pedetes capensis (Rodentia, Pedetidae). Belg. J. Zool. 123, 231–261.

Ogden, R., Shuttleworth, C., McEwing, R. & Cesarini, S. (2005). Genetic management of the red squirrel, Sciurus vulgaris: a practical approach to regional conservation. Conserv. Genet. 6, 511–525.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2019). vegan: Community ecology package. https://cran.r-project.org/web/packages/vegan/index.html

Pergams, O.R.W. & Ashley, M.V. (1999). Rapid morphological evolution in Channel Island deer mice. Evolution 53, 1573–1581.

Pergams, O.R.W. & Ashley, M.V. (2001). Microevolution in island rodents. Genetica 112–113, 245–256.

Pergams, O.R.W. & Lacy, R.C. (2008). Rapid morphological and genetic change in Chicago-area Peromyscus. Mol. Ecol. 17, 450–463.

Pergams, O.R.W. & Lawler, J.J. (2009). Recent and widespread rapid morphological change in rodents. PLoS One 4, e6452.

Pergams, O.R.W., Byrn, D., Lee, K.L.Y. & Jackson, R. (2015). Rapid morphological change in black rat (Rattus rattus) after an island introduction. PeerJ 3, e812.

Poole, A. & Lawton, C. (2009). The translocation and post release settlement of red squirrels Sciurus vulgaris to a previously uninhabited woodland. Biodivers. Conserv. 18, 3205–3218.

R Core Team. (2017). R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/

Renaud, S. & Auffray, J.-C. (2010). Adaptation and plasticity in insular evolution of the house mouse mandible. J. Zool. Syst. Evol. Res. 48, 138–150.

Renaud, S. & Millien, V. (2001). Intra- and interspecific morphological variation in the field mouse species Apodemus sylvaticus. Evolution 55, 2200–2217.
argenteus and A. speciosus in the Japanese archipelago: the role of insular isolation and biogeographic gradients. *Biol. J. Linn. Soc.* 74, 557–569.

Renaud, S., Hardouin, E.A., Pisanu, B. & Chapuis, J.-L. (2013). Invasive house mice facing a changing environment on the Sub-Antarctic Guéou Island (Kerguelen Archipelago). *J. Evol. Biol.* 26, 612–624.

Renaud, S., Gomes Rodrigues, H.G., Ledevin, R., Pisanu, B., Chapuis, J.-L. & Hardouin, E.A. (2015). Fast evolutionary response of house mice to anthropogenic disturbance on a Sub-Antarctic island. *Biol. J. Linn. Soc.* 114, 513–526.

Rice-Oxley, S.B. (1993). Caching behaviour of red squirrels *Sciurus vulgaris* under conditions of high food availability. *Mammal. Rev.* 23, 93–100.

Rohlf, F.J. (2018). *tpsDig2, digitize landmarks and outlines, version 2.31*. Stony Brook: Department of Ecology and Evolution, State University of New York.

Rushton, S.P., Gurnell, J., Lurz, P.W.W. & Fuller, R.M. (2002). Modeling impacts and costs of gray squirrel control regimes on the viability of red squirrel populations. *J. Wildl. Manag.* 66, 683–697.

Schaefer, K., Lauc, T., Mitteroecker, P., Gunz, P. & Bookstein, F. (2006). Dental arch asymmetry in an isolated Adriatic community. *Am. J. Phys. Anthropol.* 129, 132–142.

Schmidt, N.M. & Jensen, P.M. (2003). Changes in mammalian body length over 175 years – adaptations to a fragmented landscape? *Conserv. Ecol.* 7, 6.

Shorten, M. (1954). *Squirrels*. London: Collins.

Shuttleworth, C.M. (1996). The effect of supplemental feeding on the red squirrel (*Sciurus vulgaris*). PhD thesis. The University of London, London, UK.

Shuttleworth, C.M. (1997). The effect of supplemental feeding in the diet, population density and reproduction of red squirrels (*Sciurus vulgaris*). In *The conservation of red squirrels*: 13–24. Gurnell, J. & Lurz, P. (Eds). London: People’s Trust for Endangered Species.

Shuttleworth, C.M. (2000). The foraging behaviour and diet of red squirrels *Sciurus vulgaris* receiving supplemental feeding. *Wildl. Biol.* 6, 149–156.

Shuttleworth, C.M. (2010). Turning the grey tide – progress in red squirrel recovery. *Ecos* 31, 27–35.

Simpson, V.R., Hargreaves, J., Everest, D.J., Baker, A.S., Booth, P.A., Butler, H.M. & Blackett, T. (2010). Mortality in red squirrels (*Sciurus vulgaris*) associated with exudative dermatitis. *Vet. Rec.* 167, 59–62.

Snorrasson, S.S., Skúlason, S., Jonsson, B., Malmquist, H.J., Jónasson, P.M., Sandlund, O.T. & Lindem, T. (1994). Trophic specialization in Arctic char *Salvelinus alpinus* (Pisces: Salmonidae): morphological divergence and ontogenetic niche shifts. *Biol. J. Linn. Soc.* 52, 1–18.

Stumpf, R., Fuzessy, L. & Paglia, A.P. (2018). Environment drivers acting on rodent rapid morphological change. *J. Mammal. Evol.* 25, 131–140.

Sumner, J., Moritz, C. & Shine, R. (1999). Shrinking forest shrinks skink: morphological change in response to rainforest fragmentation in the prickly forest skink (*Gnypetosiscinus queenslandiae*). *Biol. Conserv.* 91, 159–167.

Swiderski, D.L. & Zelditch, M.L. (2010). Morphological diversity despite isometric scaling of lever arms. *Evol. Biol.* 37, 1–18.

Tittensor, A.M. (1970). The red squirrel (*Sciurus vulgaris* L.) in relation to its food resource. PhD thesis. University of Edinburgh, Edinburgh, UK.

Tomkins, D.M., Sainsbury, A.W., Nettleton, P., Buxton, D. & Gurnell, J. (2002). Parapoxvirus causes a deleterious disease in red squirrels associated with UK population declines. *Proc. R. Soc. Lond. B* 269, 529–533.

Tonkin, J.M. (1983). Activity patterns of the red squirrel (*Sciurus vulgaris*). *Mammal Rev.* 13, 99–111.

Turnbull, W.D. (1970). Mammalian masticatory apparatus. *Fieldiana (Geol.)* 18, 147–356.

Velhagen, W.A. & Roth, V.L. (1997). Scaling of the mandible in squirrels. *J. Morphol.* 232, 107–132.

Venning, T., Sainsbury, A.W. & Gurnell, J. (1997). Red squirrel translocation and population reinforcement as a conservation tactic. In *The conservation of red squirrels Sciurus vulgaris*: 134–144. Gurnell, J. & Lurz, P.W.W. (Eds). London: People’s Trust for Endangered Species.

Weijs, W.A. & Dantuma, R. (1975). Electromyography and mechanics of mastication in the albino rat. *J. Morphol.* 146, 1–34.

West, A.G. & King, C.M. (2018). Variation in mandible shape and body size of house mice *Mus musculus* in five separate New Zealand forest habitats. *New Zealand J. Zool.* 45, 136–153.

Yazdi, T. & Adriaens, D. (2011). Patterns of skull shape variation in *Meriones persicus* (Rodentia: Muridae) in relation to geoclimatic conditions. *Iran. J. Animal Biosyst.* 7, 129–142.

Yom-Tov, Y., Yom-Tov, S. & Moller, H. (1999). Competition, coexistence, and adaption amongst rodent invaders to Pacific and New Zealand islands. *J. Biogeogr.* 26, 947–958.

Yom-Tov, Y., Yom-Tov, S. & Jarrell, G. (2008). Recent increase in body size of the American marten *Martes americana* in Alaska. *Biol. J. Linn. Soc.* 93, 701–707.

Zelditch, M.L., Wood, A.R., Bonett, R.M. & Swiderski, D.L. (2008). Modularity of the rodent mandible: integrating bones, muscles, and teeth. *Evol. Dev.* 10, 756–768.

Zelditch, M.L., Li, J., Tran, L.A.P. & Swiderski, D.L. (2015). Relationships of diversity, disparity, and their evolutionary rates in squirrels (*Sciuridae*). *Evolution* 69, 1284–1300.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Mean log centroid size (± standard deviation) of mandibles of red squirrel populations.
Table S2. P values from post-hoc pairwise permutation tests of mandible log centroid size between populations. Significant comparisons highlighted.

Table S3. Mean mechanical advantage (± standard deviation) of masticatory muscles for red squirrel populations. DM, deep masseter; SM superficial masseter; T, temporalis.

Table S4. P values from post-hoc pairwise permutation tests of Td MA between populations. Significant comparisons highlighted.

Table S5. P values from post-hoc pairwise permutation tests of Tv MA between populations. Significant comparisons highlighted.

Table S6. P values from post-hoc pairwise permutation tests of Tv MA between populations. Significant comparisons highlighted.

Data File S1.