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

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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 muroid 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

Eurasian red squirrels (*Sciurus vulgaris*). Once widespread across Great Britain (Shorten, 1954; Lloyd, 1983), the red squirrel suffered a severe population decline from the 1920s onwards (Gurnell, 1987). This has been attributed to various factors, such as loss of woodland habitat and competition with the introduced Eastern grey squirrel (*S. carolinensis*) (Gurnell & Pepper, 1993; Gurnell *et al.*, 2004). In recent decades, squirrelpox virus, carried by grey squirrels, is also known to have played an important role in the disappearance of red squirrels from many parts of Great Britain (Tompkins *et al.*, 2002; LaRose *et al.*, 2010). The population decline has resulted in a highly reduced and fragmented distribution of British red squirrels (Gurnell & Pepper, 1993; Barratt *et al.*, 1999; Fig. 1). Currently, red squirrels are found in most parts of Scotland except for the Central Belt, and across the northernmost counties of England, that is Northumberland and Cumbria (Gurnell, Lurz & Bertoldi, 2014). There are also isolated populations in County Durham, the Yorkshire Dales, the National Trust reserve at Formby on the Lancashire coast, and in some coniferous forests in mid Wales (Cartmel, 1997; Shuttleworth, 2000; Hobbs, 2005; Harris & Yalden, 2008). A population of red squirrels also existed in the lowland pine forest at Thetford in East Anglia until at least the early 21st century (Gurnell *et al.*, 2002; Rushton *et al.*, 2002), but now appears to be extinct (Mathews *et al.*, 2018). Beyond mainland Great Britain, red squirrels are found on Anglesey, the Isle of Wight and five islands in Poole Harbour, as well as on Jersey in the Channel Islands (Harris & Yalden, 2008; Simpson *et al.*, 2010; Shuttleworth, 2010).

Historically, the British red squirrel has been considered a subspecies, *S. v. leucourus*, distinguished from the central European red squirrel, *S. v. fuscoater*, by bleaching of the tail pelage during summer (Hale & Lurz, 2003). However, the validity of these subspecies has been called into question (Marr & MacLeod, 2019), not least because of the numerous introductions of red squirrels from continental Europe over the last 150 years (Hale, Lurz & Wolff, 2004). In particular, the Jersey population was introduced from Europe, probably France, and southern England in the 1880s (Magris & Gurnell, 2002), and the Formby population was introduced from Europe, possibly Scandinavia, in the early to middle 20th century (Lowe & Gardiner, 1983; Gurnell & Pepper, 1993). The red squirrels in the western half of northern England appear to be native, whereas in the east there have been some introductions from continental Europe (Hale & Lurz, 2003). Finally, the Scottish population seems to be mainly derived from re-introductions from England and, to a lesser extent, Scandinavia (Harvie-Brown, 1880-1881).

The aim of this study is to determine whether the remaining populations of British red squirrels vary in morphology from one another, and to assess the functional impact of any morphological differences. The first hypothesis is that populations will differ in mandibular shape. This is predicted as British red squirrel populations are isolated from one another in different habitats and climatic conditions, and rodent mandibular morphology has been shown to be responsive to variations in mechanical properties of dietary items (Michaux, Chevret & Renaud, 2007; Renaud & Auffray, 2010; Anderson, Renaud &
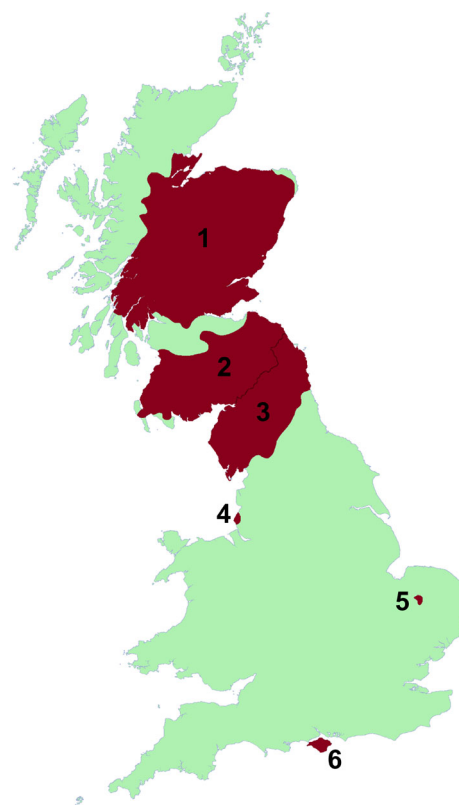

Figure 1 Map showing British red squirrel populations analysed in this study. Key: 1, North Scotland; 2, South Scotland; 3, North England; 4, Formby; 5, Thetford; and 6, Isle of Wight. Jersey and Germany not shown. [Colour figure can be viewed at zslpublications. onlinelibrary.wiley.com].

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 *geomorph* 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,
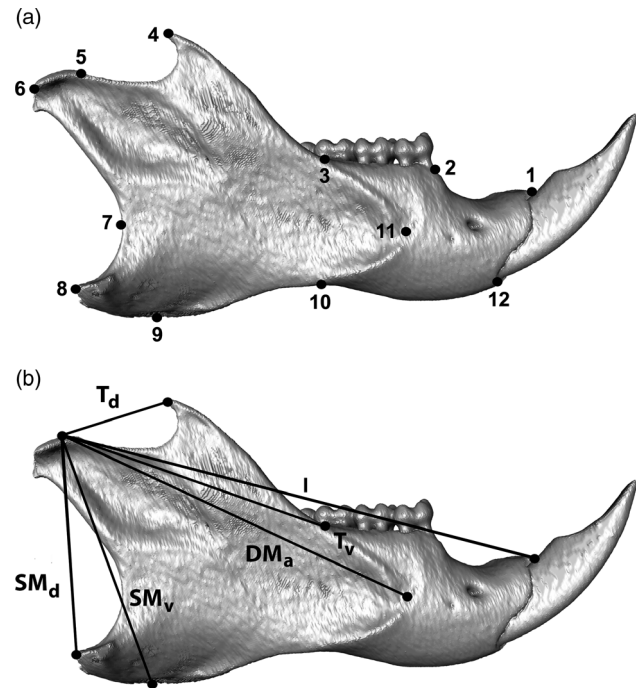

Figure 2 Right hemi-mandible of *Sciurus vulgaris* in lateral view showing (a) landmarks used in GMM and (b) in- and out-levers used in biomechanical analysis. Landmarks: 1, dorsal-most point on incisor alveolar margin; 2, anterior margin of premolar alveolus; 3, base of coronoid process where it crosses molar alveolar margin; 4, tip of coronoid process; 5, anterior-most point of condyle articular surface; 6, posterior-most point of condyle articular surface; 7, anterior-most point on curve between condyle and angular process; 8, posterior tip of angular process; 9, ventral-most point on angular process; 10, dorsal-most point on ventral border of ramus; 11, anterior-most point on masseteric ridge; and 12, ventral-most point on incisor alveolar margin. Levers: DM_a, anterior deep masseter in-lever; I, 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.

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 (as in Casanovas-Vilar & van Dam, 2013; Gomes Rodrigues, Šumbera & Hautier, 2016). In- and out-levers are illustrated in Fig. 2b, and the calculated MAs for each specimen are given in Data File S1.

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 sciurognathous 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 each 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 (T_d: $F = 25.7$, $P = 0.0001$; T_v: $F = 15.8$, $P = 0.0001$; 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
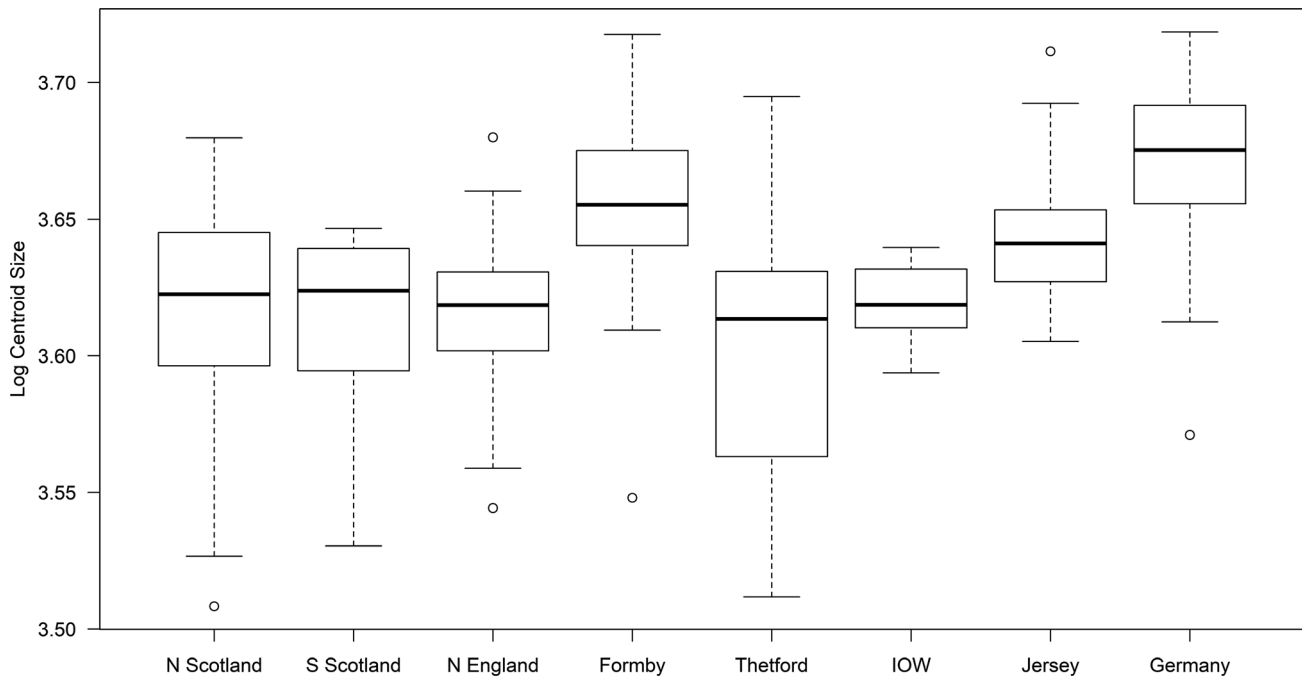
Figure 3 Box plot of mandible log centroid size in red squirrel populations. Black line represents median, box represents interquartile range, and open circles represent data points lying further than 1.5 times the interquartile range from the box.

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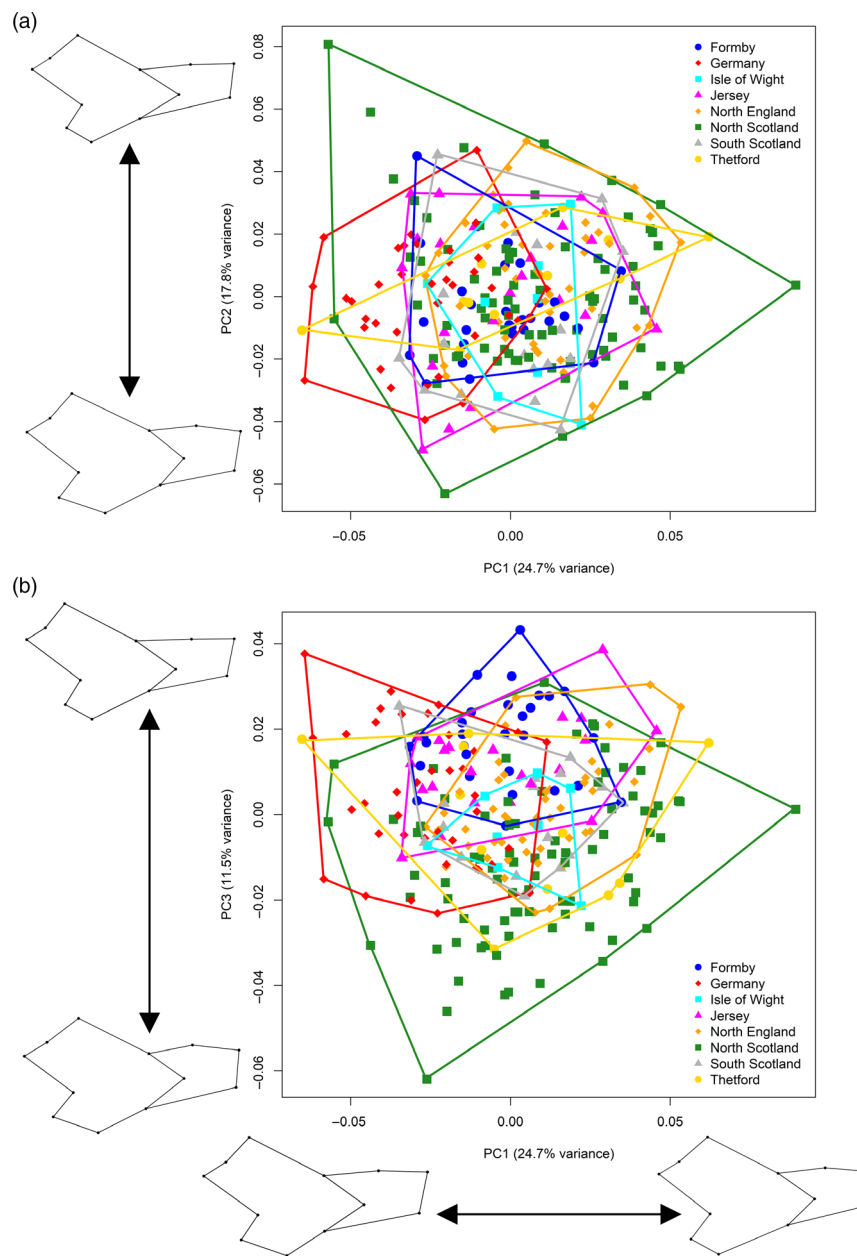
Figure 4 Principal components analysis of red squirrel mandibular shape. (a) PC1 versus PC2; (b) PC1 versus PC3. Convex polygons drawn around individuals from each population. Wireframes illustrate shape changes along each principal component from −0.05 to 0.05. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com].

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

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.

this relationship (Markow, 1995), and a recent study on mice found no impact of inbreeding depression on bite force (Ginot, Agret & Claude, 2018).

The remaining red squirrel populations – North Scotland, South Scotland, North England, Jersey and Formby – show some degree of separation across PC3 from negative to positive (Fig. 4b), although there is still a great deal of overlap between them. In particular, the populations from North England and South Scotland overlap almost completely, which may suggest that there is mixing between these two populations and/or that the habitat and climate in which they live are largely similar. The North Scotland population also overlaps with these two populations, but extends much further to the extreme negative end of PC3. This may reflect the wide range of this population, encompassing areas just north of the Central Belt, which have a similar climate to the South Scotland region, to areas much further north with a much more extreme climate.

The Jersey and Formby specimens are found towards the positive end of PC3. This represents a mandibular morphology in which the coronoid process is situated much closer to the condyle than in the Scottish and Northern English squirrels. This morphology reduces the temporalis in-lever, which is reflected in the lower temporalis MA of the Formby and Jersey populations. In particular, the dorsally inserting fibres of the temporalis are notably lower in the Formby squirrels than in all other populations studied here (Fig. 5). This clear difference is intriguing as previous research has shown muscle MAs shown to be highly stable between sciurid species (Velhagen & Roth, 1997; Swiderski & Zelditch, 2010). The temporalis muscle in

rodents is a powerful elevator of the jaw (Hiiemae, 1971; Gorniak, 1977) and forms a greater proportion of the total jaw adductor musculature in species that use their incisors in mechanically demanding activities such as squirrels (Ball & Roth, 1995; Druzinsky, 2010) beavers (Cox & Baverstock, 2016), and mole-rats (Cox, Faulkes & Bennett, 2020), compared to other rodents (Turnbull, 1970; Cox & Jeffery, 2011). Furthermore, the MA of the temporalis is greater in African mole-rats that dig with their incisors than in closely related species that do not (McIntosh & Cox, 2016). Thus, the mandibular morphology of the Formby and Jersey squirrels appears to be less efficient at incisor gnawing than that of other squirrel populations in this sample – a finding that could be related to a number of (not necessarily mutually exclusive) factors.

The biomechanical distinctiveness of Formby and Jersey red squirrels could be a founder effect stemming from their different genetic backgrounds. The Jersey squirrels are thought to derive mostly from a French population (Magris and Gurnell, 2002), and those from Formby may originate from a European (possibly Scandinavian) population introduced to Ainsdale, Lancashire, in the early 1930s (Lowe & Gardiner, 1983; Gurnell & Pepper, 1993). However, the history of red squirrels in Britain has included numerous translocations from Europe over the last 200 years, leading to a complex phylogenetic relationship between current populations and no clear phylogeographical pattern (Barratt *et al.*, 1999; Hale *et al.*, 2004). Additionally, inbreeding resulting from a small founder population could be playing a part, at least in the case of the Formby squirrels. It should be noted that much of the inbreeding in Formby results from a severe population decline following an outbreak of squirrelpox virus in 2008 (Chantry *et al.*, 2014), which post-dates the majority of specimens in this analysis. However, it is likely that the isolation of the Formby red squirrels had also resulted in some degree of inbreeding prior to the 2008 outbreak.

The reduction in temporalis MA could also be a result of relaxed selection, owing to the larger mandibular size of the Formby and Jersey populations. As mentioned above in relation to German red squirrels, the larger masticatory muscles likely associated with larger mandibles may offset the lower muscle MAs, thus maintaining maximum bite force. However, it is notable that, in the Formby and Jersey red squirrels, only the temporalis MA is reduced, not the MA of all masticatory

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	2.085	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	4.28	6.464	5.734	1.128	0.368	5.082	

Significant comparisons highlighted.
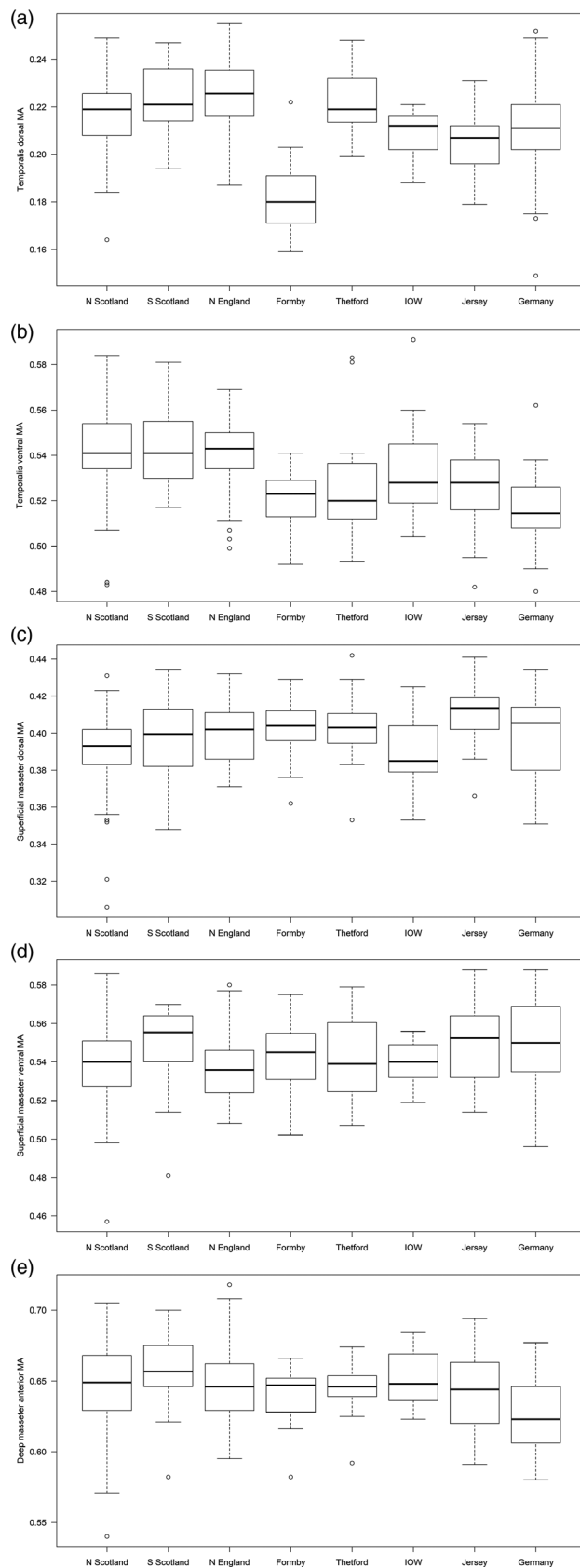
Figure 5 Box plots of masticatory muscle MA in red squirrel populations. (a) Most dorsally inserting fibres of the temporalis; (b) most ventrally inserting fibres of the temporalis; (c) most dorsally inserting fibres of the superficial masseter; (d) most ventrally inserting fibres of the superficial 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.

muscles. Furthermore, permutational ANOVA tests showed population to have a significant effect on T_d 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 masseter MAs, as those muscles have greater importance in protraction of the mandible (Hiiemae, 1971) and molar chewing (Weijs & Dantuma, 1975; Offermans & De Vree, 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 granivorous diets have less well-developed coronoid processes (Michaux *et al.*, 2007).

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

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.

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Supporting Information

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

Table S1. Mean log centroid size ($\pm$ 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.