Better sturdy or slender? Eurasian otter skull plasticity in response to feeding ecology

Luca Francesco Russo¹, Carlo Meloro²*, Mara De Silvestri¹, Elizabeth A. Chadwick³, Anna Loy¹

¹ EnvixLab, Department of Biosciences and Territory, Università degli Studi del Molise, Pesche, Italy, ² Research Centre in Evolutionary Anthropology and Palaeoecology, Liverpool John Moores University, Liverpool, United Kingdom, ³ Cardiff University, Biomedical Science Building, Museum Avenue, Cardiff, United Kingdom

* C.Meloro@ljmu.ac.uk

Abstract

Otters are semi-aquatic mammals specialized in feeding on aquatic prey. The Eurasian otter Lutra lutra is the most widely distributed otter species. Despite a low degree of genetic variation across its European range, the population from Great Britain exhibits distinct genetic structuring. We examined 43 skulls of adult Eurasian otters belonging to 18 sampling localities and three genetic clusters (Shetlands, Wales and Scotland). For each sample location, information regarding climate was described using bioclimatic variables from WorldClim, and information on otter diet was extracted from the literature. By using photogrammetry, 3D models were obtained for each skull. To explore any evidence of adaptive divergence within these areas we used a three dimensional geometric morphometric approach to test differences in skull size and shape between areas with genetically distinct populations, as well as the influence of diet, isolation by distance and climate. Males were significantly larger in skull size than females across all the three genetic clusters. Skull shape, but not size, appeared to differ significantly among genetic clusters, with otters from Shetland exhibiting wider zygomatic arches and longer snouts compared to otters from Wales, whereas otters from Scotland displayed intermediate traits. A significant relationship could also be found between skull shape variation, diet as well as climate. Specifically, otters feeding on freshwater fish had more slender and short-snouted skulls compared to otters feeding mostly on marine fish. Individuals living along the coast are characterized by a mixed feeding regime based on marine fish and crustaceans and their skull showed an intermediate shape. Coastal and island otters also had larger orbits and eyes more oriented toward the ground, a larger nasal cavity, and a larger distance between postorbital processes and zygomatic arch. These functional traits could also represent an adaptation to favor the duration and depth of diving, while the slender skull of freshwater feeding otters could improve the hydrodynamics.
Introduction

Otters are semi-aquatic mammals found in a wide variety of aquatic environments, including rivers, lakes, reservoirs, swamps, and marshes [1]. The Eurasian otter *Lutra lutra* is the most widely distributed species within the subfamily Lutrinae, with 11 subspecies occurring from Europe throughout Asia and North Africa [2, 3]. The European populations belong to the nominal subspecies *L. l. lutra* and are currently recovering after a strong decline in the last century due to direct persecution, hunting for pelts, water pollution, and habitat degradation [2]. Wide-ranging mammals often show differences in both shape and size related to ecological gradients [4–6], which are more likely to occur in highly specialized species such as the semi-aquatic otters [7]. Research at range-wide scales has revealed only a low degree of phenotypic and genetic variation across Europe (e.g. [8, 9]), but smaller scale studies have shown evidence both for cranial differentiation (e.g. in Denmark, Germany and East Asia [10–12]), and genetic sub-structuring (e.g. in UK [13, 14], and southern Italy [15]). The skull is a complex anatomical adaptive structure, enclosing the central nervous system and the specialized sense organs and hosting the teeth used to capture, kill the prey, process and manipulate food [16]. Several studies have shown that in mammals, skull shape is closely related to diet [17–21], and shape changes in response to feeding habits can occur among different populations of the same species [22]. The Eurasian otter is an opportunistic feeder able to exploit different aquatic prey depending on their availability and catchability [23]. Its diet includes mainly fish, but also crustaceans, amphibians, reptiles, and, to a lesser extent, birds and mammals [23–27]. This high feeding plasticity is expected to be reflected in the cranial and mandibular morphology with modifications expected, especially in the dentition and masticatory muscle attachment area, as observed for members of the order Carnivora [21, 28]. Furthermore, differences in diet may also be reflected in sexual dimorphism of the skull with respect to size and shape [29–32], as specific adaptations can improve the fitness and reduce intraspecific resource competition [33]. Although mustelids are known usually to show sexual dimorphism only with respect to size, and not shape [7, 29–31], advances in methodology may now permit more nuanced analyses. The advent of geometric morphometric (GM) approaches offer a powerful tool to investigate the role of geographical and ecological gradients in influencing the size and the shape of biological structures, especially the highly informative skull, as well as to explore the role of sexual dimorphism and allometry [34–37]. The study of geographic variation is particularly interesting when dealing with islands, as isolation from the mainland may accelerate the emergence of adaptive traits in highly specialized feeders like the Eurasian otter [38]. Here, we investigate the morphological variation of otters across the mainland and the islands of Great Britain to explore the ultimate drivers of the observed patterns in terms of the genetic, latitudinal and ecological differentiation revealed by recent studies. Otter populations in Britain are ecologically heterogeneous, with high levels of genetic sub-structuring (e.g. [13, 14]), differences in scent gland secretions between genetically distinct regions (‘odour dialects’ [39]), and regional variation in diet [26]. Farnell et al. [40] gave a first insight into 3D morphological variation of skulls across Great Britain, suggesting that the observed changes in size and shape might reflect genetic differences among populations. However, the authors suggested that regional differences could be driven by other potentially confounding factors and that more rigorous research was needed.

We specifically used a 3D GM approach to examine the size and shape variation of Eurasian otter skulls in order to: i) evaluate the occurrence of sexual size (SSD) and sexual shape (SShD) dimorphism; ii) detect any pattern in the size and shape of skull that could reflect genetic clustering; iii) evaluate the role of diet and isolation by distance on size and shape variation; iv) evaluate climate has a proxy of diet adaptation as reported in many studies.
Methods

Data collection

We examined 43 skulls of adult Eurasian otters stored in the National Museums of Scotland. Specimens belong to 18 sampling localities (S1 and S2 Tables). Each sample locality was assigned to inland, coastal, or island waters, and pooled in three likely genetic clusters based on Hobbs et al. [14] and Stanton et al. [13]. Stanton et al. [13] describe a distinct contrast in genetic structure between ‘Northern Britain’ and ‘Southern Britain’, but do not include samples from the Shetland Isles, which were previously shown distinct from the Scottish mainland by Hobbs et al. [14]. In the current study we used samples originating from the Shetland Islands (F = 8, M = 7, hereafter referred to as ‘Shetland’), mainland Scotland, the outer Hebrides, and the Orkney Islands (hereafter referred to as ‘Scotland’ F = 6, M = 12) and from Wales and central England (hereafter referred to as ‘Wales’, F = 3; M = 7) (Fig 1).

Genetically, we assume that the ‘Wales’ samples are part of the Southern Britain population defined by Stanton et al. [13] while the ‘Scotland’ samples are assumed to be part of the ‘Northern Britain’ population, although note that samples from the outer Hebrides were not included in either Hobbs et al. [14], or Stanton et al. [13] and may be distinct.

Each skull was placed on a turntable and photographed every 10˚ on the dorsal, ventral, and vertical projections, for a total of 108 pictures. All pictures were taken using a Canon 30EOS SLR with a fixed 50mm lens, placed on the tripod at a fixed distance (50 cm) from the turntable and activated with a remote control to avoid blurring [41].

Three dimensional pictures were reconstructed using the photogrammetry method [42] by means of Agisoft PhotoScan software (Agisoft PhotoScan, http://www.agisoft.ru/). The resulting models were scaled to real size using Tpsdig [43] and Meshlab [44]. Previous studies have abundantly demonstrated that the level of accuracy generated by 3D photogrammetry models in quantifying size and shape using GM is as high as the one provided by CT scan or laser scanner [45, 46], and these techniques can be implemented within the same analysis without generating significant intra-individual error in GM data [41, 47]. On each 3D model, we positioned thirty 3D landmarks (LM, S1 File) on clearly distinguishable, homologous, and significant anatomical regions, using the software Meshlab (Fig 2).

Morphometric analyses

A Generalized Procrustes Alignment (GPA) analysis was run to translate, scale and rotate original coordinates [34], using the gpagen() function implemented in the R Geomorph package [48, 49]. We used the log transformed centroid size (CS hereafter), i.e. the square root of the sum of squared distances of each landmark, as a proxy of skull size [34]. To avoid noise due to either directional or fluctuating asymmetry, shape changes were explored retaining only the symmetric component of skull shape [50]. The symmetric component was extracted using geomorph’s bilat.symmetry() function.

A Principal Components Analysis (PCA) was run on the aligned coordinates (i.e. shape variables) of the symmetric component to explore shape changes among genetic clusters and sexes, using the geomorph’s gm.prcomp() function.

ANOVA on CS and Procrustes ANOVA on shape variables were run to test for the effect of sex, genetic cluster, and their interaction, on size and shape respectively. Procrustes ANOVA was also used to test for static allometry (sensu Klingenberg, [37]), by considering the effect of size (lnCS) and genetic clusters, and their interaction on shape. All analyses were run through the function procd.lm(). To analyse the relationship between either size or shape, we used the mean size and the mean shape for each sample locality. Specifically, the mean shape was
computed through the geomorph mshape() function as recommended in previous ecogeographical studies [4, 51–53].

**Shape and diet**

We explored the existing literature to gather information on otter diet at each sample location. When information was not available, we used references from the closest areas within the

![Fig 1. Geographic origin of otter skulls.](https://doi.org/10.1371/journal.pone.0274893.g001)
same river basin (S3 Table). We specifically recorded the frequencies of occurrence of seven prey categories: marine fish, freshwater fish, crustaceans, amphibians, birds, mammals, plants, and insects (S3 Table). A PCA was then run on the resulting data matrix to explore the variation in the diet of otters living in inland freshwater, mainland coasts, and islands.

We further explored the diet composition by quantifying the frequency of 31 fish families (S3 Table).

The correlation between one or the other diet matrix (i.e. the whole prey categories and the fish families) and either the skull size or shape was explored through a Partial Least Squares regression (PLS, [54]) using `two.b pls()` function in geomorph, an approach successfully used in previous studies on other mammals and bird species [18, 55, 56].

To explore if the climate can be used as proxy of diet in the Eurasian otter, the results of PLS on climate and PLS on diet were compared using the function `compare.pls()` of geomorph to assess possible difference or parallelism in shape co-variation with dietary or climatic factors [57].

**Shape and climate**

We extracted 19 bioclimatic variables at each sample locality (S4 Table). Variables were recorded at 5min (~ 10 km²) resolution from WorldClim [58]. We used the `vif()` function of the usdm package [59] to account for autocorrelation. The final six uncorrelated variables were standardised for further analyses. To explore climatic pattern across the sample localities and avoid bias due to spatial autocorrelation [60] we used the function Principal Coordinates of Neighbor Matrices (PCNM) [60] implemented in the `pcnm()` function of the vegan package [61] to extract spatial vectors based on specimen locations. Partial Least Squares regression (PLS) was then run between cranial size or shape of each sample locality vs a matrix including the first PCNM scores and the selected bioclimatic variables [62], using geomorph function `two.b pls()`.

All Procrustes ANOVA and PLS analyses were run using randomized residuals permutation procedures (RRPP) with 1000 permutations.

**Results**

**Sexual dimorphism**

ANOVA showed a significant difference in size between males and females (Table 1), with male skulls always being larger than females in each of the three genetic clusters (Fig 3). These differences were consistent among genetic clusters since the interaction term "sex: genetic clusters" was not significant.

In contrast, no sexual dimorphism in skull shape could be identified by Procrustes ANOVA, nor in the sex:genetic clusters interaction (Table 1). These results allowed pooling of sexes and including the undetermined specimens for subsequent analyses of shape variation.

**Geographic variation**

Otters belonging to the three genetic clusters i.e. Wales, Scotland, and Shetland, showed significant differences in their cranial morphology, accounting for ca. 16% of skull shape variation (Table 1). The Principal Components Analysis (PCA) on shape variables showed a clear separation of genetic clusters along PC1 (16.88% of variance) (Fig 4). 3D wireframe plots related to variation along PC1 indicate that the most northern cluster (otters from Shetland) exhibited a squatter skull with wider zygomatic arches and longer snout, compared to the most southern cluster (from Wales), whereas otters from Scotland displayed an intermediate shape between the two former clusters (Fig 4).
These shape differences were not due to allometric shape changes (Table 2) since variation explained by size was minimal and non-significant when compared to that due to genetic clustering.

**Shape and diet**

PCA run on the frequency of occurrence of eight prey categories along nine sample localities showed a clear distinction among the diet of otters living in coastal, islands, or mainland freshwater habitats, with the first axis explaining the 97.74% of cumulative variance (S1 Fig). The diet of otters living along the mainland coasts differed from that of otters living on islands or in mainland freshwaters. PC1 axis was mainly influenced by the relative frequency of freshwater (negative extreme) and marine fish (positive extreme), whereas PC2 axis was positively associated with the relative frequency of crustaceans (S1 Fig). Otters living in coastal areas and
islands fed predominantly on marine fish, those along coasts also on crustaceans, whereas otters living in mainland freshwaters fed primarily on freshwater fish.

PLS regression revealed a significant association between shape and diet (PLS, based on a matrix of 37 prey (i.e., 31 fish species plus 6 non-fish taxa): r-PLS = 0.86, p = 0.03), with otters

Table 1. Association of A. Skull size (lnCS) and B. Shape, with genetic cluster, sex, and their interaction. Results are based on ANOVA model for size, and Procrustes ANOVA for shape.

| A. lnCS (ANOVA)          | Df | SS      | MS      | Rsq   | F      | Z      | Pr(>F) |
|--------------------------|----|---------|---------|-------|--------|--------|--------|
| Genetic cluster          | 2  | 0.019   | 0.009   | 0.083 | 2.228  | 1.235  | 0.107  |
| Sex                      | 1  | 0.049   | 0.049   | 0.217 | 11.630 | 2.660  | 0.003  |
| Genetic cluster:Sex      | 2  | 0.002   | 0.001   | 0.008 | 0.223  | -0.859 | 0.799  |
| Residuals                | 37 | 0.157   | 0.004   | 0.691 |        |        |        |
| Total                    | 42 | 0.227   |         |       |        |        |        |

| B. Shape (Procrustes ANOVA) | Genetic cluster | Df | SS      | MS      | Rsq   | F      | Z      | Pr(>F) |
|-----------------------------|-----------------|----|---------|---------|-------|--------|--------|--------|
| Genetic cluster             | 2               | 0.010 | 0.005 | 0.164 | 4.054 | 5.824  | 0.001  |
| Sex                         | 1               | 0.002 | 0.002 | 0.031 | 1.553 | 1.573  | 0.069  |
| Genetic cluster:Sex         | 2               | 0.003 | 0.002 | 0.058 | 1.430 | 1.672  | 0.053  |
| Residuals                   | 37              | 0.045 | 0.001 | 0.747 |        |        |        |
| Total                       | 42              | 0.060 |        |       |        |        |        |

https://doi.org/10.1371/journal.pone.0274893.t001

Fig 3. Box plots of cranial size variation (= lnCS) in males and females from three genetic clusters. Horizontal lines within each box indicate the median, upper and lower limits the inter-quartile range, while 'whiskers' indicate the minimum and the maximum.

https://doi.org/10.1371/journal.pone.0274893.g003
feeding in mainland freshwaters positioned at the positive extreme of both PLS1 and PLS2 axes, whereas marine fish feeders were found at the negative extreme of both axes, with the only exception of the sample from North East Scotland (Grampian). Samples from coastal habitats were either slightly distinguished or overlapped with the marine fish feeders from islands (Fig 5). No significant association found between cranial size and diet ($r_{PLS} = 0.32$, $p = 0.83$).

https://doi.org/10.1371/journal.pone.0274893.g004

Fig 4. Skull shape variation along the first two Principal Component axes (PCs) of Procrustes coordinates. A. Colours indicate different genetic clusters, Scotland (green), Shetland (blue) and Wales (red). Individual data points represent the first two component scores from PCA carried out on all shape variables. B. A visualisation of the 3D contours related to extremes of variation along PC1, which describes 16.88% of the variance.
The skulls of freshwater feeders were relatively slender and short-snouted compared to the skulls of otters feeding on marine fish, whereas the coastal otters, feeding on marine fish and crustaceans, showed an intermediate shape. Coastal and island otters also had larger orbits and eyes more oriented toward the bottom (LMs 3–5, 9–11 in Fig 2), a larger nasal cavity, and a larger distance between postorbital processes and zygomatic arch (Fig 6).

Shape and climate

The variance inflation factors (VIF) identified significant correlation among most of the 19 bioclimatic variables. To avoid violating the assumption of independence, six unrelated variables were retained for further analyses: Annual Mean Temperature (BIO1), Isothermality (BIO3), Temperature Seasonality (BIO4), Mean Temperature of Wettest Quarter (BIO8), Mean Temperature of Driest Quarter (BIO9), Precipitation of Driest Month (BIO14) and Precipitation Seasonality (BIO15). PCA run on the six variables showed clear climate differences between the genetic clusters along the first two PC axes, expressing 99.11% of cumulative variation, and especially along PC1 (88.32% of cumulative variation) (S2 Fig). PC1 was mainly influenced by Temperature Seasonality (BIO4), whereas Precipitation of Driest Month (BIO14) and Precipitation Seasonality (BIO15) mainly affected variation along PC2 (S2 Fig). The Wales localities are clearly distinguished from Scotland and Shetland, being characterized by high temperature seasonality (BIO4). The remaining Scottish localities showed the maximum values. Moreover, samples from Shetland and one Scottish locality were characterized by low rainfall in the driest month.

Results from PLS analyses showed no correlation between cranial size and climate (r-PLS = -0.4966, P = 0.684), whereas climate was significantly related with the shape of the skull (r-PLS = 0.86, P = 0.003). Specifically, otters living in areas with a highest seasonality of temperature and precipitations had a more slender and long-snouted skull compared to otters living areas with low seasonality (Fig 7).

Shape changes from freshwater to marine fish feeding habits were similar to those observed in relation to climate, and along the geographic gradient. Comparison of PLS results indicated that although a larger effect size is associated with climate (PLS Z = 2.64) than with diet (PLS Z = 1.76) the difference between the two was not statistically significant (p = 0.75).

Discussion

Sexual dimorphism

As observed in most Musteloidea [7, 31, 32, 63, 64], we found a significant Sexual Size Dimorphism (SSD) in otter skulls from Great Britain, with males larger than females. Differences in skull size are usually related to bite force [30]. In species with polygyny mating systems, like otters, larger males with greater bite force are likely able to better defend their territory and mate with more females [65–68]. Another known advantage of SSD is the differentiation of trophic niches between males and females, lowering the resource competition between sexes.
In fact, it has been observed that both sexes and different age groups of otters feed on different types of prey or on prey of different sizes. Within this context, a larger size and a greater bite force could allow the males to hunt larger prey that are inaccessible to females. Although some previous studies using linear morphometry found a difference in the

---

**Fig 5. Relationships between mean shape and diet as shown by the PLS regression of mean skull shape on diet matrices for 18 geographically distinct sampling locations.** Colours indicate the feeding areas of otters living in that locality. Below the x-axis are shown the wireframes corresponding to shape changes at the extremes of the axis. Nodes represent the landmarks, edges the anatomical connections between landmarks.

https://doi.org/10.1371/journal.pone.0274893.g005
shape of the skulls of males and females [11, 40, 70], we did not find any significant Sexual Shape Dimorphism (SShD). These contrasting results may reflect the different approaches used in the analyses, as GM is able to better capture the information on the shape compared to linear morphometry [71]. However, since shape differences between males and females were close to significance (p = 0.069), further analyses on a larger sample size might likely identify sexual related traits also in skull shape.

**Geographic variation**

**Size.** In contrast to the Bergmann’s rule [72, 73] and body size variation of otters from Sweden [74], we did not find any latitudinal gradient in the skull size of otters from Great

Fig 6. Comparison of 3D models of a skull of an otter living in an island (right, Shetland, Coll ID: 1990.104.021) and an otter living in mainland freshwater (left, Wales, Coll ID: CARDIFF-733). The arrow A indicates the zygomatic arch and B shows the postorbital processes.

https://doi.org/10.1371/journal.pone.0274893.g006
Britain. Exceptions to Bergmann’s rule have been found in many mammals [72, 75] and our sample now adds to previous evidences. The Shetland specimens could be subject to the island rule (i.e. smallest size [38]) and bias the gradient in size predicted by Bergmann’s rule. Indeed, the lnCS of Scotland population appears to be larger on average than Wales.

Fig 7. Association between skull shape and climate. Each point indicates one of the 18 sampling locations; colours indicate the genetic clusters. Data represent PLS scores from Block1 (representing mean shape variables for each sample locality) and Block2 (climate variables). Below the x-axis are shown the wireframes corresponding to shape changes at the extremes of the axis. Nodes represent the landmarks, edges the anatomical connections between landmarks.

https://doi.org/10.1371/journal.pone.0274893.g007
**Shape.** Shape variation revealed a clear distinction between the three genetically distinct clusters (i.e., Shetland, Scotland and Wales), which are areas that support differences also in diet and climate.

We propose that the most likely driver of changes in skull shape are differences associated with the available diet. Our results evidenced that otters feeding on freshwater fish have a slender and short-snouted skull compared to marine fish feeders, whereas the increase of crustaceans in the diet of coastal otters is reflected in less marked ‘marine’ shape. A slender skull shape in freshwater feeding otters suggests a wider gape and a faster closure of the jaws [76]. Nevertheless, a more elongated braincase allows attachment for posterior and anterior temporalis muscles, increasing the horizontal force [77]. These traits are functional to capture fast swimming and soft prey like freshwater fish, especially salmonids. In contrast, otters feeding mainly in the marine environment have a sturdy skull with wider zygomatic arches. The wider zygomatic arches and taller crania in marine feeders can allow an increased area for the attachment of the masseter and temporalis jaw adductor muscles [30, 78]. These muscles function primarily to close the jaw [77] and the resulting larger temporalis mass in marine feeders can allow a stronger bite force [78, 22]. These characteristics are often attributed to durophagous otter species like the sea otter *Enhydra lutris* [76]. The diet of otters hunting in marine environments is mainly composed of benthic and slower swimming species (e.g., Zoarcidae and Lotidae (see S3 Table), with tougher skins, and reaching larger sizes compared to freshwater fish.

Marine and coastal feeding otters also differed in their larger orbits, larger nasal cavity and more bottom oriented eyes, which might be associated with a distinct hunting strategy compared to the inland feeders [79]. It seems plausible that the diurnal habit of the Shetland population [80] means that sight may be more important than in areas where nocturnal feeding is more typical and where water is characterized by higher turbidity. The compact shape of the skull and the larger orbits and large nasal cavity of marine feeding otters from Shetland may also represent an adaptation to favour the duration and depth of diving, as observed in pinnipeds [81]. Otters from Shetland have been observed diving to depths of more than 15m [82, 83]. In contrast, otters living in European freshwaters are mainly nocturnal [84–86], and are known to use their whiskers for hunting, as sight is impeded by both murky waters and darkness [87]. Also, freshwaters are not very deep but the currents can be rapid, and a slender skull may improve hydrodynamics and favour a higher swimming speed needed to catch fast swimming fish.

Skull traits associated with diet variation were similar to those associated with climate variation, as confirmed by comparison of PLS results. Climate is commonly used as a proxy of diet when this information is not available (see [52, 62, 88]) and our evidence suggests that climate may be used as a proxy for diet adaptation also in the Eurasian otter. On the other hand, Tseng and Flynn [79] indicated that skull shape variation in carnivores is correlated with precipitation, as this latter drives modifications in the sensory systems. In our case, the larger nasal cavity observed in otters living in the coldest areas, could increase oxygen assumption. This association is supported by evidence from Yom-Tov et al. [89] which shows negative association between water temperature and oxygen consumption in Eurasian otters.

**Concluding remarks.** Our study has highlighted how 3D morphometrics of the skull of otters across Great Britain mainland and islands was able to clearly differentiate the morphological characteristics of three distinct genetic clusters identified by Hobbs et al. [14] and Stanton et al. [13], and to identify the functional traits and possible drivers involved in the morphological shift within those areas. Our results suggest that the morphology of the otter skull can respond to adaptive pressures, that are likely related to the availability and accessibility of prey resources. That is, otters living in mainland freshwater, coastal areas and islands showed a clear distinction in the morphology of the skull, suggesting adaptive plasticity in...
response to feeding resources. These findings are in contrast to the general belief that European otters are characterized by high homogeneity in their genetic and morphological traits, and highlight the need for more extensive investigations to identify any Evolutionary Significant Unit in need of conservation efforts to preserve the evolutionary potential in the species [90]. This issue is particularly relevant for a species like the Eurasian otter that lost most of its European populations in the past decades due to multiple anthropogenic pressures [2].

**Supporting information**

**S1 File. Thirty 3D landmarks.**
(XLSX)

**S1 Fig. Spatial variation in the UK otters’ diet.** Points represent 9 sampling locations for which diet composition was available, colours indicate genetic clusters. The first two axes of a Principal Component Analysis for percentage of seven prey categories (marine fish, freshwater fish, crustaceans, amphibians, reptiles, birds, mammals, and insects) are used to summarise variation between points.
(DOCX)

**S2 Fig. Spatial variation in climate between the three genetic clusters.** Each point is a location along Principal Component scores (PC1 vs PC2) from a PCA on the six bioclimatic variables overlapped in a bi-plot. PC1 and PC2 are primarily influenced by Temperature Seasonality (BIO4) and Precipitation of Driest Month (BIO 14), respectively.
(DOCX)

**S1 Table. Number of skull specimens analysed.** The sample is partitioned by sex for each geographic location.
(DOCX)

**S2 Table. Specimen list of the analysed otter skulls.** Abbreviations: NMS = National Museums of Scotland, M = male, F = female, A = Adult.
(DOCX)

**S3 Table. Percentage of dietary items from 9 sample localities.** Dietary data are based on.
(DOCX)

**S4 Table. Definition of climatic variables used in the analyses.** Abbreviations: BIO = bioclimate.
(DOCX)

**Acknowledgments**

We are particularly grateful to Andrew Kitchener, principal curator of vertebrate biology at the National Museums Scotland who provided assistance and access to the otter collection.

**Author Contributions**

**Conceptualization:** Luca Francesco Russo, Carlo Meloro, Elizabeth A. Chadwick, Anna Loy.

**Data curation:** Luca Francesco Russo, Mara De Silvestri, Anna Loy.

**Formal analysis:** Luca Francesco Russo, Carlo Meloro, Mara De Silvestri.

**Investigation:** Luca Francesco Russo, Carlo Meloro, Mara De Silvestri.
Methodology: Luca Francesco Russo, Carlo Meloro, Mara De Silvestri, Elizabeth A. Chadwick, Anna Loy.

Project administration: Carlo Meloro, Anna Loy.

Software: Luca Francesco Russo.

Supervision: Carlo Meloro, Anna Loy.

Validation: Carlo Meloro.

Writing – original draft: Luca Francesco Russo, Carlo Meloro.

Writing – review & editing: Luca Francesco Russo, Carlo Meloro, Elizabeth A. Chadwick, Anna Loy.

References

1. Kruuk H. Otters: Ecology, behaviour and conservation. New York: Oxford University Press; 2006. https://doi.org/10.1093/acprof:oso/9780198565871.001.0001

2. Roos A, Loy A, de Silva P, Hajkova P, Zemanová B. Lutra lutra. The IUCN Red List of Threatened Species. Basel: Birkhäuser Basel; 2015. https://doi.org/10.1007/978-3-0348-7753-4_77

3. Hung N, Law CJ. Lutra Lutra (Carnivora: Mustelidae). Mamm Species. 2016; 48: 109–122. https://doi.org/10.1093/mspecies/sew011

4. Cardini A, Jansson AU, Elton S. A geometric morphometric approach to the study of ecoregographical and clinal variation in vervet monkeys. J Biogeogr. 2007; 34: 1663–1678. https://doi.org/10.1111/j.1365-2699.2007.01731.x

5. Milenković M, Šipetić VJ, Blagojević J, Tatović S, Vujčić M. Skull variation in Dinaric-Balkan and Carpathian gray wolf populations revealed by geometric morphometric approaches. J Mammal. 2010; 91: 376–386. https://doi.org/10.1644/09-MAMM-A-265.1

6. Mazák JH. Cranio metric variation in the tiger (Panthera tigris): Implications for patterns of diversity, taxonomy and conservation. Mamm Biol. 2010; 75: 45–68. https://doi.org/10.1016/j.mambio.2008.06.003

7. Hernández-Romero PC, Guerrero JA, Valdespino C. Morphological variability of the cranium of Lontra longicaudis (Carnivora: Mustelidae): A morphometric and geographic analysis. Zool Stud. 2015; 54. https://doi.org/10.1186/s40555-015-0127-6 PMID: 31966137

8. Ranyuk M, Ansorge H. Low epigenetic variability of the Eurasian otter Lutra lutra (L.) from Europe to Kamchatka, Russ J Ecol. 2015; 46: 195–201. https://doi.org/10.1134/S1067413615020095

9. Randi E, Davoli F, Pierpaoli M, Pertoldi C, Madsen AB, Loeschcke V. Genetic structure in otter (Lutra lutra) populations in Europe: Implications for conservation. Anim Conserv. 2003; 6: 93–100. https://doi.org/10.1017/S1367943003003123

10. Lau ACC, Asahara M, Han SY, Kimura J. Geographic variation of craniodental morphology of the Eurasian otter (Lutra lutra) in East Asia. J Vet Med Sci. 2017; 79: 144−152. https://doi.org/10.1292/jvms.16-0250 PMID: 27644315

11. Lynch JM, Conroy JW, Kitchener AC, Jeffries DJ, Hayden TJ. Variation in cranial form and sexual dimorphism among five European populations of the otter Lutra lutra. J Zool. 1996; 238: 81–96. https://doi.org/10.1111/j.1469-7998.1996.tb05380.x

12. Pertoldi C, Madsen AB, Randi E, Braun A, Loeschcke V. Variation of skull morphometry of Eurasian otters (Lutra lutra) in Denmark and Germany. Ann Zool Fennici. 1998; 35: 87–94.

13. Stanton DWG, Hobbs GI, McCafferty DJ, Chadwick EA, Philibey AW, Saccheri IJ, et al. Contrasting genetic structure of the Eurasian otter (Lutra lutra) across a latitudinal divide. J Mammal. 2014; 95: 814–823. https://doi.org/10.1644/13-MAMM-A-201

14. Hobbs GI, Chadwick EA, Bruford MW, Slater FM. Bayesian clustering techniques and progressive partitioning to identify population structuring within a recovering otter population in the UK. J Appl Ecol. 2011; 48: 1206–1217. https://doi.org/10.1111/j.1365-2664.2011.02028.x

15. Buglione M, Petrelli S, Troiano C, Notomista T, Petrella A, De L, et al. Spatial genetic structure in the Eurasian otter (Lutra lutra) meta-population from its core range in Italy. Contrib to Zool. 2021; 90: 70–92. https://doi.org/10.1163/18759866-BJA10012

16. Hanken J, Hall BK. The skull, volume 3: functional and evolutionary mechanisms. Vol. 3. University of Chicago Press; 1993.
17. Giacomini G, Herrel A, Chaverri G, Brown RP, Russo D, Scaravelli D, et al. Functional correlates of skull shape in Chiroptera: feeding and echolocation adaptations. Integr Zool. 2021; 1–13. https://doi.org/10.1111/1749-4877.12564 PMID: 34047457

18. Hendges CD, Patterson BD, Cáceres NC, Gasparini GM, Ross CF. Skull shape and the demands of feeding: A biomechanical study of peccaries (Mammalia, Cetartiodactyla). J Mammal. 2019; 100: 475–486. https://doi.org/10.1093/jmammal/gy261

19. Law CJ, Mehta RS. Carnivory maintains cranial dimorphism between males and females: Evidence for niche divergence in extant Musteloidae. Evolution (N Y). 2018; 72: 1950–1961. https://doi.org/10.1111/evo.13514 PMID: 29882586

20. McLean BS, Helgen KM, Goodwin HT, Cook JA. Trait-specific processes of convergence and conservatism shape ecomorphological evolution in ground-dwelling squirrels. Evolution (N Y). 2018; 72: 473–489. https://doi.org/10.1111/evo.13422 PMID: 29319883

21. Meloro C, Tamagnini D. Macroevolutionary ecomorphology of the Carnivora skull: adaptations and constraints in the extant species. Zool J Linn Soc. 2021; 1–15. https://doi.org/10.1093/zoolinnean/zlab075

22. Campbell KM, Santana SE. Do differences in skull morphology and bite performance explain dietary specialization in sea otters? J Mammal. 2017; 98: 1408–1416. https://doi.org/10.1093/jmammal/gyx091

23. Remonti L, Prigioni C, Balestrieri A, Sgrosso S, Piori G. Eurasian otter (Lutra lutra) prey selection in response to a variation of fish abundance. Ital J Zool. 2010; 77: 331–338. https://doi.org/10.1080/11250000903229809

24. Clavero M, Prenda J, Delibes M. Trophic diversity of the otter (Lutra lutra L.) in temperate and Mediterranean freshwater habitats. J Biogeogr. 2003; 30: 761–769. https://doi.org/10.1046/j.1365-2699.2003.00865.x

25. Kruuk H, Moorhouse A. The spatial organization of otters (Lutra lutra) in Shetland. J Zool. 1991; 224: 41–57. https://doi.org/10.1111/j.1469-7998.1991.tb04787.x

26. Moorhouse-Gann RJ, Keany EF, Marnell F, MacDonald WI. Dietary complexity and hidden costs of prey switching in a generalist top predator. Ecol Evol. 2020; 10: 6395–6408. https://doi.org/10.1002/eco.3.3274521

27. Reid N, Thompson D, Hayden B, Marnell F, Montgomery WI. Review and quantitative meta-analysis of diet suggests the Eurasian otter (Lutra lutra) is likely to be a poor bioindicator. Ecol Indic. 2013; 26: 5–13. https://doi.org/10.1016/j.ecolind.2012.10.017

28. Meloro C O’Higgins P. Ecological Adaptations of Mandible Form in Fissiped Carnivora. J Mamm Evol. 2011; 18: 190–200. https://doi.org/10.1007/s10914-011-9156-z

29. Noonan MJ, Johnson PJ, Kitchener AC, Harrington LA, Newman C, Macdonald DW. Sexual size dimorphism in musteloids: An anomalous allometric pattern is explained by feeding ecology. Ecol Evol. 2016; 6: 8495–8501. https://doi.org/10.1002/eco.3.2480 PMID: 28031801

30. Law CJ, Duran E, Hung N, Richards E, Santillan I, Mehta RS. Effects of diet on cranial morphology and biting ability in musteloid mammals. J Evol Biol. 2018; 31: 1918–1931. https://doi.org/10.1111/jeb.13385 PMID: 30270461

31. Loy A, Spinosi O, Carlino R. Cranial morphology of Martes foina and M. martes (Mammalia, Carnivora, Mustelidae): The role of size and shape in sexual dimorphism and interspecific differentiation. Ital J Zool. 2004; 71: 27–34. https://doi.org/10.1080/11250000409356547

32. Migliorini RP, Forneì R, Kasper CB. Geographic variation in the skull morphology of the lesser grison (Galictis cuja: Carnivora, Mustelidae) from two Brazilian ecoregions. PeerJ. 2020; 8: 1–19. https://doi.org/10.7717/peerj.9388 PMID: 33194321

33. Brown WL, Wilson EO. Character Displacement. Syst Biol. 1956; 5: 49–64. https://doi.org/10.2307/2411924

34. Rohlf FJ, Slice D. Extensions of the procrustes method for the optimal superimposition of landmarks. Syst Zool. 1990; 39: 40–59. https://doi.org/10.2307/2411920

35. Zelditch M, Swiderski D, Sheets H, Fink W. Geometric Morphometrics for Biologists. New York: Elsevier Academic Press; 2004. https://doi.org/10.1016/B978-0-12-778480-1.X5000-5

36. Mitteroecker P, Gunz P. Advances in Geometric morphometrics. Evol Biol. 2008; 36: 235–247. https://doi.org/10.1007/s11692-009-9055-x

37. Klingenberg CP. Size, shape, and form: concepts of allometry in geometric morphometrics. Dev Genes Evol. 2016; 226: 113–137. https://doi.org/10.1007/s00427-016-0539-2 PMID: 27038023

38. Raia P, Meiri S. the Island Rule in Large Mammals: Paleontology Meets Ecology. Evolution (N Y). 2006; 60: 1731. https://doi.org/10.1554/05-664.1 PMID: 17017072

39. Kean EF, Bruford MW, Russo IRM, Müller CT, Chadwick EA. Odour dialects among wild mammals. Sci Rep. 2017; 7: 1–6. https://doi.org/10.1038/s41598-017-12706-8 PMID: 29051500
40. Farnell DJJ, Khor C, Ayre WN, Doyle Z, Chadwick EA. Initial investigations of the cranial size and shape of adult eurasian otters (Lutra lutra) in great Britain. J Imaging. 2020; 6. https://doi.org/10.3390/JIMAGING6010016 PMID: 34460547

41. Loy A, Ciucci P, Guidarelli G, Roccotelli E, Colangelo P. Developmental instability and phenotypic evolution in a small and isolated bear population. Biol Lett. 2021; 17. https://doi.org/10.1098/rsbl.2020.0729 PMID: 33878275

42. Falkingham PL. Acquisition of high resolution three-dimensional models using free, open-source, photogrammetric software. Palaeontol Electron. 2012; 15. https://doi.org/10.26879/264

43. Rohlf FJ. The tps series of software. Hystrix. 2015; 26: 1–4. https://doi.org/10.4404/hystrix-26.1–11264

44. Cignoni P, Callieri M, Corsini M, Dellepiane M, Ganovelli F, Ranzuglia G. MeshLab: An open-source mesh processing tool. 6th Eurographics Ital Chapter Conf 2008—Proc. 2008; 129–136. https://doi.org/10.2312/LocalChapterEvents/Ital Chap/ItalianChapConf2008/129-136

45. Evin A, Souter T, Hulme-Beaman A, Ameen C, Allen R, Viacava P, et al. The use of close-range photogrammetry in zooarchaeology: Creating accurate 3D models of wolf crania to study dog domestication. J Archaeol Sci Reports. 2016; 9: 87–93. https://doi.org/10.1016/j.jasrep.2016.06.028

46. Giacomini G, Scaravelli D, Herrel A, Veneziano A, Russo D, Brown RP, et al. 3D Photogrammetry of Bat Skulls: Perspectives for Macro-evolutionary Analyses. Evol Biol. 2019; 46: 249–259. https://doi.org/10.1007/s11692-017-9478-6

47. Bright JA, Marugán-Lobón J, Cobb SN, Rayfield EJ. The shapes of bird beaks are highly controlled by nondietary factors. Proc Natl Acad Sci U S A. 2016; 113: 5352–5357. https://doi.org/10.1073/pnas.1602683113 PMID: 27125856

48. Adams DC. Collyer ML. Package “geomorph” Title Geometric Morphometric Analyses of 2D/3D Landmark Data. 2021. Available: https://github.com/geomorphR/geomorph

49. Collyer ML, Adams DC. {RRPP}: Linear Model Evaluation with Randomized Residuals in a Permutation Procedure, R package version 0.6.2. 2021. Available: https://cran.r-project.org/package=RRPP

50. Cardini A. Lost in the Other Half: Improving Accuracy in Geometric Morphometric Analyses of One Side of Bilaterally Symmetric Structures. Syst Biol. 2016; 65: 1096–1106. https://doi.org/10.1093/sysbio/syw043 PMID: 27288476

51. de Moura Bubadue J, Cáceres N, dos Santos Carvalho R, Meloro C. Ecogeographical Variation in Skull Shape of South-American Canids: Abiotic or Biotic Processes? Evol Biol. 2016; 43: 145–159. https://doi.org/10.1007/s11692-015-9362-3 PMID: 27217595

52. Cáceres N, Meloro C, Carotenuto F, Passaro F, Sponchiado J, Melo GL, et al. Ecogeographical variation in skull shape of capuchin monkeys. J Biogeogr. 2014; 41: 501–512. https://doi.org/10.1111/jbi.12203

53. Meloro C, Guidarelli G, Colangelo P, Ciucci P, Loy A. Mandible size and shape in extant Ursidae (Carnivora, Mammalia): A tool for taxonomy and ecogeography. J Zool Syst Evol Res. 2017; 55: 269–287. https://doi.org/10.1111/jzs.12171

54. Rohlf FJ, Corti M. Use of two-block partial least-squares to study covariation in shape. Syst Biol. 2000; 49: 740–753. https://doi.org/10.1038/106351500750049806 PMID: 12116437

55. Ledevin R, Koyabu D. Patterns and Constraints of Craniofacial Variation in Colobine Monkeys: Disentangling the Effects of Phylogeny, Allometry and Diet. Evol Biol. 2019; 46: 14–34. https://doi.org/10.1007/s11692-019-09469-7

56. Olsen AM. Feeding ecology is the primary driver of beak shape diversification in waterfowl. Funct Ecol. 2017; 31: 1985–1995. https://doi.org/10.1111/1365-2435.12890

57. Adams DC, Collyer ML. On the comparison of the strength of morphological integration across morphometric datasets. Evolution (N Y). 2016; 70: 2623–2631. https://doi.org/10.1111/evo.13045 PMID: 27592864

58. Fick SE, Hijmans RJ. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int J Climatol. 2017; 37: 4302–4315. https://doi.org/10.1002/joc.5086

59. Naimi B, a.s. Hamm N, Groen TA, Skidmore AK, Toxopeus AG. Where is positional uncertainty a problem for species distribution modelling. Ecography (Cop). 2014; 37: 191–203. https://doi.org/10.1111/j.1600-0587.2013.02050.x

60. Diniz-Filho JAF, Bini LM, Hawkins BA. Spatial autocorrelation and red herrings in geographical ecology. Glob Ecol Biogeogr. 2003; 12: 53–64. https://doi.org/10.1046/j.1466-822X.2003.00322.x

61. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, R.B. O, et al. vegan: Community ecology package. R Package version 23–5. 2016. Available: https://cran.r-project.org/package=vegan
62. Bubadué J, Meloro C, Hendges C, Battistella T, Carvalho R, Cáceres N. Clinal and Allometric Variation in the Skull of Sexually Dimorphic Opossums. J Mamm Evol. 2021; 28: 185–198. https://doi.org/10.1007/s10914-020-09513-w

63. Law CJ, Venkatram V, Mehta RS. Sexual dimorphism in craniomandibular morphology of southern sea otters (Enhydra lutris nereis). J Mammal. 2016; 97: 1764–1773. https://doi.org/10.1093/jmammal/gyw148

64. Lindenfors P, Gittleman JL, Jones KE. Sexual size dimorphism in mammals. Sex, Size Gend Roles Evol Stud Sex Size Dimorphism. 2007; 2005. https://doi.org/10.1093/acprof:oso/9780199208784.003.0003

65. Law CJ. Solitary meat-eaters: solitary, carnivorous carnivorans exhibit the highest degree of sexual size dimorphism. Sci Rep. 2019; 9: 1–10. https://doi.org/10.1038/s41598-019-51943-x PMID: 31653949

66. Pérez-Barbería FJ, Gordon IJ, Pagel M. The origins of sexual dimorphism in body size in ungulates. Evolution (N Y). 2002; 56: 1276–1285. https://doi.org/10.1111/j.0014-3820.2002.tb01438.x PMID: 12144026

67. Weckerly FW. Sexual-size dimorphism: Influence of mass and mating systems in the most dimorphic mammals. J Mammal. 1998; 79: 33–52. https://doi.org/10.2307/1382840

68. Emlen ST, Oring LW. Ecology, sexual selection, and the evolution of mating systems. Science (80-). 1977; 197: 215–223. https://doi.org/10.1126/science.327542 PMID: 327542

69. Kruuk H, Moorhouse A. Seasonal and spatial differences in food selection by otters Lutra lutra in Shetland. J Zool. 1990; 221: 621–637. https://doi.org/10.1111/j.1469-7998.1990.tb0421.x

70. Lynch JM O’Sullivan WM. Cranial form and sexual dimorphism in the Irish otter Lutra lutra L. Biol Environ Proc R Irish Acad. 1993; 93B: 98; 97–105. Available: https://www.jstor.org/stable/20499883

71. James Rohlf, Marcus LF. A revolution morphometrics. Trends Ecol Evol. 1993; 8: 129–132. https://doi.org/10.1016/0169-5347(93)90024-J PMID: 21236128

72. Meiri S, Dayan T. On the validity of Bergmann’s rule. J Biogeogr. 2003; 30: 331–351. https://doi.org/10.1046/j.1365-2699.2003.00837.x

73. Claus M, Dittmann MT, Müller DWH, Meloro C, Codron D. Bergmann’s rule in mammals: A cross-species interspecific pattern. Oikos. Paper manu. 2013; 122: 1465–1472. https://doi.org/10.1111/j.1600-0706.2013.00463.x

74. Yom-Tov Y, Roos A, Mortensen P, Wiig Ø, Yom-Tov S, Heggberget TM. Recent Changes in Body Size of the Eurasian Otter Lutra lutra in Sweden. Ambio. 2010; 39: 496. https://doi.org/10.1007/s13280-010-0074-8 PMID: 21090004

75. Ashton KG, Tracy MC, De Queiroz A. Is Bergmann’s rule valid for mammals? Am Nat. 2000; 156: 390–415. https://doi.org/10.1086/303400 PMID: 2992141

76. Timm-Davis LL, DeWitt TJ, Marshall CD. Divergent skull morphology supports two trophic specializations in otters (lutrinae). PLoS One. 2015; 10: 1–18. https://doi.org/10.1371/journal.pone.0143236 PMID: 26649575

77. Kitchener AC, Meloro C, Williams TM. Form and function of the musteloids. Biology and Conservation of Musteloids. 2018. https://doi.org/10.1093/oso/9780198759805.003.0003

78. Law CJ, Young C, Mehta RS. Ontogenetic scaling of theoretical bite force in southern sea otters (Enhydra lutris nereis). Physiol Biochem Zool. 2016; 89: 347–363. https://doi.org/10.1086/688313 PMID: 27617357

79. Tseng ZJ, Flynn JJ. Structure-function covariation with nonfeeding ecological variables influences evolution of feeding specialization in Carnivora. Sci Adv. 2018; 4. https://doi.org/10.1126/sciadv.aao5441 PMID: 29441363

80. White P, Kruuk H. Wild Otters: Predation and Populations. J Anim Ecol. 1996; 65: 131. https://doi.org/10.2307/5710

81. Deby LB, Pyenson ND. Osteological correlates and phylogenetic analysis of deep diving in living and extinct pinnipeds: What good are big eyes? Mar Mammal Sci. 2013; 29: 48–83. https://doi.org/10.1111/j.1748-7692.2011.00545.x

82. Nolet BA, Kruuk H. Grooming and resting of otters Lutra lutra in a marine habitat. J Zool. 1989; 218: 343–344. https://doi.org/10.1111/j.1469-7998.1989.tb05555.x

83. Nolet BA, Wansink DEH, Kruuk H. Diving of Otters (Lutra lutra) in a Marine Habitat: Use of Depths by a Single-Prey Loader. J Anim Ecol. 1993; 62: 22. https://doi.org/10.2307/5479

84. Durbin LS. Habitat selection by five otters Lutra lutra in rivers of northern Scotland. J Zool. 1998; 245: 85–92. https://doi.org/10.1017/j.1469-7998.1998.tb00075.x
85. Garcia De Leaniz C, Forman DW, Davies S, Thomson A. Non-intrusive monitoring of otters (Lutra lutra) using infrared technology. J Zool. 2006; 270: 577–584. https://doi.org/10.1111/J.1469-7998.2006.00124.X

86. Quaglietta L, Mira A, Boitani L. Extrinsic and intrinsic factors affecting the daily rhythms of a semiaquatic carnivore in a Mediterranean environment. Hystrix, Ital J Mammal. 2018; 29: 128–136. https://doi.org/10.4404/HYSTRIX-00022-2017

87. Loy A. Mustelidae Morphology. Encyclopedia of Animal Cognition and Behavior. Cham: Springer International Publishing; 2018. pp. 1–13. https://doi.org/10.1007/978-3-319-47829-6_1210–1

88. Schiaffini MI, Segura V, Prevosti FJ. Geographic variation in skull shape and size of the Pampas fox Lycalopex gymnocercus (Carnivora: Canidae) in Argentina. Mamm Biol. 2019; 97: 50–58. https://doi.org/10.1016/j.mambio.2019.04.001

89. Yom-Tov Y, Heggberget TM, Wiig Ø, Yom-Tov S. Body size changes among otters, Lutra lutra, in Norway: The possible effects of food availability and global warming. Oecologia. 2006; 150: 155–160. https://doi.org/10.1007/s00442-006-0499-8 PMID: 16868759

90. Crandall KA, Bininda-Emonds ORR, Mace GM, Wayne RK. Considering evolutionary processes in conservation biology. Trends Ecol Evol. 2000; 15: 290–295. https://doi.org/10.1016/s0169-5347(00)01876-0 PMID: 10856956