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Ancient DNA analysis indicates the first English lions originated from North Africa

Ross Barnett[1], Nobuyuki Yamaguchi[2], Beth Shapiro[1], Richard Sabin[3]

[1] *Henry Wellcome Ancient Biomolecules Centre, University of Oxford, South Parks Road, Oxford, OX1 3PS UK*
[2] *Wildlife Conservation Research Unit, University of Oxford, Tubney House, Abingdon Road, Tubney, Abingdon, OX13 5QL UK, nobuyuki.yamaguchi@zoo.ox.ac.uk; present address: Department of Biological Sciences, University of Qatar, P.O. Box 2713, Doha, Qatar, yamaguchi@qu.edu.qa (corresponding author)*
[3] *Department of Zoology, Natural History Museum, Cromwell Road, London, SW7 5BD UK*

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Abstract

The Royal Menagerie of England was established at the Tower of London in the 13th Century and served as a home of exotic animals until it was closed on behalf of the Duke of Wellington in 1835. Two well-preserved lion skulls recovered from the moat of the Tower of London were recently radiocarbon-dated to AD 1280-1385 and AD 1420-1480, making them the earliest confirmed lion remains in the British Isles since the extinction of the Pleistocene cave lion. Using ancient DNA techniques and cranio-morphometric analysis, we identify the source of these first English lions to lie in North Africa, where no natural lion population remains today.

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Introduction

The lion *Panthera leo* (Linnaeus, 1758) is a charismatic large cat that has been imported into Europe since early historic times. Lions were amongst the many exotic animals that were imported to Rome during the early Imperial Period for the gladiatorial games, although towards the end of the Roman Empire the increased scarcity of these animals in the wild forced combat shows to be largely replaced by exhibitions (Baratay and Hardouin-Fugier, 2002). Exotic wild animals do not appear to have been kept regularly in western Europe until the 13th century, when they were rediscovered through Western contact with the Byzantine and Muslim worlds (Baratay and Hardouin-Fugier, 2002). In England, the Royal Menagerie was established in 12th-13th centuries in Woodstock near Oxford, and slightly later was relocated to the Tower of London, where the first residents were three leopards sent to Henry III by the Holy Roman Emperor Frederick II in 1235 (Hahn, 2003).

Although the Royal Menagerie and its animals are known from documentary records, few physical remains survive (O'Regan *et al.,* 2005). Amongst the rare exceptions are two lion skulls that were recovered from the moat of the Tower of London during excavations in 1936-1937. These skulls were recently radiocarbon-dated to AD1280-1385 and AD1420-1480, making them the earliest confirmed lion remains in the British Isles since the extinction of the Pleistocene cave lion (*P. l. spelaea*) (O'Regan *et al.,* 2005). The discovery of these first English lions attracted significant media attention (BBC, 2005). However, the geographical origin of these animals has not yet been investigated. Such knowledge would provide novel insights not only into the history of the Royal Menagerie, but also into patterns of animal trafficking during the Medieval period. Direct animal trade between Europe and sub-Saharan Africa was not well developed until the 18th century (Anonymus, 1876). Therefore, it may be reasonable to presume that the Tower lions were unlikely to have originated from sub-Saharan regions. Nevertheless, there is an undeniable possibility that sub-Saharan lions reached Europe as they could have

reached shipping ports in North Africa and the Middle East through trans-Saharan trade routes that were well established by the early Medieval period (Yamaguchi, 2000b). Apart from a tiny population in northwest India, lions had been practically exterminated outside sub-Saharan Africa by the turn of the 20th century (Yamaguchi and Haddane, 2002; Patterson, 2004). In this context, if the foregoing first hypothesis turned out to be the case, the Tower lion skulls would possess significant value for the history of the lion, as well as the history of Medieval England.

Recent advances in ancient DNA (aDNA) techniques (e.g. Shapiro *et al.,* 2004), in association with the available data concerning genetic profiles of the lion across its natural range (Dubach *et al.,* 2005; Barnett *et al.,* 2006a, 2006b) have made it possible to identify the origins of unprovenanced lion specimens, such as the Tower lions (Barnett *et al.,* 2007).

In this paper we use aDNA techniques to extract and amplify mitochondrial DNA (mtDNA) from the two Tower lions, and compare the results with those of Barnett *et al.* (2006a, 2006b). We also conduct a cranio-morphometric analysis to investigate which lion population the Tower lions are morphologically similar to. Then, by combining both molecular and morphological results, we will try to determine the geographic origin of the first lions in England.

Materials and methods

Sampling and laboratory procedures

Small pieces of cortical bone (c. 5 × 5 × 2 mm) were sampled from the mandibles of the two Tower lion skulls (registration numbers NHM1952.10.20.15 and NHM1952.10.20.16) at the Natural History Museum, London, UK. Laboratory procedures were carried out as described in Barnett *et al*. (2006a) at the Henry Wellcome Ancient Biomolecules Centre (ABC), Oxford University, which is geographically isolated from modern molecular biology work and DNA amplification by polymerase chain reaction (PCR).

Data authenticity and analysis

Extraction of specimens NHM1952.10.20.15 and NHM 1952.10.20.16 took place in the ABC and was performed along with negative extraction controls. PCR amplification of a small hypervariable fragment of the mitochondrial control region was performed twice for each sample, each time incorporating negative amplification controls. The four resulting amplification products were then cloned using the TOPO TA system (Invitrogen, Carlsbad CA USA), sequenced on ABI377 automated sequencers (Perkin-Elmer, Wellesley MA USA), and aligned with previously published lion sequences (Barnett *et al.,* 2006a, 2006b). A summary of the cloning results is presented in the Appendix. A total of 12 clones were sequenced from sample NHM 1952.10.20.15, and 10 from NHM1952. 10.20.16. Of these, only three sequences show evidence of postmortem DNA damage (E4, E6, and F5 in Table S1) and, in each instance, the damage occurs at nucleotide sites that are not known to be polymorphic in lions. A median-joining network was constructed from the resulting sequences using Network v4.1.0.3 (Bandelt *et al.,* 1999).

Skull measurements

To investigate the origins of the Tower lions independently of the molecular results, morphological investigation was undertaken using Asiatic and North African Barbary lion skulls kept in natural history collections in the UK and Europe. A skull was classified as subadult if cemento-enamel junctions of all canines were already visible above the alveoli of the cleaned skull and yet the basioccipital-basisphenoid suture, and/or frontal suture, was still open. If those sutures were closed, a skull was classified adult. Seventy five craniometric measurements were taken of the cranium and mandible, modified from Yamaguchi *et al*. (2004), using a metal caliper to the nearest 0.02 mm, except for those of 10 larger variables that were measured to the nearest 0.05 mm using a larger metal calliper (for details see Appendix). To test the measurement errors, five skulls were randomly selected and each measurement was taken three times on each skull. The coefficient of variation for each of the 75 variables was calculated, and the variables with average coefficient of variations of more than 2% were excluded from the analysis by accepting the arbitrary cut-off line for reliability and consistency in measurements used in Yamaguchi *et al.* (2004) (see Appendix). We have measured all Asiatic and North African Barbary lion skulls kept, and available for measurement, in major natural history collections in the UK and Europe. However, as not every skull was intact, some variables were excluded from the analysis for maximising both Asiatic and North African Barbary lion specimens to be included into the analysis whilst retaining as many variables as possible. We retained 57 variables with 23 individuals (Table 1).

Statistics for morphological analysis

All statistical analyses were carried out using the SPSS statistics package (version 13: SPSS Inc., Chicago, USA). A principal component analysis (PCA) was carried out to reduce the numbers of variables for the subsequent analyses, which were based on extracted principal components whose eigenvalues were larger than 1 (Tabachnick and Fidell, 2007). Then, a discriminant analyses (DA) was carried out to investigate if Asiatic and North African Barbary lion skulls could be distinguished, with the prior probabilities computed from the group sizes. A DA is designed to develop classification functions to classify each specimen best by following *a priori* groupings, so that it will usually result in a fairly good discrimination between the groups (Tabachnick and Fidell, 2007). Therefore, a cross-validation test was also carried out to check which group each case would be classified into if it was classified by the functions derived from all cases other than itself. Then, we tested if the Tower lions would be classified as either Asiatic or North African Barbary, or both.

In addition to sexual size dimorphism that is common in the Felidae, it has been suggested that the skull morphological characteristics of captive lions differ from those of wild animals (Hollister, 1917). While a preliminary morphometric analysis suggested that both Tower lions were males based on the greatest length of skull and canine size (Gittleman and Van Valkenburgh, 1997), specimens did not have their sex recorded. Additionally, the specimens had spent at least some time in captivity. We therefore included into the analysis both male and female, and both captive and wild, individuals in our comparative data set (see Table 1). We deliberately did so to find out if a discriminant analysis (DA) would be able to separate the North African Barbary lion from the Asiatic lion regardless of sex and whether an animal was captive or wild.

Table 1. Lion skulls used for the morphological analysis. Museums are Natural History Museum London, Muséum National d'Histoire Naturelle Paris, Museum für Naturkunde der Humboldt-Universität, Berlin, Natural History Museum, University of Oxford, Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, Musée Zoologique, Strasbourg, and female is indicated by (f), male (m), adult (a), and subadult (sa). Individual ID numbers (e.g. P1, L1 or T1) are corresponding to those in Fig 2.

subspecies	sex	age	origin	museum	museum ID	comments
Panthera leo persica						
P1	f	a	India	London	1931.4.13.2	wild
P2	f	a	India	London	1945.136	captive
P3	f	a	India	London	1857.2.24.1	captive
P4	f	a	India	Oxford	14174	wild
P5	f	a	India	Paris	A-1884	captive
P6	f	a	India	Paris	I-1460	captive
P7	f	a	Iran	Paris	1962.2847	captive
P8	m	a	India	London	1930.6.6.1	wild
P9	m	a	India	London	1931.1.5.1	wild
P10	m	a	India	London	1931.1.5.2	wild
P11	m	a	India	London	1931.4.13.1	wild
P12	m	a	India	Paris	1873.556	captive
P13	m	a	Asia?	Frankfurt	1366	captive?
P14	m	a	Iran	Paris	1962.2854	captive
P. l. leo						
L1	f	a	Algeria	Paris	A-1873	captive
L2	f	a	Algeria	Paris	1862.54	captive
L3	f	a	N. Africa	Berlin	15960	captive
L4	f	a	N. Africa	Frankfurt	15766	captive
L5	f	a	N. Africa	Strasbourg	939	captive
L6	m	a	N. Africa	Paris	A-7912	captive
L7	m	a	N. Africa	Paris	1882.502	captive
Tower lion						
T1		a		London	NHM1952.10.20.15	captive
T2		sa		London	NHM1952.10.20.16	captive

Table 2. Classification results obtained by a discriminant analysis. The only one misclassified case in the cross-validation test was P6 in Table 1.

Original classifications	Predicted classifications	
	Panthera leo persica	*P. l. leo*
P. l. persica	14	0
P. l. leo	0	7
	Cross-validation	
P. l. persica	13	1
P. l. leo	0	7
	Tower lion	
1952.10.20.15	0	1
1952.10.20.16	0	1

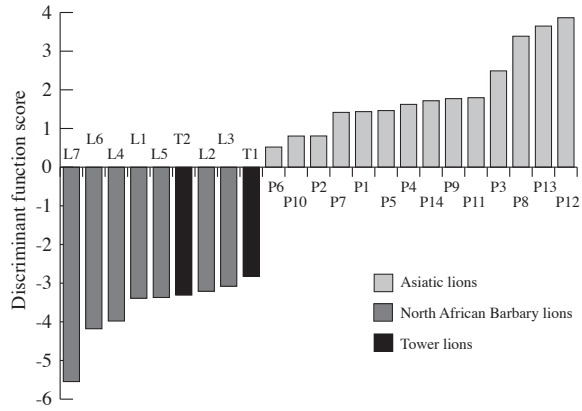

Fig. 1. Discriminant function scores of the specimens analysed. Individual ID numbers are corresponding to those in Table 1. Note that the discriminant function was extracted to distinguish lions from Asia and North Africa, and not to separate sexes. Therefore, although both the Tower lions place themselves amongst female Barbary lions, this does not indicate that they are females.

Results

Molecular phylogeny

Approximately 130bp of the mtDNA control region (HVR1) was amplified from both samples. Although the Felidae are known to contain macrosatellites (numts) resulting from nuclear translocation of the mtDNA (Cracraft *et al.,* 1998; Lopez *et al.,* 1997), visual comparison of the sequences obtained from the Tower lions with previously published numts and lion mitochondrial sequences clearly indicated that the Tower lion sequences were indeed mitochondrial (for details see Barnett *et al.,* 2006a, b). Median-joining network analysis clarified that both consensus clone sequences were identical to the unique haplotype of the North African Barbary lion out of the 11 distinct haplotypes previously identified in the lion (Barnett *et al.,* 2007). The sequences obtained from the Tower lions were also distinguishable from two haplotypes that characterised lions from India and Iran, which were the next most closely related haplotypes (Barnett *et al.,* 2006a, b).

Morphological similarity

A PCA based on the retained 57 variables resulted in seven principal components. Then, a DA based on those seven principal components extracted one canonical discriminant function, which was used in the analysis. The DA successfully separated Asiatic and North African Barbary lions, and the cross-validation test classified most (20 out of 21) specimens into the right groups (Table 2). The two Tower lions were both classified as the North African Barbary lion (Table 2 and Fig. 1).

Discussion

Our results demonstrate that the two Tower lions share a unique mtDNA haplotype with the North African Barbary lion. Whilst written records have previously suggested that most lions brought into Medieval Europe originated from the region between North Africa and India (Anonymus, 1769; Baratay and Hardouin-Fugier, 2002), such records have not been able to distinguish lions which actually originated from North Africa and lions that had been brought from sub-Saharan Africa and then shipped to Europe from North African ports. Our results are the first genetic evidence to clearly confirm that the former was the case.

Previous work has shown that lions inhabiting the extensive stretch of land between northwest Africa and India are very closely related: characterised by a simple mtDNA haplotype structure, in particular in comparison to the ancestral population in sub-Saharan Africa, with the distance between the former and the latter being three to seven substitutions whilst only less than two within the former (Barnett *et al.,* 2006a). Amongst the North African - Asian lion populations, our results (both genetic and morphological) suggest that the Tower lions belong to the North African Barbary lion in comparison to those originating from India and Iran.

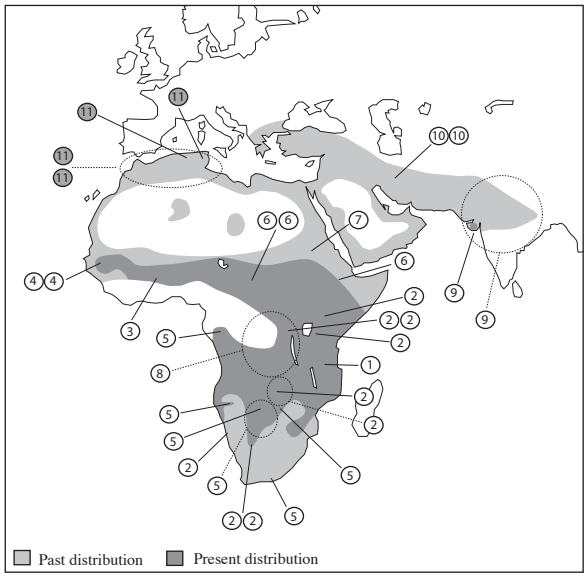

Fig. 2. Map showing approximate sampling sites of the lion specimens analysed in Barnett *et al.* (2006a, b), with mtDNA haplotypes (see Table 1). If the exact sampling location is not known, a dashed circle and dashed line are used. Two Tower lions possess the haplotype-11 that is unique to, and invariable within, the North African Barbary lion.

We expected that the skull morphometric analysis would not clearly separate North African Barbary lions from Asiatic lions if we included all the skulls measured into the analysis. This was because the well-known sexual dimorphism (e.g. Gittleman and Van Valkenburgh, 1997) and morphological difference between wild and captive individuals (Hollister, 1917) might make a clear-cut separation of the two geographic populations unlikely. Nevertheless, the discriminant analysis (DA) succeeded in doing so, suggesting that those two geographic populations would be distinguished from each other regardless of sex and whether an animal was wild or captive. We have not overlooked that the two Tower lions, which are likely to be males, appear amongst female Barbary lions in Figure 1. However, the DA was carried out to investigate if North African Barbary lions could be separated from Asiatic lions, and vice versa, and therefore, the analysis was not optimised for separating sexes.

Unfortunately, in spite of our extensive search across major natural history collections in Europe, Russia, Central Asia, and North America, no lion sample has been identified from the region between Libya and Iraq, from which lions totally disappeared by the mid 20th century (Nowell and Jackson, 1996; Patterson, 2004) although lions originated from that region were apparently imported into Europe during the 19th century (Edwards, 1996; Jardine, 1834). This makes it impossible to determine either mtDNA haplotype(s) or quantitative morphological characteristics that were present in this region, and potentially complicating the identification of the geographic origin of the Tower lions.

Historic records suggest that a single, contiguous population of lions existed from North Africa through the Middle East to India until the growth of civilisations along the Egyptian Nile and Sinai Peninsula (the narrow connection between Africa and Eurasia) some 4,000 years ago. The development of a human-dominated, narrow, land bridge effectively severed gene flow between North Africa and Near East Asia, which are also separated by more than 4,000 km of arid land (Nowell and Jackson, 1996; Yamaguchi, 2000a), and isolated lion populations to the west and east of this barrier. In the Near East, lions survived in southeastern Turkey until 1870, northern Syria (up until c. 1890s) and Iraq (the last record was in 1918) (Nowell and Jackson, 1996; Patterson, 2004). As we mentioned above, no genetic/quantitative morphological information is known for these eastern lions because no provenanced specimen is known to exist for investigation. However, their geographic proximity to lions in Iran (Nowell and Jackson, 1996), and their isolation from lion populations further west, may suggest that they are likely to belong to the Iranian haplotype (as well as Iranian/Indian morphological group), rather than those of the North African Barbary, and were not the source of the Tower lions.

Arid conditions in the eastern part of North Africa (Libya and Egypt) limited the size of the lion population in this region, even prior to the early 18th century, when lions finally disappeared from the eastern North African Mediterranean littoral zone (Nowell and Jackson, 1996; Yamaguchi and Haddane, 2002). Western North Africa (Morocco, Algeria and Tunisia), on the other hand, supported a relatively large lion population until fairy recently, and was one of the most important regions to supply lions to Europe between the 17th and first half of the 19th centuries (Anonymus, 1769; Yamaguchi and Haddane, 2002). Furthermore, western North Africa was the nearest region to Europe to sustain lion populations until the early 20th century, making it an obvious and practical source for medieval merchants. The foregoing argument may suggest that, while further evidence will be required to draw a firm conclusion, the first English lions originated in North Africa - probably North-west Africa.

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Appendix

List of craniometric parameters measured

All measurements are taken from the left side of the skull wherever possible. Numbers and letters in the square brackets are corresponding to those in the Figure. Measurement errors are indicated by coefficient of variation (%: *Mean* ± *Standard Error*: *N* = 5, 3 repeats each) in the round brackets.

	Parameter	Coefficient of variation
01	Cranial volume	(0.35 ± 0.084)
02	Frontal breadth [21]	(0.08 ± 0.021)
03	Greatest length [A-B]	(0.04 ± 0.012)
04	Condylobasal length [A-C]	(0.03 ± 0.002)
05	Parlate-inion [V-B]	(0.14 ± 0.039)
06	Nasal-inion [E-B]	(0.14 ± 0.035)
07	Facial length [A-G]	(0.17 ± 0.034)
08	Head length [G-B]	(0.08 ± 0.015)
09	Bizygomatic breadth [24]	(0.04 ± 0.009)
10	Zygomatic length [K-M]	(0.11 ± 0.028)
11	Zygomatic length anterior [K-L]	(0.30 ± 0.048)
12	Zygomatic length posterior [L-M]	(0.31 ± 0.064)
13	Orbit vertical [4]	(0.50 ± 0.195)
14	Orbit horizontal [3]	(0.94 ± 0.198)
15	Postorbital bar [2]	(0.28 ± 0.043)
16	Facial length anterior [A-E]	(0.59 ± 0.085)
17	Facial length posterior [E-G]	(0.73 ± 0.166)
18	Sagittal crest [H-B]	(0.23 ± 0.064)
19	Cranial height-I [N-H]	(0.19 ± 0.027)
20	Cranial height-II [N-H']	(0.40 ± 0.116)
21	Cranial height-III [N-H'']	(0.33 ± 0.078)
22	Cranial height-IV [N-B]	(0.34 ± 0.049)
23	Interorbital breadth [20]	(0.10 ± 0.016)
24	Postorbital breadth [22]	(0.15 ± 0.073)
25	Nasal length-I [D-F]	(0.33 ± 0.144)
26	Nasal length-II [S-F]	(0.42 ± 0.202)
27	Nasal breadth [D-D]	(0.57 ± 0.110)
28	Breadth between infra orbital foramina [19]	(0.17 ± 0.055)
29	Rostral depth-I [1]	(0.75 ± 0.140)
30	Rostral depth-II (E - most posterior end of canine alveolus)	(0.53 ± 0.057)
31	Rostral breadth [17]	(0.16 ± 0.036)
32	Nasal aperture [18]	(0.80 ± 0.448)
33	Upper jaw [A-U]	(0.10 ± 0.018)
34	Palate length [T-V]	(0.14 ± 0.041)
35	Palate breadth-I [29]	(0.14 ± 0.034)
36	Palate breadth-II [28]	(0.16 ± 0.045)
37	Canine - Pm^4 (alveolus – alveolus)	(0.46 ± 0.136)
38	Pm^2 - Pm^4 (alveolus – alveolus)	(0.25 ± 0.129)
39	Upper canine height [5]	(0.36 ± 0.180)
40	Upper canine diameter antero-posterior [25]	(0.53 ± 0.160)
41	Upper canine diameter medio-lateral [26]	(0.57 ± 0.171)
42	Upper canine alveolus diameter antero-posterior	(1.00 ± 0.233)
43	Upper canine alveolus diameter medio-lateral	(2.02 ± 0.702)
44	Pm^4 length [6]	(0.18 ± 0.040)
45	Pm^4 breadth-I [27]	(0.83 ± 0.153)
46	Pm^4 breadth -II [27]	(0.37 ± 0.092)
47	Mastoid breadth [23]	(0.04 ± 0.009)
48	Skull height-I [W-B]	(0.79 ± 0.410)
49	Skull height-II [7]	(0.55 ± 0.320)
50	Foramen magnum breadth	(0.16 ± 0.022)
51	Foramen magnum height [greatest distance: usually oblique]	(0.65 ± 0.188)
52	Occipital condyles breadth [33]	(0.08 ± 0.043)
53	Tympanic bulla length [31]	(0.79 ± 0.137)
54	Tympanic bulla breadth-I [30]	(1.42 ± 0.559)
55	Tympanic bulla breadth-II [32]	(2.96 ± 1.269)
56	Mandible length [O-Q]	(0.18 ± 0.042)
57	Mandible length coronoid process[O-Q']	(0.19 ± 0.058)
58	Mandible length angular process [O-Q'']	(0.07 ± 0.019)
59	Mandible height [13]	(0.23 ± 0.069)
60	Mandible height angular process [14]	(0.57 ± 0.182)
61	Mandible height coronoid process [15]	(0.16 ± 0.040)
62	Maximum width of mandibular condyle [16]	(0.08 ± 0.020)
63	Mandible depth-I [11]	(0.51 ± 0.237)
64	Mandible depth-II [12]	(0.20 ± 0.043)
65	Canine - M_1 (alveolus – alveolus)	(0.27 ± 0.088)
66	Pm_3 - M_1 (alveolus – alveolus)	(0.30 ± 0.077)
67	Lower canine height [8]	(1.33 ± 0.463)
68	Lower canine diameter antero-posterior	(0.78 ± 0.222)
69	Lower canine diameter medio-lateral	(1.56 ± 0.290)
70	Lower canine alveolus diameter antero-posterior	(1.03 ± 0.189)
71	Lower canine alveolus diameter medio-lateral	(1.72 ± 0.674)
72	Pm_4 length	(0.13 ± 0.022)
73	Pm_4 breadth (largest breadth usually towards the posterior end)	(0.54 ± 0.178)
74	M_1 length [9]	(0.95 ± 0.342)
75	M_1 breadth (largest breadth usually around the middle)	(0.40 ± 0.117)

Definitions for the points and measurements in the Figure

Definitions of general terms are as follows.

Outer Furthest from the mesion

Inner Closest to the mesion

Vertical and Horizontal

These can be defined when a cranium or a mandible(s) is placed in the way shown in the Figure. For the mandible(s), simply place it on a horizontal surface. For the cranium, adjust the cranium to make the surface including the posterior ends of alveoli of both canines and both Pm^4s horizontal.

Definitions for points

A. Prosthion: the most anterior point of the skull
B. Inion: the most posterior point of the skull
C. The line connecting the most posterior points of occipital condyles
D. The most anterior points of nasals
E. The highest points on the vaults of the anterior ends of dorsal parts of nasals
F. The most posterior point of the inter nasal suture
G. The point where the line connecting the most outer points of postorbital process of frontal meets the mesion
H. Bregma: where the coronal suture meets sagittal suture (if the sagittal crest is very well developed, use the place where the coronal suture reaches the top of the sagittal crest in the mesion)
H' One third distance point between bregma and inion
H'' Two third distance point between bregma and inion
I. Vertical lines including the most outer points of the alveoli of I^3s
J. The most anterior point of alveolus of the upper canine
K. The most dorsal point of infraorbital foramen (in case if there are more than one foramen, the most dorsal point of the foramina)
L. The most outer point of the zygomatic arch (usually just above the malar - temporal suture)
M. A point where a vertical section including the most dorsal point of auditory meatus cuts the outer curve of the zygomatic process of temporal
N. The most dorsal point of auditory meatus
O. Pogonion: the most anterior point of mandible on the inter mandible suture
P. The most ventral point around the angular process. If it is not obvious, use the point where the extended line of the middle line of inferior notch crosses the ventral edge of angular process as being shown in the Figure. This may sound difficult, but in practice there is little problem and a subtle difference of the position of "P" does not seem to affect the result.
Q. The point where the extended line of the ventral end of superior notch crosses the posterior edge of condyle (approximately the middle of condyle)
Q'. The furthest point on the coronoid process from the pogonion
Q". The furthest point on the angular process from the pogonion
R. The line connecting the highest points on the vaults of the anterior ends of dorsal parts of nasals
S. The most anterior point of the inter nasal suture. Often the inter nasal suture of some skulls may be slightly opened towards the anterior end, and it may be difficult to assess where is "S". In such case, ignore the part where the inner lines of the nasals forms a sallow angle (almost parallel) to the mesion, and find the point where the angle start to change.

Table S1. A total of 12 clones were sequenced from sample NHM1952.10.20.15, and 10 from NHM1952.10.20.16, and compared to those of Barbary, Indian, and Iranian lions. Only three sequences show evidence of postmortem DNA damage (E4, E6, and F5) and in each instance the damage occurs at nucleotide sites that are not polymorphic in lions.

	1	11	21	31	41	51	
Barbary	CTTATTCCCC	ACGAAAAGCA	AGTGAAAATC	CCCAACCTCC	ACAGCACAAA	CGCACAATGT	
India	....	....	....	....	....	....	
Iran	....	....	....	....	....	....	
'C1_1952.10.20.15_Amp1'	....	....	....	....	....	....	
'G10_1952.10.20.15_Amp1'	....	....	....	....	....	....	
'G11_1952.10.20.15_Amp1'	....	....	....	....	....	....	
'G12_1952.10.20.15_Amp1'	....	....	....	....	....	....	
'F1_1952.10.20.15_Amp2'	....	....	....	....	....	....	
'F3_1952.10.20.15_Amp2'	....	....	....	....	....	....	
'F4_1952.10.20.15_Amp2'	....	....	....	....	....	....	
'F5_1952.10.20.15_Amp2'	....	....	....	....C.....	....	....	
'F7_1952.10.20.15_Amp2'	....	....	....	....	....	....	
'F8_1952.10.20.15_Amp2'	....	....	....	....	....	....	
'F9_1952.10.20.15_Amp2'	....	....	....	....	....	....	
'H12_1952.10.20.15_Amp2'	....	....	....	....	....	....	
'B12_1952.10.20.16_Amp1'	....	....	....	....	....	....	
'G6_1952.10.20.16_Amp1'	....	....	....	....	....	....	
'G8_1952.10.20.16_Amp1'	....	....	....	....	....	....	
'E4_1952.10.20.16_Amp2'	....	....	....	....	....	....	
'E6_1952.10.20.16_Amp2'	....	....	....	....	....	....	
'E8_1952.10.20.16_Amp2'	....	....	....	....	....	....	
'E10_1952.10.20.16_Amp2'	....	....	....	....	....	....	
'E11_1952.10.20.16_Amp2'	....	....	....	....	....	....	
'E12_1952.10.20.16_Amp2'	....	....	....	....	....	....	
'H6_1952.10.20.16_Amp2'	....	....	....	....N.....	....	....	

T. Orale: the point where the line connecting the most posterior points of I^1 alveoli meets the mesion
U. The middle point along the posterior part of the alveolus of M^1
V. The most posterior point of the palate on the mesion
W. The most ventral point in the mesion between occipital condyles

Definition for measurements

1. The distance from R to the most posterior point of the upper canine alveolus
2. The shortest distance between the most dorsal point of the malar - temporal suture and the most ventral edge of the zygomatic arch
3. The distance between the tip of postorbital process of malar and the point where lacrimal - frontal suture crosses the edge of orbit
4. The longest distance between the tip of postorbital process of frontal and the edge of orbit (if the tip of postorbital process of frontal is not pointy enough, use the middle point of the rounded tip)
5. The distance along the buccal surface of an upper canine between the unworn tip and the point on the cemento - enamel junction where the medio-lateral breadth of the canine becomes its maximum
6. The distance between the most anterior point of the buccal part of Pm^4 and the most posterior point of the tooth
7. The greatest distance between W and the sagittal crest around its posterior end. In most case, it is easy to measure this distance. However, if the sagittal crest of the skull is very well developed, measure the distance between W and the sagittal crest around the middle point between H" and B.
8. The distance along the buccal surface of a lower canine between the unworn tip and the point on the cemento - enamel junction where the medio-lateral breadth of the canine becomes its maximum
9. The distance between most anterior and posterior points of M_1
10. The distance between the most anterior point of canine alveolus and the most posterior point of M_1 alveolus
11. The smallest of the greatest diameter of the section which cuts the mandible in front of the most anterior point of Pm_3 alveolus: usually an oblique measurement like the one shown in the Figure
12. The smallest of the greatest diameter of the section which cuts the mandible just behind the most posterior point of M_1 alveolus: usually a vertical or slightly oblique measurement shown in the Figure
13. The greatest distance between P and the dorsal part of coronoid process
14. The greatest distance between P and the dorsal part of condyle just outside the place where superior notch

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61         71         81         91         101        111              121
|          |          |          |          |          |                |
AAAATAACCA GTCAACTTTC TTTTTTCCC- ACATACACTG TATCATCGAC TGCCCTCCCA TGAATATTAG
.......... .......... .........C .......... .......... .......... .........A
.......... .......... .........C .......... .......... .......... ..........
.......... .......... .......... .......... .......... .......... ..........
.......... .......... .......... .......... .......... .......... ..........
.......... .......... ..........
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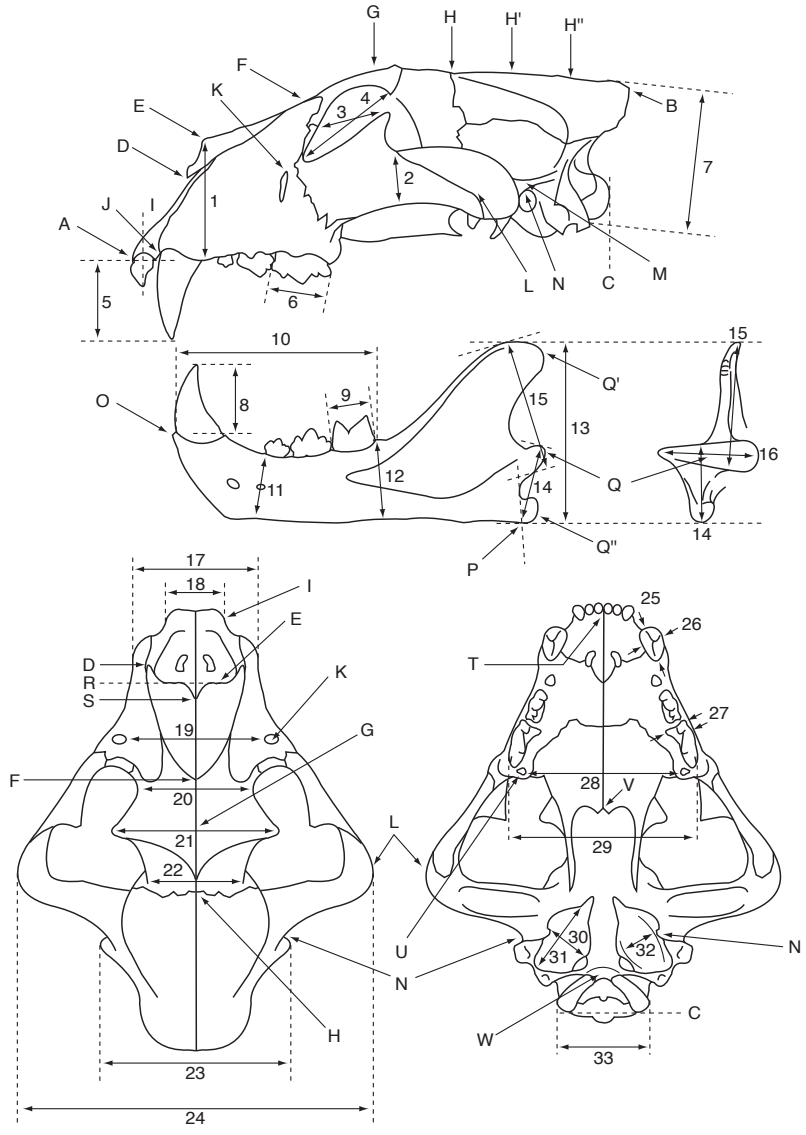
crosses the condyle: usually an oblique measurement shown in the Figure

15. The greatest distance between the ventral part of condyle just inside the place where the condyle meets inferior notch and the dorsal part of coronoid process: usually an oblique measurement shown in the Figure - in most cases use the point on the coronoid process that was used to measurement 13
16. Maximum width of mandibular condyle
17. The greatest breadth of the rostrum just above the canine alveoli
18. The breadth of nasal aperture above the most outer points of I^3 alveoli
19. The smallest distance between infraorbital foramina
20. Interorbital breadth: the smallest distance between the orbits
21. The distance between the most outer points of postorbital process of frontal
22. Postorbital breadth: the smallest breadth of the postorbital constriction
23. Mastoid breadth: the distance between the most outer points of mastoidal processes
24. The distance between zygions: the most outer points of zygomatic arches
25. The greatest antero-posterior length of an upper canine at the cemento-enamel junction: the greater diameter of the canine
26. The greatest medio-lateral length of an upper canine at the cemento-enamel junction: the smaller diameter of the canine
27. Pm^4-I: between the inner process and the most anterior outer process, and Pm^4-II: between the former and the second most anterior outer process of the tooth
28. The smallest distance between M^1 alveoli
29. The greatest distance between Pm^4 alveoli
30. The distance between the most anterior/inner meeting point between tympanic bulla and external auditory meatus and the most anterior meeting point between tympanic bulla and foramen lacerum posterius
31. The greatest length of tympanic bulla excluding styloid process and other processes attached to the tympanic bulla: fix one end of the caliper at the point where foramen lacerum medius meets the most prominent styloid process, and measure the greatest distance between that point and the posterior part of the tympanic bulla
32. The greatest mediolateral distance of the vault of the tympanic bulla: the smaller diameter of the vault of the tympanic bulla
33. The greatest breadth of occipital condyles