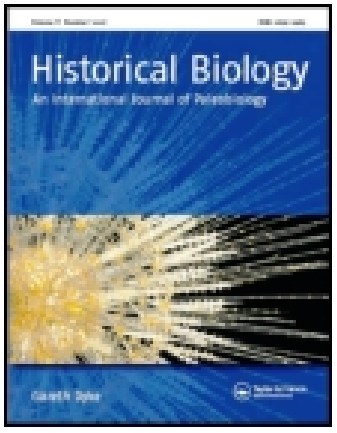

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Multidisciplinary investigation of a 'British big cat': a lynx killed in southern England c. 1903

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The alleged presence of non-native felid species in the British countryside – popularly, though in part erroneously, known as 'British big cats' or 'alien big cats' – is a long-standing and controversial topic, perennially of interest to both the mass media and amateur naturalists, and with little apparent acceptance from the technical zoological community. Nevertheless, a number of carcasses and captured live specimens have demonstrated the occasional presence within the region of escapees that potentially explain at least some 'British big cat' eyewitness records. We report here the existence of a probable Canada lynx, *Lynx canadensis*, shot in Newton Abbot, Devon, England, in or prior to 1903, and then accessioned to Bristol Museum and Art Gallery. The specimen (represented by extensive skeletal material and a stuffed taxidermy mount) is Bobcat-like in some respects but is identified as a Canada lynx on the basis of skeletal morphology with a high degree of support; attempts to extract DNA were unsuccessful. Stable strontium isotope analysis supports either a recent introduction from western Canada or long-term acclimation to the local area of Devon where it was collected. Although the specimen was undoubtedly an 'alien' (an escapee or release from a collection), it is significant as material evidence in demonstrating, for the first time, the presence of a wild-caught, feral, exotic felid dating to the early years of the twentieth century.

Keywords: *Lynx canadensis*; *Lynx lynx*; ancient DNA; isotopes; linear discrimenant analysis; Devon

1. Introduction

The presence within the modern British ecosystem of non-native cats remains controversial. A substantial number of eyewitness accounts describe animals that have been interpreted as Puma (*Puma concolor*), Leopard (*Panthera pardus*), Eurasian lynx (*Lynx lynx*), Jungle cat (*Felis chaus*) and Leopard cat (*Prionailurus bengalensis*). Taken alone, it might be possible to dismiss these numerous sightings as misidentifications, hoaxes or hallucinations. However, photographic and field evidence provide support for the presence of non-native felids – popularly dubbed 'British big cats' (even though most are not big cats in the strict sense of the term) – within the British countryside. Tracks, hairs and scat reportedly left by non-native felids have been reported, whereas the carcasses of deer, sheep and other species have also been discovered and suggested to provide evidence for the presence of non-native cats in Britain (McGowan 2007). Unfortunately, there has thus far been little effort to present these data within the peer-reviewed literature. Coard (2007), however, showed that bite marks present on Welsh sheep bones correspond to the dentition of a 'medium-sized felid' and hence provide support for the existence of exotic felids in the British fauna.

Several carcasses and even captured specimens further demonstrate the occasional presence of non-native cats within the British countryside. Although it can be argued that these individuals represent mere rare escapees – present in the British countryside for a fleeting span of time – it remains little appreciated that the existence of such escapees both verifies and potentially explains – in part – the 'British big cat' phenomenon. Two lynxes were reportedly shot in Scotland during the 1920s and apparently sent to London Zoo (Shuker 1989); their current whereabouts are unknown. A Eurasian lynx was shot by a farmer in Suffolk, 1991: the carcass was photographed (Shuker 1995) but was buried in an unknown location. Five Leopard cats have been killed or captured in Britain and two dead Jungle cats (Hayling Island, 1988 and Shropshire, 1989) have been recovered (Shuker 1989; Minter 2011). In 1980, a live Puma was captured at Cannich, Inverness-shire; the animal's scat showed that it had been living wild for an extended period (Shuker 1989). A live Eurasian lynx was captured in London in 2001 (Minter 2011). It is not doubted that these animals were escapes (or, in cases, possibly deliberate releases) from captivity. A popular hypothesis is that exotic felids were only released into the British countryside following the introduction of the 1976 Dangerous

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Wild Animals Act. However, sightings that substantially pre-date 1976 cast doubt on the idea that this one piece of legislation explains all exotic felid releases in the UK: it seems more likely that escapes and releases have occurred throughout history, and that this continual presence of aliens explains the 'British big cat' phenomenon.

Over the years, several skeletal elements claimed to represent the remains of 'British big cats' have been discovered within Britain, but (to date) all can be explained as fraudulent. They include a large *Panthera* skull found near Newton Abbot, Devon, in 1988, fragments of a tiger skull discovered in Exmoor in 1993 and a leopard skull discovered near the River Fowey, Devon, in 1995 (Shuker 1989; Minter 2011). The latter specimen was presented as definitive evidence for the presence of non-native felids in Britain but subsequent investigation revealed the presence of a tropical cockroach ootheca within the skull's nasal cavity, thereby demonstrating an origin in the tropics.

We report here the discovery of a lynx, a medium-sized felid represented by much of the skeleton and by the skin (incorporated into a taxidermy mount) and accessioned as specimen Ab4458 (Figures 1 and 2) in the collections of the Bristol Museum and Art Gallery. This individual was donated by a Mr J. Niblet of Newton Abbot, Devon, south-west England, and accessioned to the collection on 26 February 1903 (Figure 3). The specimen's locality is given in the museum records as 'Newton Abbot' (Figure 4). We are confident that this is a reference to the place where it was shot since foreign specimens are clearly marked with their place and country of origin. The associated hand-written records are difficult to read, but it seems that the specimen was shot by a Mr Heb (?) after killing two dogs. Despite a search of local newspaper archives and biological records, we have thus far been unable to find any additional reference to this animal.

Figure 1. Specimen Ab4458 (Bristol Museum and Art Gallery), as mounted. The specimen's tail markings, proportionally short legs, silvery-brown pelt and black markings on the ventral parts of its facial ruff support identification as Canada lynx.

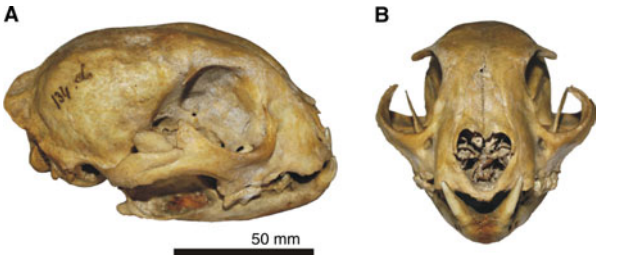

Figure 2. Skull of specimen Ab4458 (Bristol Museum and Art Gallery) in (A) right lateral and (B) anterior view.

We present the results of a multidisciplinary analysis of specimen Ab4458, utilising morphometrics, ancient DNA (aDNA) and strontium stable isotopes to investigate species status, potential source population and length of residency in the wild.

2. Materials and methods

2.1 Gross morphology

The taxidermied mount and skeletal elements of specimen Ab4458 were carefully examined to find features that could provide evidence for a species-level identification of the animal. In addition, the material was scrutinised for any evidence of unusual features, including congenital or acquired pathologies.

2.2 Ancient DNA analysis

Samples of hair from the taxidermied specimen were analysed in a dedicated aDNA laboratory at Durham University. The aDNA workspace is separated from any molecular biology work, and employs a strict regime of sterilisation to avoid potential routes of contamination

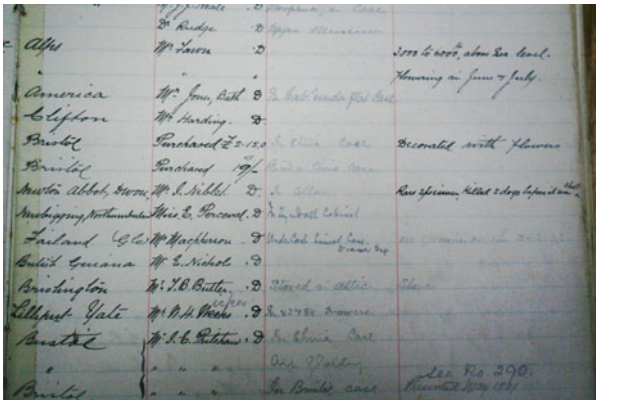

Figure 3. Photo of Bristol Museum and Art Gallery catalogue page documenting accession of specimen Ab4458.

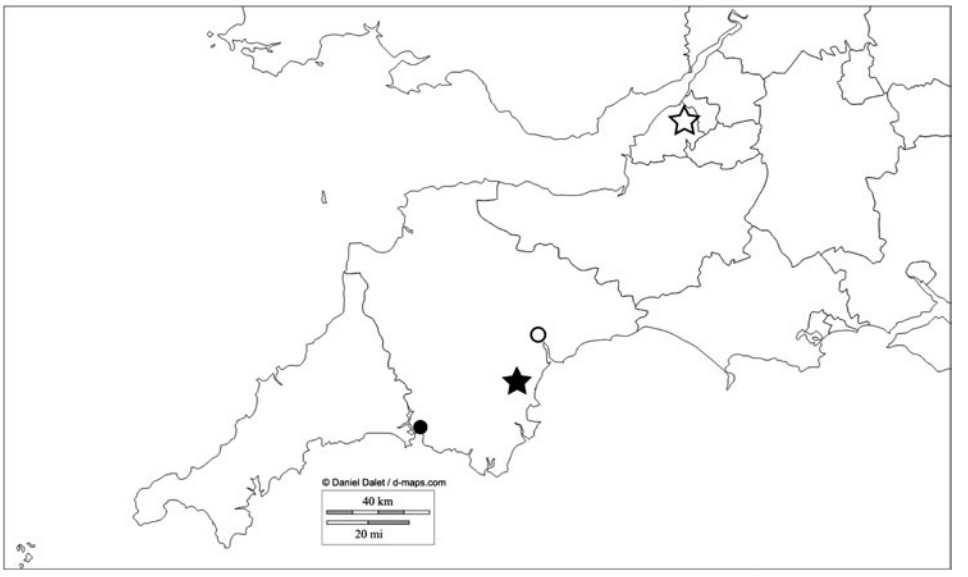

Figure 4. Map of Devon, south-west England, showing location of Newton Abbot (black star), the location at which specimen Ab4458 was shot in or prior to February 1903. Also shown are the cities of Bristol (white star), Plymouth (black circle) and Exeter (white circle).

(Gilbert et al. 2005). All open surfaces and glove-boxes are cleaned daily with bleach solution (20% sodium hypochlorite) and ethanol (100%), and work areas, equipment and appropriate reagents are regularly exposed to sterilising UV radiation via a crosslinker or handheld lamp (Cooper and Poinar 2000). All pipette tips are filtered and all plastic ware is used once.

Three separate extractions of lynx hair were attempted, using 1, 5 and 20 hairs, respectively. Each extraction was done in parallel with a negative extraction control (Gilbert et al. 2005). DNA extraction involved a modified version of Kim's ion-exchange column method (Rohland and Hofreiter 2007; Kim et al. 2008). Hairs were manually washed in 20% bleach solution and then rinsed with 100% ethanol to remove any absorbed DNA contaminants before being digested overnight at 50°C in 1 ml of buffer (0.425 M ethylene diamine tetra-acetic acid pH 8, 1 mM Tris–HCl, pH 8, 0.05% (w/v) sodium dodecyl sulphate, 0.33 mg/ml Proteinase K, 40 mM dithiothreitol, 5 mM $CaCl_2$) under constant rotation (Gilbert et al. 2007). The digested solution was then concentrated to approximately 500 µl using centrifugal filters with a molecular weight cut-off of 30 kDa (Amicon® Ultra, Millipore, Abingdon, UK). The concentrated solution was then passed through a silica column (QIAquick®, Qiagen, Manchester, UK) following the manufacturer's protocol, before final elution in 100 µl of Tris-EDTA buffer. Two microlitres of extract were measured for total DNA concentration using the Qubit® platform (Invitrogen, Paisley, UK). Extracts were stored at −20°C.

Primers were chosen that were suitable for Felidae, gave products of <150 bp (ATP8_1F/ATP8_3R and ND5_6F/ND5_7R) and had been shown previously to work with low template copy extracts (Barnett et al. 2005). polymerase chain reactions (PCRs) used KAPA2G Robust HotStart (KAPA Biosystems, Woburn, MA, USA) according to manufacturer's guidelines, with a 90-s activation step at 95°C, followed by 45 cycles of 95°C for 45 s, 56°C for 45 s and 72°C for 45 s with a final extension at 72°C for 5 min. Negative PCR controls were also included (with the addition of water instead of DNA extract) with each experiment. PCRs were visualised on an agarose gel.

2.3 *Strontium stable isotope analysis*

Strontium isotope analysis is a well-established method used to research issues of migration and origins. The ratio of $^{87}Sr/^{86}Sr$ varies between different geological formations of the Earth's crust according to the age and composition of the rock. An animal's food and water sources have $^{87}Sr/^{86}Sr$ values which reflect the underlying geology of their areas of origin due to the weathering of strontium from ground-rock into soils and water (Bentley 2006). These ratios are transferred unfractionated into the body's tissues upon ingestion (Knudson et al. 2010), with strontium substituting easily for calcium in the mineral lattice of bone and dental enamel due to its similar size and valency (Ericson 1985). Strontium isotope ratios are 'fixed' in bone and enamel during formation of the bone. For dental enamel, this means that they are set during infancy. Bone, however, remodels throughout life and, therefore, Sr isotope ratios from bone reflect habits up to time of death (e.g. Parfitt 1983).

Strontium isotope ratios in bone can be used to show whether a specimen was living in the area, in which it was found prior to its time of death. The area surrounding Newton Abbot (the provenance of this sample) is formed

Table 1. Expected Sr values for Canadian/Devon origins based on previous geological studies.

Area of origin	Formation/geology	Expected $^{86}Sr/^{87}Sr$	Study
Canada	Canadian shield Grenville province granites	0.7098–0.7200	Millot et al. (2002)
	Canadian shield Slave province granites	0.7200–0.7517	Millot et al. (2002)
	Western Canadian sedimentary basins	0.7074–0.709260	Holmden et al. (1997)
	Northwestern marine sediments (Upper Tindir Group)	~0.7065	Kaufman et al. (1992)
	Devonian Canadian dolomites	0.7080–0.7083	Mountjoy and Qing (1992)
Newton Abbot	Dartmoor granite	0.7130–0.7166	Darbyshire and Shepherd (1985)
	Devonian–Carboniferous limestones, slates, etc.	~0.7080	Popp et al. (1986) (seawater Sr data)
	Permian marine formations	~0.7070	Popp et al. (1986) (seawater Sr data)
	Cretaceous greensands	0.70730–0.70744	Jones et al. (1994) (seawater Sr data)

primarily of marine shales, slates, limestones and greensands ranging in age from Devonian to Cretaceous (Selwood et al. 1984). Lying just to the north, but potentially within the home range of a lynx (up to 35 km^2 (Parker et al. 1983)), is the Devonian–Carboniferous Dartmoor Granite complex, comprising S-type granite and dolomite (Selwood et al. 1984).

In contrast, the natural range of the Canada lynx in Canada covers a wide area comprising many different rock types of different ages. Most notable are the granites/gneisses of the Canadian shield. These date from the Precambrian and are, in general, highly felsic (Millot et al. 2002). In the west of the range, however, we find Devonian sedimentary basins comprising dolomites, limestones and greensands (e.g. Holmden et al. 1997). Table 1 gives expected Sr values of the two possible areas of origin based on previous isotopic study of the underlying geology/groundwater.

Analysis was conducted upon two bone fragments removed using a surgical scalpel from the second right metatarsal. The bone had not been subject to diagenetic processes as it was never exposed on the ground's surface, thus intensive treatment to remove diagenetic strontium was not necessary. Nevertheless, as a precaution both fragments were leached overnight in 10 vol.% acetic acid, before removal of the leachate and washing with MilliQ, then drying down (cf. Koch et al. 1997). One fragment was also ashed in a muffler furnace at 500°C for 12 h, in order to assess whether any appreciable difference in Sr isotope ratio was achieved through further treatment for diagenesis.

Bone samples were dissolved in 3 N HNO_3; strontium was purified from this solution by running through columns of Sr spec resin prior to analysis. Purified strontium was analysed for $^{87}Sr/^{86}Sr$ ratio using a ThermoFinnigan Neptune plasma ionisation MC-ICP-MS at the Northern Centre for Isotope Analysis, University of Durham. $^{87}Sr/^{86}Sr$ was normalised using repeated measurements of the NBS 987 standard ($^{87}Sr/^{86}Sr = 0.71024$) which gave an average $^{87}Sr/^{86}Sr$ value of 0.71027 ± 0.000017 (2SD, $n = 9$). To ensure contamination of samples was not occurring during sample preparation, we prepared and ran point calibrations of a blank. This contained only 13 pg of Sr.

2.4 *Morphometric analysis*

Traditional morphometrics (linear measurements) adjusted for size through Mosimann transformation (Mosimann 1970) have recently been shown to reliably predict a priori lineage-level taxonomic delimitations (Sakamoto and Ruta 2012). Here, we take the same 29 cranial variables, and conducted a linear discriminant analysis (LDA) at the species level on a training set of 310 cranial specimens covering 33 extant felid species. We performed a leave-one-out cross-validation to assess the accuracy of the discriminant functions in correctly classifying individuals of known taxonomic identity. The discriminant functions derived from the training set were then used to predict the taxonomic affinity of Ab4458 based on the 29 Mosimann-transformed variables for this specimen. See Sakamoto and Ruta (2012) for further details on the morphometric variables, LDA and the measurements for the 310 specimens including Ab4458. LDA and all data processing were conducted in R (R Core Development Team 2011).

3. Results

3.1 *Gross morphology results*

Ab4458 (Figure 1) is clearly a lynx, but in the four modern species: Bobcat, *L. rufus*; Iberian, Spanish or Pardel lynx, *L. pardinus*; Canada lynx, *L. canadensis* and Eurasian or Northern lynx, *L. lynx*, there is a degree of overlap in external morphology that can make specific identification difficult. Of these species, the Iberian and Eurasian lynx can be excluded from comparison in view of a tawny ground colour and extensive amount of dark spotting. The specimen's proportionally short legs, silvery-brown pelt and black markings on the ventral parts of its facial ruff tentatively suggest identification as a Canada lynx, *L. canadensis* Kerr, 1792. However, it is also similar to a plain-coated morph of Bobcat in possessing dark facial markings ventral to the eyes and an apparently relatively long tail that is whitish on its ventral surface (Hunter and Barrett 2011). Unlike a Bobcat, however, the specimen lacks obvious dark dorsal markings on the tail, and the

Table 2. DNA extracts from hair specimen Ab4458.

Extract number	Number of hairs	DNA concentration (ng/μl)	Amplifications ATP8 (result)	Amplifications ND5 (result)
RB441	1	0.336	n/a	n/a
RB442	0 (Control)	0.145	X69.4 (pig)	n/a
RB443	1	0.121	n/a	n/a
RB444	0 (Control)	0.162	n/a	n/a
RB456	5	<0.0.5	X72.1 (pig?)/X69.1 (dog)	n/a
RB457	0 (Control)	<0.0.5	n/a	n/a
Lynx1	20	<0.0.5	n/a	X73.9 (pig)
LynxCex	0 (Control)	<0.0.5	n/a	n/a

posterior surfaces of its hind feet are not noticeably dark. The specimen's size is in potential agreement with either identification (shoulder height 39 cm; total length 74 cm). In view of this ambiguity, we are unable to determine from pelage characters whether Ab4458 should be identified as either a Canada lynx or Bobcat.

The taxiderm mount is associated with the better part of a skeleton, assumed to belong to the same individual (the mount does not appear to contain any original bone). The appearance of the skull is consistent with identification as *L. canadensis*: as is characteristic for *Lynx* (Russell et al. 1995; Garcia-Perea 1996), the rostrum appears proportionally short and P2 is absent. Analysis of the skull (Figure 2) showed that the specimen had lost all upper and lower incisors, with the resulting alveoli replaced by new bone. Investigation of the teeth showed that all upper and lower premolars (P3, P4, p3 and p4) were covered with an abundant build-up of dental calculus.

3.2 *Ancient DNA results*

All PCR negative controls were clean (Table 2). Only one extraction control produced an amplification product. All sequences resulting from amplifications appear to be the result of reagent contaminants introduced during the extraction or PCR process. The presence of domestic animal contaminants in aDNA PCRs has been shown to occur due to combination of carrier effect and exposure of reagents to animal products (Leonard et al. 2007) (e.g. pork gelatin during *Taq* enzyme purification, dNTPs derived from animal sources). The inability to amplify endogenous DNA from the lynx hair is surprising, but may be due to treatment of the pelt during taxidermy with unknown chemicals that could have acted to oxidise, crosslink or hydrolyse the DNA.

3.3 *Strontium isotope results*

Isotopic analysis gave a $^{87}Sr/^{86}Sr$ ratio of 0.70936 ± 0.000015 (2SD) for the sample which was not ashed prior to dissolution and 0.70936 ± 0.000013 (2SD) for the ashed sample. There is little appreciable difference between the two values, which indicates that very little diagenetic material was present within the sample.

This isotopic value unfortunately cannot be used to provenance the lynx precisely. The values obtained certainly preclude an origin within the Precambrian Canadian shield, as Sr ratios from the shield would be far more radiogenic than we find here. The value could, however, be explained either with a western Canadian origin or with a mixing between Dartmoor granite and marine sediments of the Newton Abbot area (cf. Montgomery et al. 2002). This is shown graphically in Figure 5.

3.4 *LDA results*

The overall classification accuracy assessed through leave-one-out cross-validation was 90.6%, ranging from 60% in *Caracal aurata* to 100% in a number of species including *L. canadensis* and *L. pardinus*. An 88.9% of *L. lynx* and 75% of *L. rufus* specimens were correctly assigned to their respective taxonomic identities (Table S1; see Table S2 for posterior probabilities of individual specimens). Using the linear discriminant functions derived from this training set, our specimen of interest, Ab4458, was classified as *L. canadensis* with a posterior probability of 99.9%.

4. Discussion

Misidentification of historic specimens is a common problem in museum collections (e.g. Barnett et al. 2007).

Figure 5. Lateral view of P3–P4 in specimen Ab4458 showing build-up of dental calculus. Scale bar = 10 mm.

As mounted, Ab4458 appears unusual and several of the features that ordinarily enable plain-coated Bobcats to be distinguished from Canada lynx (e.g. greater relative tail length, white belly and dark posterior surfaces to the hind feet in the Bobcat) have been distorted or are not obvious: the belly is not obviously white and the posterior surfaces of the hind feet are not obviously dark, for example. The dark facial streaks in Ab4458 are reminiscent of the facial markings seen in Bobcat; the tail is partially white ventrally (as is typical of Bobcat and not of Canada lynx), but the dark dorsal tail markings characteristic of Bobcat are not unambiguously present. Owing to this morphological ambiguity, external morphological characters alone do not allow us to identify the specimen as either a plain-coated Bobcat or a Canada lynx. If we are correct in associating the taxiderm mount with the lynx bones accessioned together as Ab4458, analysis of the bones provides an independent test of the specimen's species-level identification. Traditional morphometric analyses have recently been demonstrated to correctly assign taxonomic affinity with a high level of accuracy (Sakamoto and Ruta 2012). Our LDA results lend very strong support for a *L. canadensis* classification for Ab4458 over any other felid species.

Our initial interest in the lynx specimen analysed here was inspired by the possibility that evidence for late-surviving Eurasian lynx, *L. lynx*, specimens might be sought in extant museum collections. Until recently, *L. lynx* was regarded as a prehistoric component of the British fauna that failed to survive beyond the end of the Pleistocene. Since 1999, radiocarbon dating of several British lynx specimens has shown that Eurasian lynxes persisted until far more recently. Specimens from Devon and Derbyshire have provided dates of 8800–9500 years before present (Coard and Chamberlain 1999; Bronk Ramsey et al. 2002), whereas a lynx discovered at Reindeer Cave, Sutherland, is approximately 1770 years old and was hence alive in the third century AD (Kitchener and Bonsall 1997). Lynx bones from North Yorkshire revealed even younger ages of about 1550 years old, dating these specimens to the fifth or sixth century (Hetherington et al. 2006). Hetherington et al. (2006) noted that references to the lynx in seventh century lullabies and stories hinted at its survival within the region at this date, a possibility perhaps strengthened by the fact that north-west England was densely forested at time.

Several authors have informally mooted the possibility that *L. lynx* might have survived from early historic times to as recently as the eighteenth, nineteenth or twentieth century (Heuvelmans 1986; Shuker 1989). Evidence for such late survival is lacking and also appears inconsistent with the substantial exploitation of the British large mammal fauna and thorough exploration of Britain's remaining wild habitat following the Agricultural Revolution. In the absence of further evidence, we feel that it is safe to assume that the Eurasian lynx was most likely extinct as a British native beyond the seventh century. Ergo, any lynx living wild in Britain in more recent times must be assumed introduced.

The discovery of a 'historic', non-native lynx specimen is, although undoubtedly of great interest, predominantly a historical curiosity. Those who endorse the reality of British big cats have argued (based on anecdotal observations, possibly extending back to the 1800s and to dubious references in folklore) that such animals may have been present in the country for an extended period of time (decades or centuries). The presence of a non-native species from the early 1900s could endorse this hypothesis of extended residence time and establishment of a possible breeding population; however, although the individual represented by Ab4458 may well have lived in the wild for an extended period, the possibility that it was a recent escapee remains equally plausible. Nevertheless, the animal remains are significant in representing the first 'historic big cat' from Britain, verifying the existence of such escapees.

We hoped to determine whether the lynx had been resident in the wild for an extended time, because it is sometimes possible to determine, from osteology alone, whether a felid has lived for years in captivity: Duckler (1998) showed that captive tigers had deeper occipital regions than wild tigers (apparently due to excessive grooming), whereas O'Regan (2001) found that the skulls of captive big cats were wider across the zygomatic arches than those of wild-living animals (for reasons unknown). Loss of the lynx's incisors (Figure 2) and overgrowth of the incisive alveoli by additional bone suggest that periodontal disease may have affected the individual. This condition is known to affect domestic cats and other carnivorans fed on soft or wet diets (Watson 1994). In addition, both the upper and lower premolars (P3, P4, p3 and p4) show extensive calculus accumulation, i.e. plaque build up, to the point that the buccal surfaces of the premolars are completely encrusted (Figures 6 and 7).

Figure 6. Ventral view of P3–P4 in specimen Ab4458 showing build-up of dental calculus. Scale bar = 10 mm.

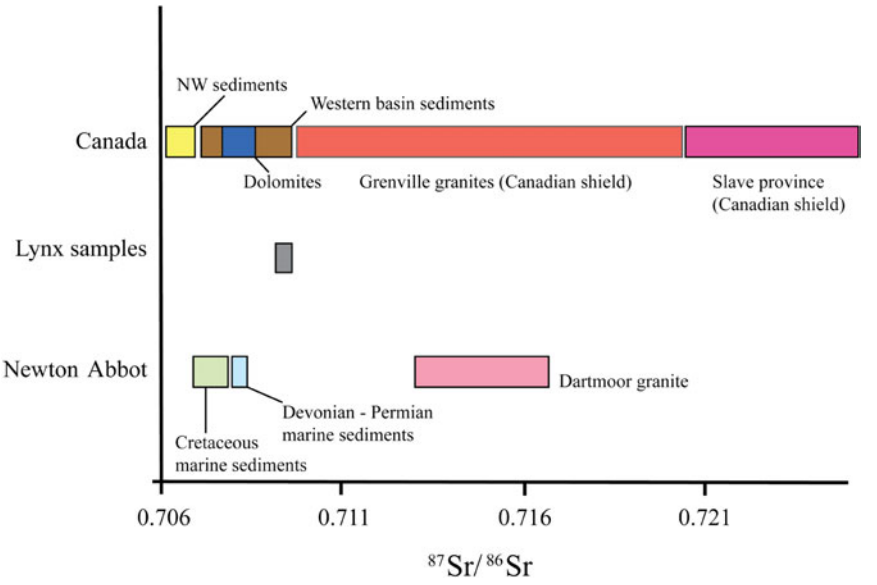

Figure 7. Showing how isotopic values from this study are consistent with both western Canadian origins and mixing between Dartmoor granite/nearby marine sediments.

Such excessive plaque calcification is common in captive animals (Glatt et al. 2008), and is also considered to be a form of periodontal disease, associated with a prolonged diet of soft/wet foods (Watson 1994). A previous study on captive Amur tigers noted that dental calculus formation is associated with age, 'beginning on the buccal surfaces of the third and fourth maxillary premolars of eight-year old animals, progressing to the maxillary canines, and finally to the buccal mandibular and lingual maxillary surfaces in animals aged 11–17 years' (Haberstroh et al. 1984, p. 143). The pattern of calculus accumulation observed in Ab4458 (buccal surfaces of the maxillary and mandibular premolars) is consistent with the later stages described by Haberstroh et al. (1984): if a similar age–calculus relationship can be assumed for lynxes, it is likely that Ab4458 was at least 10–11 years old at the time of death, and lived for most of its life in captivity, feeding on soft, non-abrasive foods (Figure 5).

Lynxes (and other felids) can undoubtedly survive in the British countryside without problem. This assertion is demonstrated not only by the lynx, puma, leopard cat and jungle cat specimens discussed here, but also by the survival in the wild of a Clouded leopard (*Neofelis nebulosa*) that escaped from Howlett's Zoo in 1975 and then survived for 9 months until shot by a farmer. We feel that the discovery of the Newton Abbot lynx is worthy of note as it verifies the presence of a medium-sized non-native cat long prior to the implementation of the 1976 Dangerous Wild Animals Act. This provides further support for the proposal that non-native felids have been an occasional but continuous presence in Britain for decades.

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