Scottish Natural Heritage

Commissioned Report 356

The Scottish wildcat:
A comparison of genetic and pelage characteristics

Scottish Natural Heritage
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COMMISSIONED REPORT

Commissioned Report No.356

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Summary

The Scottish wildcat: a comparison of genetic and pelage characteristics

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Background

The Scottish wildcat, *Felis silvestris,* is currently endangered owing to a variety of factors including hybridisation with and disease transmission from the feral cat, *F. catus*, habitat loss and fragmentation, and persecution (e.g. Macdonald *et al*., 2004). In addition enforcement of legal protection for the Scottish wildcat is problematic, because of difficulties in its clear identification, especially fragmentary remains.

In recent years advances have been made in identifying the Scottish wildcat through both pelage characteristics (Kitchener *et al.*, 2005) and genetic analysis (Driscoll *et al.,* 2007). To date these methods have not been correlated with each other, so that their effectiveness is unknown. The aims of this project are to re-evaluate the specimens of wild-living cats collected by Balharry and Daniels (1998), using the new pelage and morphometric diagnostic methods developed by Kitchener *et al.*, (2005) and compare these results with genetic data for these specimens provided by C. Driscoll (Driscoll *et al.*, 2007).

Main findings

- Of 330 pelages skins only up to 13.1% were classified as wildcat under the Strict and Relaxed IDs, thus indicating that the sample, but in particular the Balharry and Daniels (1998) sample (N = 265), is largely composed of hybrids or domestics.
- Cranial Index (CI) identified 90% of individuals as wildcats (CI < 2.75), but Total Skull Character Score (TSC) identified most as hybrids.
- Pelage and skull morphometrics, therefore, indicate that at least 70% of the total sample is a mixture of domestic and hybrid individuals.
- 3D Geometric Skull morphometrics confirmed variation in skull shape between individuals based on their pelage classification.
- Microsatellite analysis indicated some genetic differentiation between the three pelage groups with specimens being largely classified into the correct genetic cluster based on their pelage classification although there is some overlap between these, probably owing to the high levels of introgression thought to occur in Scotland.

- There were insufficient data to be confident that the results of the mtDNA analysis could be correlated with either pelage or skull classifications, but the results suggest that the sample is largely hybrid.
- In summary, the Strict pelage classification proposed by Kitchener *et al.* (2005) is sufficiently accurate to identify individuals that are genetically different from domestic cats

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1. INTRODUCTION

There has been much debate over the years as to what constitutes a wildcat and how to accurately separate wildcats from domestic cats (*F. catus*). In particular, because wildcats and domestic cats are thought to have been hybridising for at least 2,000 years, there exists a range of "wild-living" cats in Scotland that vary in physical characteristics between wildcats and domestic cats (Macdonald *et al*., 2004). The problems associated with identifying the Scottish wildcats were highlighted in 1990 when a legal case failed because an expert could not clearly identify the cats as wildcats (Balharry & Daniels, 1998). Since this there have been significant difficulties enforcing legal protection for the wildcat, largely due to difficulties in identifying between wildcat, domestic cats and hybrids (Kitchener, 1995).

In 2005, Kitchener *et al.,* proposed a methodology for identifying wildcats based on their pelage markings (see 5.1). More recently, genetic research has reported that Scottish wildcats can be separated evolutionarily from both domestic cats and the similar European wildcat (*F. silvestris*) using mtDNA (Driscoll *et al.,* 2007). In addition, studies carried out by Beaumont *et al.*, (2001) using microsatellite data indicated that there was a group of tabby "wild-living" cats in Scotland that were genetically distinct from non-tabby "wild-living" cats.

In November 2008, Scottish Natural Heritage commissioned the Wildlife Conservation Research Unit (WildCRU), University of Oxford to examine the association between morphological and genetic characteristics of the Scottish wildcat (*Felis silvestris grampia*). Cat skins and skulls collected previously (Balharry & Daniels, 1998) were categorised using the current description of a wildcat (Kitchener *et al.,* 2005) as "domestic", "domestic/wildcat hybrid" or "wildcat. These data were then compared with mitochondrial DNA (mtDNA) and microsatellite data (see section 5 for more details) produced for the same sample set by Driscoll *et al.* (2007).

2. BACKGROUND

The wildcat (*Felis silvestris* Schreber, 1777) is distributed widely throughout Europe, Africa and Asia (Nowell & Jackson, 1996). The Scottish wildcat is sometimes recognised as a distinctive subspecies, *Felis silvestris grampia,* Miller 1907, which is believed to have become separated from the European continental population some 7,000 – 9,000 years ago (Yalden, 1999).

The Scottish wildcat is Britain's only surviving native felid. Currently endangered, in the UK it has full legal protection under Schedules 5 and 6 of the Wildlife and Countryside Act, 1981 (as amended in 1988). It is a European Protected Species (EPS) under Annex IVa of the EC Habitats Directive and receives protection in the UK under Schedule 2 of the Conservation Regulations. It also features on the revised UK BAP list of Priority Species and Habitats, the Scottish Biodiversity List and, more recently, has been listed on Scottish Natural Heritage's Five Year Species Action Plan as a species for conservation action (SNH, 2007). A survey of the distribution of the Scottish wildcat is currently being carried out, the results of which may enable suitable management actions to be targeted in particular areas.

The Scottish wildcat was previously widespread across mainland Britain and is believed to have disappeared from southern Scotland, England and Wales by the mid-19th century (Taylor, 1946; Langley & Yalden, 1977). By the early 20th century the wildcat was believed to be on the brink of extinction and restricted to the far north-west Highlands (Langley & Yalden, 1977). A survey in the 1980s indicated that the wildcat had recovered much of its former distribution north of the Central Belt (Easterbee *et al.,* 1991). A more recent study in the 1990s suggested that wild-living cats were limited to the north-east of Scotland (Balharry & Daniels, 1998), but sampling was not comprehensive and was biased towards road kills, which were commoner in this area. The last published population estimate indicated that there may be between 1,000 to 4,000

Scottish wildcats, based on wildcat sightings (Harris *et al., 1995)* although a subsequent calculation based on extrapolation of a subsample of museum skins suggested that the number of individuals with classical wildcat pelage may be as low as 400 (Macdonald *et al.,* 2004). However, this number should be viewed with caution as not only is it an estimate, but it may be influenced by the fact that many of the museum samples were collected as road casualties, which may be biased towards domestic cats and hybrids (Kitchener pers. comm.). Given that population densities on higher ground away from roads are likely to be very low, this potential bias is not expected to impact this estimate significantly. However, the actual number of Scottish wildcats that currently exist has not yet been confirmed.

The initial decline of the Scottish wildcat has been attributed partly to habitat loss, in particular forested areas, and partly to hunting for sport, its fur and persecution (Kitchener 1995, Macdonald *et al.,* 2004). The development of sporting estates in Scotland from the mid-19th century led to a further decline, almost resulting in its extinction by the early 20th Century (Langley & Yalden, 1977; Tapper 1992). Currently the Scottish wildcat is threatened mainly by hybridisation with domestic/feral cats (*Felis catus* L. 1758). Although human persecution was a significant cause of decline in the early 1900's, it was thought to have declined after World War I and II (Hubbard *et al.,* 1992; Nowell & Jackson, 1996) and it is not known whether persecution still occurs. In addition to hybridisation, wildcats can suffer from a range of potentially fatal felid viruses and other diseases that are commonly carried by domestic cats (McOrist *et al.,* 1991).

3. DIFFERENCES BETWEEN THE SCOTTISH WILDCAT AND DOMESTIC CAT

There has been much debate over the characteristics that differentiate a wildcat from a domestic cat or wildcat/domestic hybrid. Typically wildcats are considered to be larger in size than domestic cats (Easterbee *et al.,* 1991; Kitchener & Easterbee, 1992; Macdonald & Barrett, 1993) (Figure 1). However, Hamilton (1986) suggested that this size difference was not as large as previously thought, and Tetley (1941) indicated that the size of the modern-day wildcat has decreased in comparison to palaeontological remains.

Wildcats are also thought to have a shorter intestinal length, longer limb bones, and a more robust skull than domestic cats () (Figure 2), with a Cranial Index (the ratio of skull length to cranial volume) of less than 2.75 (Schauenberg, 1969; Schauenberg, 1977; Daniels *et al.,* 1998; Yamaguchi *et al.*, 2004). In comparison, domestic cats have a Cranial Index (CI) greater than 2.75, representing a smaller cranial capacity, which is commonly associated with domesticated animals) (Schauenberg, 1969; Groves, 1999). According to Kitchener *et al.* (2005), wildcat pelage characteristics include a bushier tail with a thick blunt tip and 3-5 distinct tail bands, 7-11 stripes on the body and no substantial areas of white (see section 5.1 for more details). In comparison, wildcat/domestic cat hybrids exhibit various degrees of both wildcat and domestic cat characteristics and may be confused with wildcats in the field. They are generally larger than domestic cats and often have a tabby coat pattern similar to that of the wildcat. In general hybrids have a less bushy tail than that of wildcats, the dorsal line tends to run onto the tail and they may often have large patches of white, especially on their paws. They are often seen with a range of coat colours more commonly found in domestic cats (Kitchener, 1995).

As well as various morphological characteristics, Daniels *et al.,* (1998) suggested that wild-living cats more closely resembling wildcats could be geographically separated from domestic cats, preferring areas with a low mean annual temperature and land with poor potential for forestry and agriculture. More recently, Driscoll *et al.* (2007) have been able to separate Scottish wildcats from domestic cats and European wildcats evolutionarily on the basis of mitochondrial DNA (mtDNA).

Figure 1: A comparison of the tail shape and general pelage characteristics of wildcats (top), hybrids (middle) and domestic cats (bottom).

Figure 2: A comparison of the skulls wildcats (left), hybrids (middle) and domestic cats (right).

4. METHODOLOGY

The Balharry and Daniels sample set consists of 330 wild-living individuals from across Scotland (Balharry and Daniels, 1998). These are 175 registered skins and/or skulls and 95 unregistered skins/skulls collected from road carcasses or other carcasses, 44 registered live cats which were trapped, photographed and blood samples taken for genetic analysis and 16 museum wildcat samples (skins and/or skulls). The museum samples were individuals collected between 1915 and 1950 which were larger than the majority of skins collected by Balharry & Daniels and had the wildcat markings identified by Kitchener *et al.* (2005). The sample set was not complete with skins and/or skulls missing; from a total of 330 individuals, 265 were assessed by their pelage using skins or photographs (where suitable photos of dorsal, ventral and lateral aspects of the cat existed e.g. live cats or individuals missing skins) and 122 individuals were assessed by their skull characteristics.

In addition to the Balharry and Daniels dataset, samples (skins and/or skulls) from other collection periods were also included in this study if genetic information existed for them, or if they reflected an extreme of the domestic cat to wildcat spectrum, e.g. were obviously a small domestic cat. A further 65 individuals were assessed by pelage and 86 individuals by their skull characteristics bringing the total number of samples to 330 pelage and 208 complete or partially broken skulls.

Microsatellite data were also available for a total of 192 individuals (N = 190 from the Balharry & Daniels sample). Mitochondrial DNA (mtDNA) data were also available for 50 individuals from the Balharry & Daniels sample. Microsatellites are loci (or regions within DNA sequences) where short sequences of DNA are repeated one after another, in tandem. Microsatellites are useful because the number of times the DNA sequence is repeated often varies between individuals, within populations and/or between species. Each sequence with a specific number of repeats is known as an allele, so different individuals will have different alleles (i.e. different number of repeats) at the same microsatellite. Over time, when individuals within a population breed, they recombine their microsatellites so that the population as a whole will retain a variety of microsatellites that are characteristic to that population and distinct from other populations which do not interbreed. Microsatellites are therefore often used by biologists to assess the genetic variability between different species and in population analyses, in particular to examine whether individuals show evidence of hybridisation by sharing microsatellites from two or more genetically distinct populations (Randi *et al.,* 2003; Lecis *et al.,* 2006; Verardi *et al.,* 2006). In this study, microsatellites were used to determine whether individuals identified as “wildcat”, “hybrid” or “domestic” by their pelage were genetically different from each other.

In comparison to microsatellites, MtDNA is a specific type of genetic material that is found in mitochondria, a small organelle (a specialised subunit with a cell that has a specific function) (Lodish *et al.,* 1995). MtDNA is maternally inherited and therefore only represents a small proportion of an individuals DNA. However, because of this, mtDNA is particularly useful for assessing genetic relationships of individuals or groups within a species and also for identifying and quantifying the phylogeny (evolutionary relationships) among different species, provided they are not too distantly related (Avise, 1986). For example, Driscoll *et al.*, (2007) used mtDNA to show that the Scottish wildcat was evolutionarily different from the domestic cat and the European wildcat, and mtDNA can be used to examine whether hybridisation is occurring within a population. For example, a study on mountain hares (*Lepus timidus*) in Scandinavia proved that hybridisation had been occurring with introduced brown hares (*L. europaeus*) by finding mountain hare mtDNA in animals that looked like brown hares, indicating that two species had been interbreeding (Thulin *et al.,* 2003).

The samples were analysed as a single group to avoid complication and to maximise the robustness of the data analysis. Individuals were classified as “wildcat”, “hybrid” or “domestic” cat based on either their pelage or skull characteristics or a combination of both (see section 5.1). These classifications were then compared with the available genetic data. Statistical analyses were conducted using a combination of Minitab 15.0 (Minitab Inc.) and SPSS 12.0 (SPSS Inc.)

4.1 Morphological assessment

Morphological assessment of the cat skins and skulls was carried out using the methodology described by Kitchener *et al.* (2005). A brief overview of the methodology is described below.

4.1.1 Pelage scoring

Pelage characters were initially assessed in two ways by the same researcher in order to minimise the risk of errors occurring between different recorders. These included direct examination of preserved skins and photographs of the dead cats before skinning. The two were then compared and any discrepancies double checked before a final score was given for each individual. In particular, photographs often proved to be more reliable in assessing tail shape and ear colour because of slippage of fur owing to decay of the specimens prior to preservation. Consequently, we determined that where skins were unavailable, pelage characters could be confidently assessed from photographs (where suitable photographs exist). Where photographs were not good, did not exist or the individual was too badly damaged to be assessed, these individuals were excluded from any further analysis. In addition, individuals whose skins had been preserved in 70% IMS (Industrial Methylated Spirit often used to preserve museum specimens) were also assessed from photographs, owing to the difficulties associated with removing and drying the skins.

A total of 20 pelage characters were given a score (1 = domestic; 2 = intermediate (hybrid); 3 = wildcat) (see Appendix 1). Where the key was not applicable (e.g. black cats cannot be given a score for character 8 as they have no white or buff coloured markings on their chin; see Table 2), the character was given a score of N/A. Where the character could not be determined from either the skin or photograph, it was scored as unknown (U/K). A total pelage score (TPS) was generated and a score for seven key characteristics (7PS) (see Figure 3; Table 1) was also calculated (Kitchener *et al.,* (2005).

Kitchener *et al.* (2005) suggested that any cat with a score of 19 or more for 7PS (Table 1) with no scores of 1 should be regarded as a wildcat unless other data conflict with this. However, this definition may exclude many cats that have a high proportion of wildcat characters (both morphological and genetic) that may usefully contribute to the restoration of the wildcat (Kitchener *et al.,* 2005) and these may be difficult to assess for accurate field identification. Therefore, a more relaxed definition was proposed whereby any cat that does not have a score of one for any of the seven pelage characters or for an additional eight pelage characters (white on chin, stripes on cheek, dark spots on underside, white on flank, white on back, colour of tail tip, stripes on hind leg and colour of the back of the ear) (Table 2; Figure 4) could be considered a wildcat (Kitchener *et al.,* 2005). These additional eight pelage characters are classified as 8PC hereafter.

Figure 3: A comparison of the seven key pelage characters (7PS) that distinguish a striped-tabby domestic cat (B) from a Scottish wildcat (A) (Adapted from Kitchener et al., 2005 in Macdonald et al., 2004)

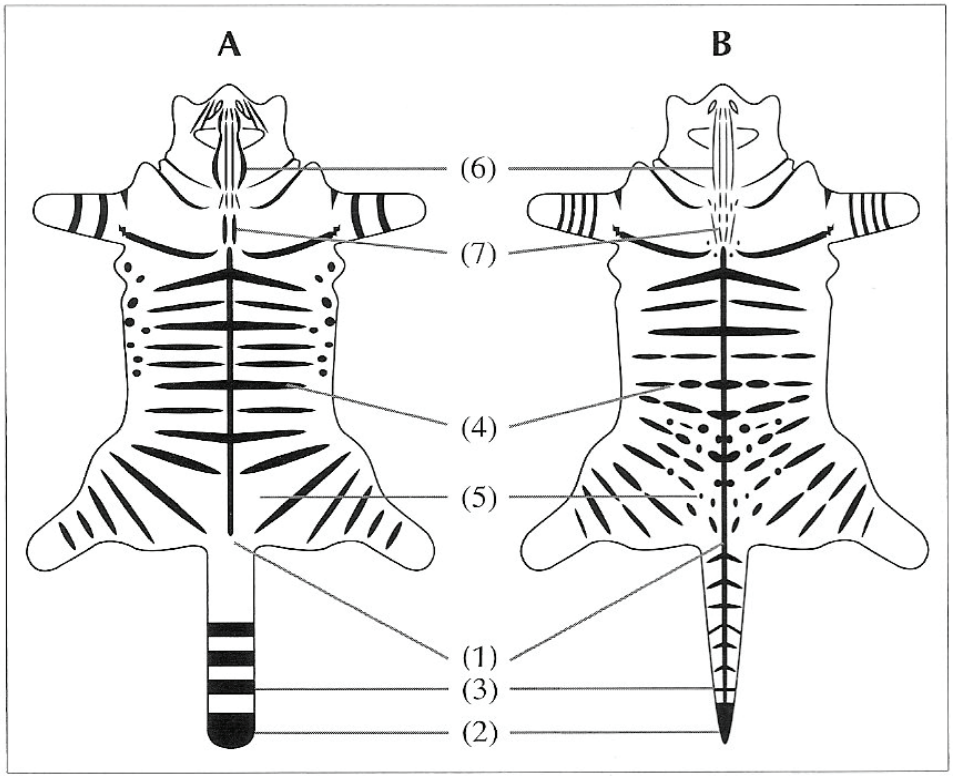


Table 1: Key to the seven pelage characteristics (7PS) (Adapted from Kitchener et al., 2005 in Macdonald et al., 2004)

(1) Extent of dorsal line
1: absent/covers entire tail
2: continues onto tail
3: stops at base of tail

(2) Shape of tail tip
1: tapered to a point
2: intermediate
3: blunt

(3) Distinctness of tail bands
1: absent/joined by dorsal line
2: indistinct or fused
3: distinct

(4) Broken stripes on flanks & hindquarters
1: > 50% broken/no marking
2: 25–50% broken
3: < 25% broken

(5) Spots on flanks and hindquarters
1: many/no marking
2: some
3: none

(6) Stripes on nape
1: thin/no stripes
2: intermediate
3: four thick stripes

(7) Stripes on shoulder
1: indistinct/no stripes
2: intermediate
3: two thick stripes

Based on the pelage scores each cat was classified as “wildcat”, “hybrid” or “domestic” using two definitions; a strict definition (Strict ID), and a more relaxed definition (Relaxed ID), described above, as follows:

Strict ID

1. **Wildcat** = 7PS score of 19 or more, no scores of 1 for any of the 7PS characters and no scores of 1 for any of other pelage characteristics.

2. **Hybrid** = scores 3 for one or more of the 7PS characters, but may also score 1 for one or more of these characters and may score 1 for one or more of the 8PC.

3. **Domestic** = no scores of 3 for any of the 7PS characteristics and scores of 1 or 2 in most of the other characteristics.

Relaxed ID

Wildcat = 7PS score of 14 or more with no scores of 1 for any of the 7PS characters and no scores of 1 for any of the 8PC.

The scoring system used here means that under the Strict ID, no individuals identified as wildcats will display any domestic cat traits and no domestic cats will display any wildcat traits. Under the Relaxed ID, wildcats will still have no domestic cat characteristics but may have some hybrid traits.

Figure 4: The eight additional pelage characters (8PC; numbered in red) that may clarify initial identification of a wildcat (Kitchener et al., 2005)

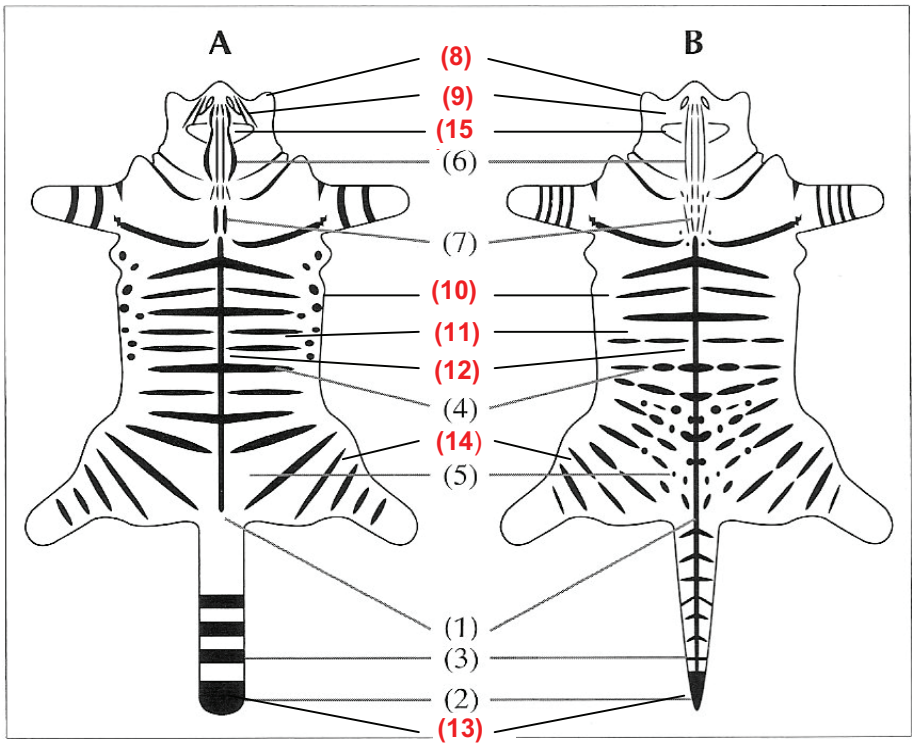


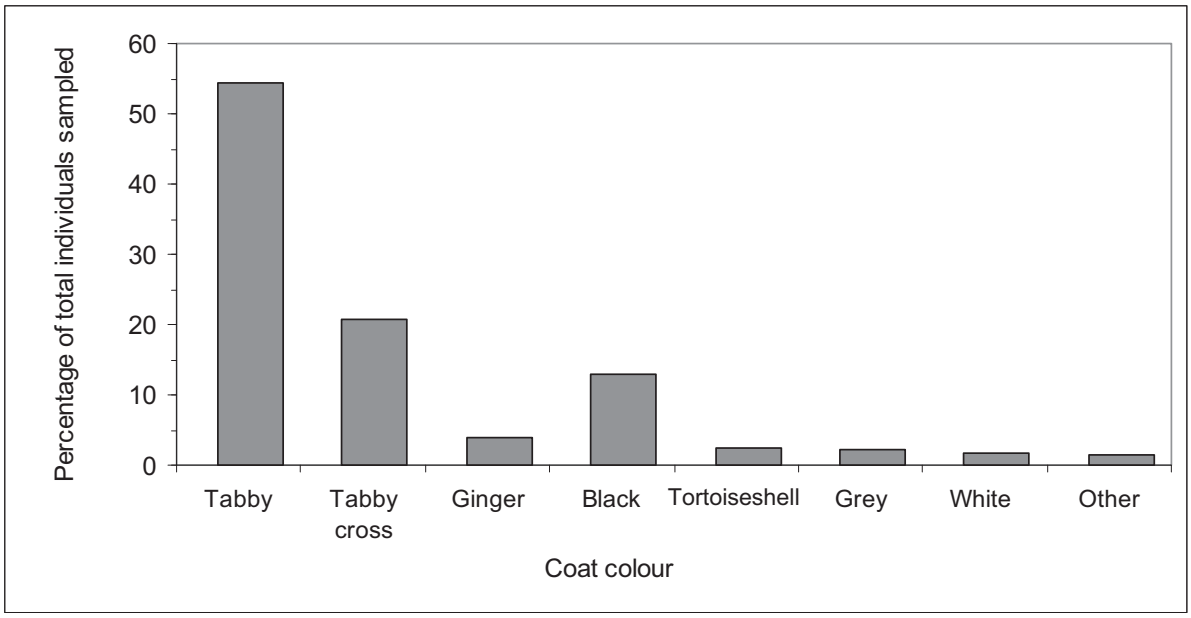
Table 2: Key for scoring the eight additional pelage characters (8PC) to distinguish wildcats from striped-tabby domestic cats and their hybrids (Kitchener et. al.., 2005)

(8) White on chin 1: white extensive on muzzle 2: white on chin 3: buff or off-white on chin	**(12) White on back** 1: present 2: - 3: absent
(9) Stripes on cheeks 1: no dark stripes 2: indistinct stripes 3: 3 clear strips (2 fused)	**(13) Colour of tail tip** 1: neither black nor dark 2: dark 3: black
(10) Dark spots underside 1: absent 2: indistinct 3: distinct	**(14) Stripes on hind leg** 1: <4 or > 7 stripes 2: - 3: 4-7 stripes
(11) White on flank 1: present 2: - 3: absent	**(15) Colour on back of ear** 1: same colour as head 2: weak ochre/reddish 3: ochre/reddish

4.1.1.1 Coat colour classification

In addition to classifying individuals based on their pelage scores, cats were also separated into two groups, tabby and non-tabby (including tabby cross). This was used to see whether dominant coat colour correlated with the genetic data. Of the examined skins/photographs 54% (N = 330) have the classical striped-tabby pelage (Figure 5). "Tabby cross" cats have various amounts of white markings on them. This includes blotched tabbies, silver tabbies and cats described as "light tabbies", where the under fur beneath the markings is very light cream unlike the darker brown normally seen in classic tabbies. Cats described as "white" have predominantly white fur but often patches of other colouring such as grey, black, ginger or tabby. Those described as "black" included cats that were black and white and "others" included Burmese cats.

Figure 5: Percentage of total sample of cats with different coat colours



4.1.2 Skull measurements

Several different skulls measurements were taken. These are described in more details in the following sections, however, in brief, these were:

1) 30 measurements to examine morphological differences between individual skulls (see 5.1.2.1);
2) a further five key characters to generate a Total Skull Character score (TSC). This was then used to determine whether the skull could be identified as either "wildcat", "domestic cat" or "hybrid" (see 5.1.2.2.);
3) Cranial Volume (CV) used to calculate a Cranial Index (CI) from the ratio of cranial volume to skull length. This was used to classify skulls as either "wildcat" or "domestic" based on their CI score (see 5.1.2.3. and 5.1.2.4.);

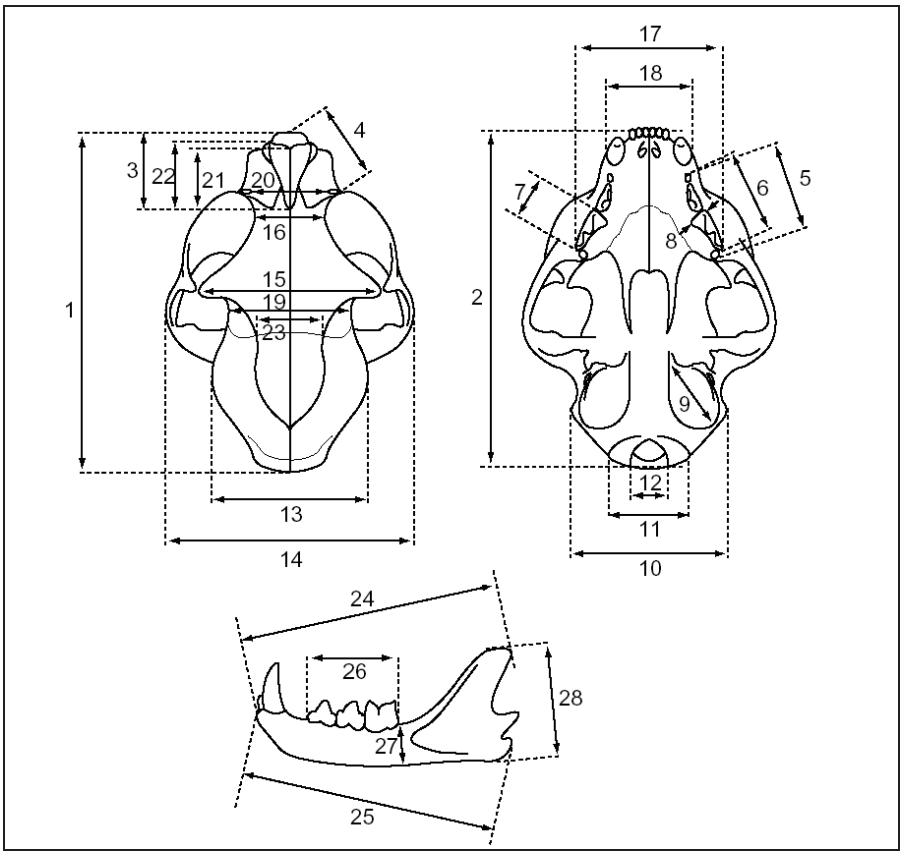
4) 60 points (landmarks) on each skull used to compare the 3D structure of each skull to its pelage classification or genetic identification (see 5.1.3). Only adult skulls were assessed; juveniles were separated from adults through a combination of size, suture fusion and epiphyseal fusion.

There were a total of 208 complete or partially broken skulls. Of these, 140 skulls had some or all of the 30 skull measurements taken as described by Yamaguchi *et al.*, (2004) and TSCs were scored for 131 individuals, CIs for 130 and 128 skulls were digitised."

4.1.2.1 Skull morphology

30 skulls variables were measured using digital callipers following the methodology of Yamaguchi *et al.*, (2004) (Figure 6 – two variables not illustrated; see Appendix 2 for full descriptions). Measurements were compared between the different groups, "wildcat", "hybrid" and "domestic" defined by the Strict ID and Relaxed ID.

Figure 6: Diagram of skull measurements taken in this study (Yamaguchi et al., 2004).

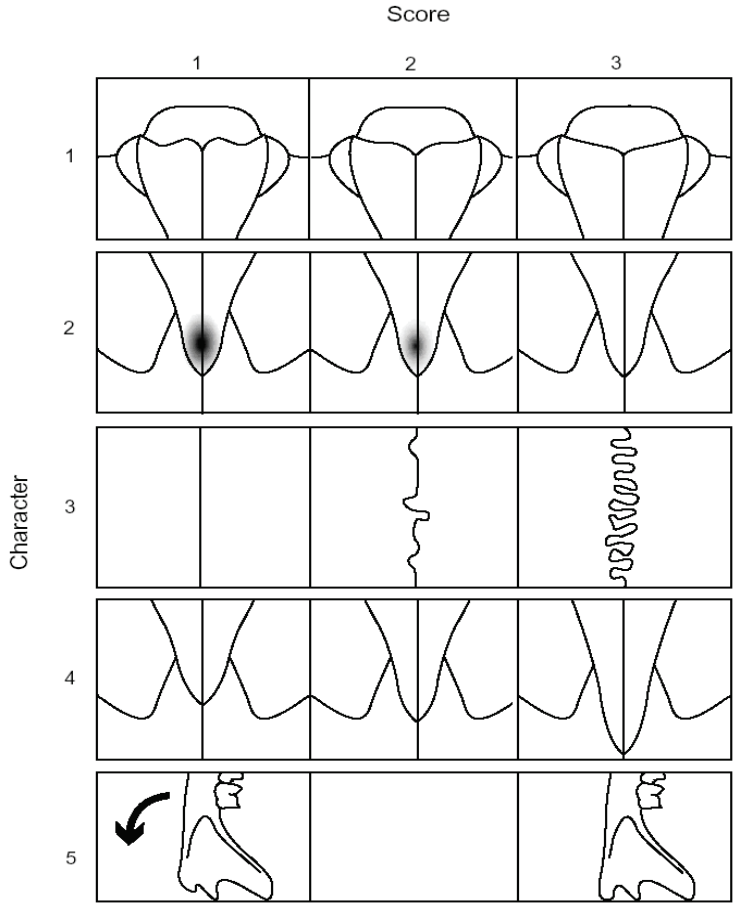


4.1.2.2 Total Skull Character Score (TSC)

Using a similar scoring to that described for the pelage characters, five key skull characters traditionally used to distinguish European wildcats from domestic cats (Pocock, 1951; Kitchener, 1995) were given a score of one for "domestic cat", two for "hybrid/intermediate" and three for "wildcat", following Yamaguchi *et al.*, (2004) (Figure 7). The score for each skull was then totalled to give a Total Skull Character (TSC) score, with wildcats scoring a total of 15, domestic cats a maximum of five and hybrids ranging in between. The five skull characters are;

1) Shape of the anterior end of the nasal bones (hereafter referred to as nasal curvature);
2) Presence or absence of a pit at the posterior end of the nasal bones (nasal pit);
3) Shape of the parietal suture (parietal suture);
4) Length of the nasal bones relative to the maxillae (nasal extension);
5) Development of the angular process of the mandible (mandible).

Figure 7: Diagram showing the character states of the five skull characters (1-5) recorded from each cat skull, the scores along the top show 1 = domestic cat, 2 = hybrid, 3 = wildcat (Yamaguchi et al., 2004).



4.1.2.3 Cranial volume

Skulls were weighed by first stopping any foramina (holes in the skull) with blu-tack, then weighing the skull (g) to calibrate the scales to zero. Glass beads were then poured into the skull, packing the beads tightly into the skull using a fingertip to ensure the beads completely filled the cranial cavity to the top of the foramen magnum. The skull containing the beads was then weighed to a hundredth of a gram. This was repeated to give a mean cranial volume, which was calculated by converting mean weight of glass beads (g) to volume (ml). A calibration curve of the weights of volumes of glass beads from 10 to 50cm^3 was calculated as a simple linear regression line: **y = mx + b,** where x is the slope of the line and b is the y-intercept. In this case:

Cranial Volume (ml) = mean weight of glass beads (g) x 0.66 + 0.47

The cranial volume was used to calculate a Cranial Index for each skull as described below (see 5.1.2.4.).

4.1.2.4 Cranial Index (CI)

CI was measured by dividing the greatest lengths of skulls (mm) by their cranial volumes (ml), following Schauenberg (1969). Schauenberg found that European wildcats had a CI of < 2.75, while that of domestic cats > 2.75. Although Schauenberg did not consider hybrids, this method is useful for distinguishing domestic cats from some hybrids and all wildcats (Kitchener pers. comm.).

4.1.3 Geometric Morphometric Analysis

Geometric 3D analysis has been carried out on Scottish "wild-living" cat skull samples before but individuals were categorised by their intestinal length and limb bone length rather than by their pelage classification (Macdonald *et al*., 2004). In this study, 128 skulls were analysed (twice as many as in the previous study) to examine the overall shape variation between individuals classified as "wildcat", "hybrid" or "domestic" under both the Strict ID and Relaxed ID and also between individuals with either wildcat or domestic cat mtDNA.

Sixty different measurements, known as osteological landmarks (specific locations on the skull), were taken using a MicroScribe® 3D digitizer to generate a 3D shape of each skull (Figure 8a and b; Appendix 3). Landmarks were chosen to give complete coverage of the cranium. The MicroScribe® 3D digitizer is a contact-based device that measures and captures 3D (x, y and z co-ordinate) data points from physical objects. The data is then downloaded to a computer where the data can be analysed with specialised software (MORPHOJ software package: Klingenberg, 2008). Each skull was digitised twice in order to reduce measurement error.

Statistical shape analysis involves summarising and comparing shapes (configurations based on data co-ordinates) of objects, in this case the shapes of skulls identified as "wildcat", "hybrid" or "domestic" by either TSC, CI, pelage or mtDNA classifications. Procrustes analysis was used to standardise the shape and variation around the mean of each skull sample, removing the variability inherent in studying skulls of different size and relative shape. This was then used to make comparisons between different skulls (Bookstein, 1996; Dryden & Mardia, 1998; Klingenberg & McIntyre, 1998).

Figure 8a: Dorsal cranial landmarks recorded by the 3-d digitizer

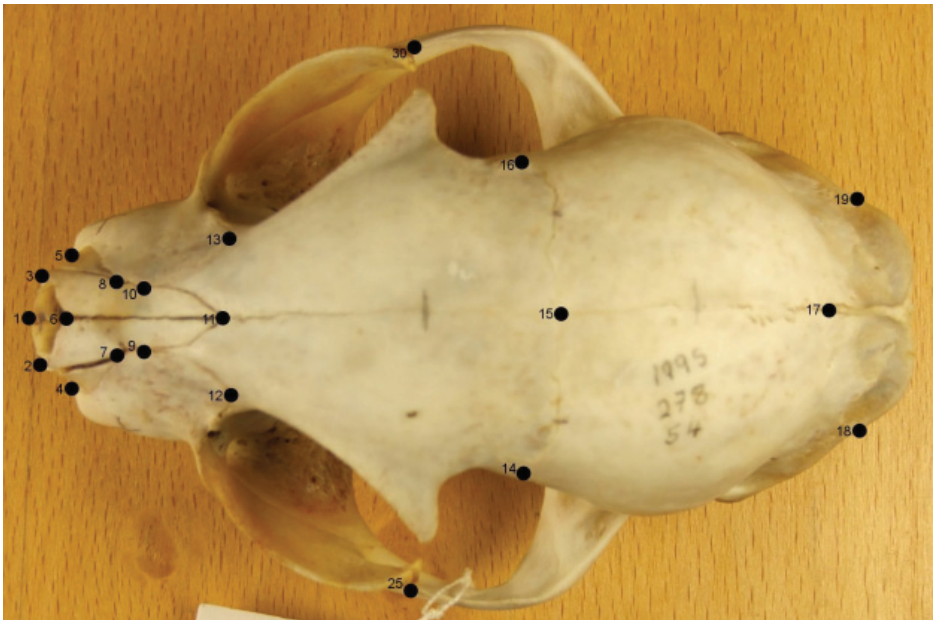


Figure 8b: Ventral cranial landmarks recorded by the 3-d digitizer

4.1.3.1 Principal Components Analysis

A principal components analysis (PCA) of the Procrustes Coordinates was conducted to investigate cranial shape variation amongst wildcats, hybrids and domestic cats. This statistical method attempts to explain correlations within a set of variables by transforming a number of possibly correlated variables into a smaller number of uncorrelated variables, called Principal Components. This reduces the complexity of a dataset so that all the variation is explained by a defined but reduced set of component variables. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. Specimens can then be described by their combined relationship to the set of principal component scores which can, in turn, be used to illustrate differences in skull shape according to the identifiable principal components.

4.1.3.2 Canonical Variates Analysis (CVA)

A canonical variates analysis (CVA) was used to assess the significance of cranial shape differences between individuals. CVA is similar to PCA in that both analyses compute new variables for each sample (e.g. Principal Components). However CVA differs in that prior information about groups and groups membership of the samples is used (i.e. in this case skulls are classified according to pelage and sex) so that the new variables (Canonical Variates) maximise the between-group variation and minimise the within-group variation (Davies & Fearn, 2008) as opposed to the variance across all individuals as described by PCA. The shape changes associated with each canonical variate (CV) describes the ways in which the groups are most differentiated. A simple Mahalanobis distance-based approach (Mahalanobis distance is a distance measure based on correlations between variables by which different patterns can be identified and analysed) is then used to determine which group each specimen belongs to, based on the canonical variate scores. The predicted group membership of each specimen based on the CVA scores is determined by assigning each specimen to the group whose mean is closest (under the Mahalanobis distance) to the specimen.

4.2 Genetic Assessment

Nine microsatellite loci, originally isolated in domestic cats (Menotti-Raymond & O'Brien 1995), were screened for in the samples (see Beaumont *et al.,* 2001) and mitochondrial DNA (mtDNA) data (i.e. wildcat or domestic cat mtDNA) were provided by Carlos Driscoll. Details on the molecular methodologies are in Beaumont *et al.* (2001) and Driscoll *et al.* (2007).

Correlations between mtDNA data and pelage and skull classifications (see 5.3) were investigated. Microsatellite data were analysed using various programmes for estimates of diversity. Genepop 3.4 (Raymond & Rousset, 1995) was used to calculate deviations from Hardy–Weinberg Equilibrium (HWE) and the significance of Weir & Cockerham's (1984) estimator of *F*IS and F_{ST} were calculated. If populations are in Hardy-Weinberg equilibrium then random mating is occurring. However, this is rarely the case as distance and other factors such as gene flow and non-random mating play a role. Deviations from HWE are measured as the differences between observed and expected genotype heterozygosity. A deviation from Hardy-Weinberg proportions indicates either selection, population mixing or nonrandom mating is occurring (Pemberton *et al*., 1995; Brookfield, 1996). *F*IS is a measure of inbreeding within a population and relates to the level of heterozygosity within a population. The greater the number of heterozygotes, the less inbred a population is. Generally, positive values of *F*IS indicate low levels of heterozygosity within a population. F_{st} is commonly used to determine whether there is structuring of sub-populations within the total population. F_{st} values are generated between 0 -1,

the closer the value is to 1 the larger the degrees of structuring and therefore barriers to gene flow. Allelic frequencies, and observed (*H*O) and expected heterozygosity (*H*E) were calculated using the programme FSTAT (Goudet, 2001).

In order to determine whether the samples could be separated into genetically different populations (e.g. wildcat or domestic) based on their genetic data, the microsatellite data was assessed using a model-based Bayesian procedure implemented in the programme STRUCTURE (Pritchard *et al.*, 2000). STRUCTURE calculates the proportion (*Q*) of each individual's genetic data (genotype) that is derived from a defined number (*K*) of different genetic populations (clusters) (e.g. wildcat or domestic cat cluster). Values are given as membership coefficients (q_{ik}) and individuals are probabilistically assigned to one cluster (the population of origin) known as the posterior probability, if $q_{ik} > 0.80$ (i.e. more than 80% of the individuals genome belongs to the inferred cluster), or jointly to more than one cluster (the parental populations), if the individual is genetically admixed as a result of hybridisation (i.e. $q_{ik} < 0.80$).

Before any modelling could take place, two steps had to be taken to set suitable parameters for the programme to run on. STRUCTURE was initially run to determine 1) burn-in length (how long to run the simulation before the programme starts collecting data to minimise the effect of starting the configuration process), 2) run length after the burn-in period in order to obtain accurate parameter estimates for the data. A suitable burn-in period is detected when the values of the summary statistics (i.e. value for F, the divergence distances among individuals) produced by the programme start to converge (Pritchard *et al.,* 2007). In this case, this occurred at a burn-in period of 20,000 runs. A suitable run length is determined by running the programme at several different values of *K* (i.e. *K* =1, 2, 3 or 4) for various different lengths until you get consistent answers. We ran simulations of between 10,000 to 1,000,000 runs setting *K* at 1-9 and found that consistent answers for each *K* could be obtained at a run length of 100,000. We therefore set STRUCTURE to run for 100,000 runs, following a 20, 000 burn-in period for all future simulations.

The second step was to calculate the number of populations (*K*) that should be assumed when running the model, the number of assumed populations does not necessarily represent the number of populations sampled. In brief, this was calculated by running STRUCTURE using the previously defined parameters (described above) at different values of *K* (*K* = 1 – 9). This generated an estimated log probability of data (Ln Pr(*X*|*K*)) at each of the different *Ks.* The *K* with the lowest Ln Pr(*X*|*K*) value that captured the major structure of the data set was chosen (i.e. most likely represented the biological data, for example, a probable wildcat population and a separate domestic cat population with hybrids being a mixture of the two) (Pritchard *et al*., 2007), in this case *K* = 2.

Using the available microsatellite data (N = 192), STRUCTURE was used to (1) infer the presence of genetically differentiated clusters, assuming that all the samples belong to a single indistinct ''population,'' independently of any prior classification, and 2) estimate the extent of genetic differentiation between cats which were pre-classified as "wildcat", "hybrid" and "domestic", using only morphological traits (i.e. Strict or Relaxed ID definition). In the first approach, STRUCTURE was run without *a priori* information, using the "admixture model" (where each individual draws some fraction of its genome from each of the two populations) and correlated allele frequencies (owing to the possibility of hybrid individuals being present in the sample set) at *K* = 2.

In the second approach STRUCTURE was run with *a priori* population information, where individuals were assigned probabilistically to one of the two groups based on their phenotypic classification as defined under the Strict ID and Relaxed ID. In this way each cat was forced to have its genotype assigned either to one of the two clusters, or, if admixed, to both clusters.

5. RESULTS

5.1 Pelage assessment

A total of 330 individuals were given a pelage score based on assessment of pelages and/or photographs. A further 14 individuals could not be given a score for any of the pelage characters, because either pelages or photographs were unavailable or too poor to record characters reliably. In some cases, several individuals were given scores for most of the characteristics, but could not be given a score for one or more of the key 7PS characters and so were classified as unknown. For example, some individual skins had no tail and no suitable photograph, and therefore could not be given a score for tail tip shape, colour or the pattern of tail bands and therefore were not able to be completely identified. Individuals were classified into three groups based on two definitions, "Strict ID" and "Relaxed ID" (Table 3).

Table 3: Percentage of individuals in the total sample and Balharry & Daniels sample that were classified as "wildcat", "hybrid" or "domestic" under both the Strict ID and Relaxed ID.

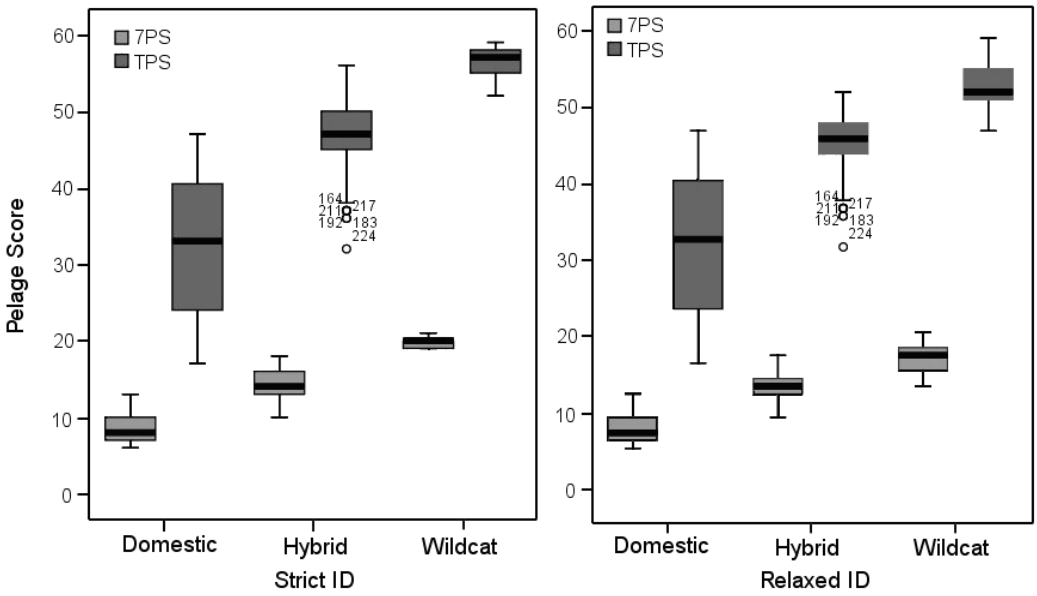
	Total sample (N = 330)		**Balharry and Daniels sample (N = 265)**	
	Strict ID	**Relaxed ID**	**Strict ID**	**Relaxed ID**
Wildcat	4.6%	13.1%	0%	6.4%
Hybrid	46.5%	38.1%	46.2%	39.6%
Domestic	44.7%	44.7%	52.1%	52.1%
Unknown	4.1%	4.1%	1.5%	1.5%

As expected, the average 7PS and TPS scores varied significantly between the three groups, with wildcats scoring higher than both domestics and hybrids. Hybrids had a greater average 7PS and TPS scores than those of domestics (Table 4). Although individuals identified as wildcats had the greatest average score in all cases, there was some overlap in range between domestics/hybrids and hybrids/wildcats (Figure 9).

Table 4: Comparison of 7PS and TPS scores for individuals classified as "wildcat", "hybrid" or "domestic" under both the Strict ID and Relaxed ID using Mann Whitney U Test.

Mann Whitney U Test	Wildcats/Domestics				Wildcats/Hybrids				Hybrids/Domestics			
	Strict ID		Relaxed ID		Strict ID		Relaxed ID		Strict ID		Relaxed ID	
	7PS	TPS	7PS	TPS	7PS	TPS	7PS	TPS	7PS	TPS	7PS	TPS
Z	-5.619	-5.519	-9.829	-9.724	-5.563	-5.397	-8378	-8.613	-13.993	-12.931	-13.013	-11.745
***P* value**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Figure 9: The median, quartiles and extreme values of 7PS and TPS for individuals classified as domestic, hybrid or wildcat under both the Strict and Relaxed IDs.



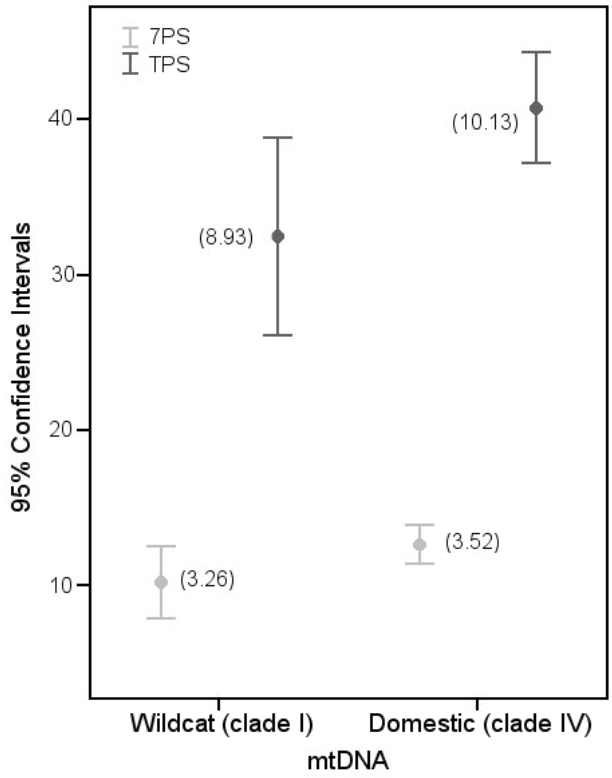
5.1.1 Pelage vs mtDNA assessment

Of 50 individuals with mtDNA data, one was a foetus that could not be assigned a pelage score and a further five could not be satisfactorily classified, leaving the remaining 44 individuals for further analysis.

5.1.2 *Comparison* of 7PS and TPS with mtDNA assignment.

Interestingly, individuals with wildcat mtDNA (clade I) (N = 10) have lower mean 7PS and TPS scores than those with domestic cat mtDNA (clade IV) (N = 34) (Figure 10). However, these differences were not significant, (T-test: $F_{7PS} = 0.087$, $p = 0.769$; $F_{TPS} = 0.089$, $p = 0.766$, N = 10, 34). In addition, post-hoc analysis using G*Power 3 (Faul *et al.,* 2007) revealed that these data have low statistical power (post hoc, t-test means: 1 – ß = 0.63, df = 43, N = 10, 35) (Lenth, 2001) and should, therefore, be reassessed with a larger sample size before any firm conclusions can be drawn.

Figure 10: Error bar plot showing the mean score and 95% confidence intervals for 7PS and TPS for individuals with the wildcat mtDNA clade 1 and domestic cat mtDNA clade IV. Values in brackets are the standard deviations of the mean.



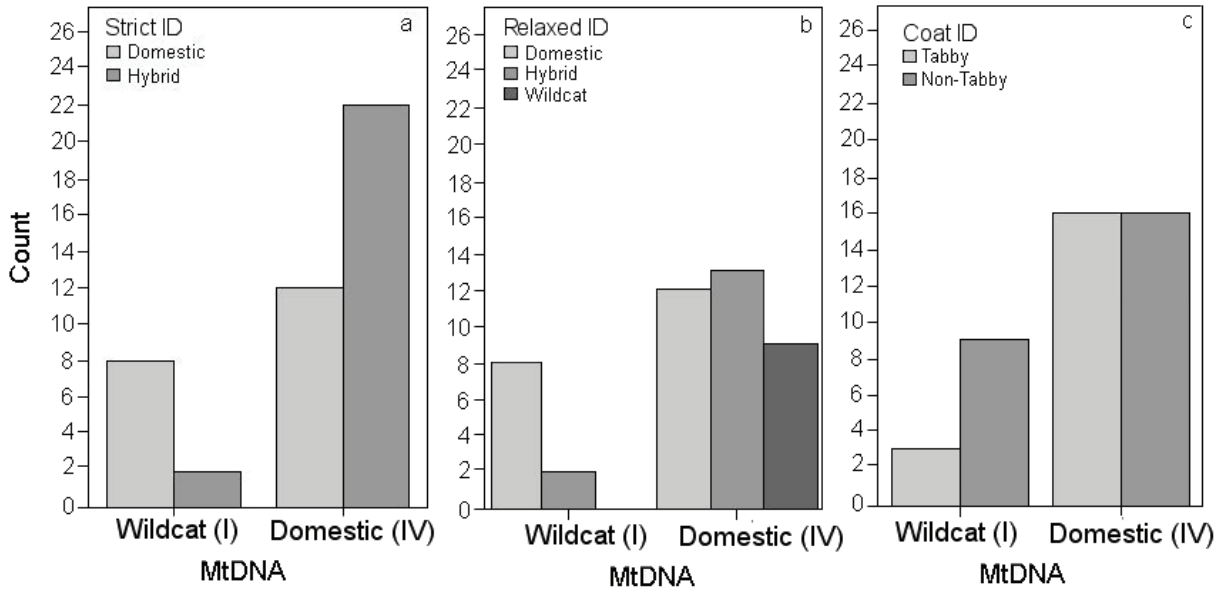
5.1.3 Comparison of pelage definition and coat colour with mtDNA assignment.

The relationship between pelage assessment/coat colour and mtDNA ID was examined. Of cats with the wildcat mtDNA, 80% were classified as domestic cats using the Strict and Relaxed IDs with the remaining 20% as hybrids (N = 10). None of the individuals with clade I mtDNA were classified as wildcats based on their pelage using either the Strict or Relaxed IDs. In comparison, using both the Strict and Relaxed IDs, a greater number of hybrids had domestic mtDNA compared to those identified as domestic cats. Furthermore, using the Relaxed ID, 26.5% of individuals with domestic mtDNA were classified as wildcats based on their pelage scores. A greater number of non-tabby cats (N = 9) had wildcat mtDNA compared to tabby cats (N = 3), whereas the number of individuals with a tabby or non-tabby coat colour and domestic mtDNA was equal (Figure 11).

There was a significant association between mtDNA classification and pelage classification under both the Strict (Pearson's Chi-square Test of Independence $\chi^2 = 6.23$, d.f. = 1, $p = 0.029$) and Relaxed IDs ($\chi^2 = 6.79$, d.f. = 1, $p = 0.030$). In both cases there were a greater number of individuals with wildcat mtDNA having a domestic-cat pelage than expected, and individuals with the domestic mtDNA were more likely to have hybrid pelage than expected. In addition using the Relaxed ID, there was also a positive association between individuals with domestic mtDNA having wildcat pelage characteristics. Although a greater number of cats with wildcat mtDNA had a non-tabby coat colour than those with a tabby coat, this association was not significant (χ^2

=2.223, d.f. =1, p = 0.139) (Figure 11). However, it should be noted that only 44 individuals had available mtDNA data, of which 12 had the wildcat mtDNA. Therefore, these results should be viewed with caution.

Figure 11: Total number of individuals assessed as wildcat, hybrid or domestic using the a) Strict ID, b) Relaxed ID, c) Coat-colour ID with either wildcat or domestic cat mtDNA.



5.1.4 Microsatellite data and pelage assessment

5.1.4.1 Genetic differentiation between inferred groups of cats

Microsatellite data were available for 192 individuals. Using this sample, all loci were polymorphic (i.e. two or more alleles at each locus) with between three and 16 alleles at each locus. The mean number of alleles per locus was 10.8. The number of alleles varied across loci and between the three groups as defined by their pelage classifications under both Strict and Relaxed IDs (Table 5).

Table 5: Total number of alleles found at each locus for each of the three groups as defined under the Strict and Relaxed ID.

	Strict ID			Relaxed ID		
	Domestic	**Hybrid**	**Wildcat**	**Domestic**	**Hybrid**	**Wildcat**
N	**91**	**93**	**8**	**91**	**66**	**27**
Loci						
fca8	11	9	3	11	9	6
fca23	12	9	3	12	9	6
fca35	7	4	1	7	4	2
fca43	8	9	2	8	9	4
fca45	16	16	4	16	16	12
fca77	12	8	3	12	8	4
fca90	10	11	3	10	11	6
fca96	9	9	2	9	9	4
fca126	9	8	3	9	8	6

Observed (HO) and expected (HE) heterozygosity ranged from *H*O = 0.325 - 0.907 and H*E* = 0.375 – 0.816 across all loci. Average values of heterozygosity were similar across all three groups as defined using both Strict and Relaxed IDs (Table 6). Using the Strict ID, both domestic and hybrid groups had lower *H*Os than expected, with average *F*IS values that were positive (0.177 and 0.094 for domestics and hybrids respectively). These positive *F*IS values indicate that each subpopulation is inbred. Both groups had a highly significant departure from Hardy-Weinberg equilibrium when results from all loci were combined using Fisher's exact test (*P* <0.0001). Further analysis revealed that this departure was probably caused by a significant heterozygote deficiency (*p* <0.0001). In comparison, individuals designated as wildcats appeared to be in Hardy Weinberg equilibrium (χ^2 = 15.13, d.f. = 16, *p* = 0.5152), although this may be as a result of the small number of individuals with wildcat phenotype using the Strict ID.

Using the Relaxed ID, domestic and hybrid groups showed a significant departure from Hardy-Weinberg equilibrium (*p* <0.0001) and, to a lesser extent, so did the wildcat group (χ^2 = 36.19, d.f. = 18, *p* = 0.006). The observed and expected levels of heterozygosity seen here are comparable to levels described in other studies of European wildcats and domestic cats (Pierpaoli *et al.,* 2003).

Table 6: Observed (HO) and expected (HE) levels of heterozygosity in each of the three groups as defined by the Strict and Relaxed ID.

Hardy Weinberg Equilibrium	Strict ID		Relaxed ID	
	HO	***HE***	***HO***	***HE***
Range	0.325 - 0.907	0.375-0.816	0.476-0.907	0.286-0.795
Domestic	0.75	0.62	0.75	0.62
Hybrid	0.72	0.65	0.72	0.66
Wildcat	0.54	0.52	0.67	0.58

Following Beaumont *et al*., (2001), the presence of private alleles was examined to assess genetic introgression between species (e.g. Gottelli *et al.,* 1994). This used a threshold frequency of 0.05, above which we can be confident of frequency differences between groups as defined by their pelage classification. Using the Strict and Relaxed IDs, there were a total of 16 private alleles at various loci within the domestic-cat group; however, 15 of these had a frequency less than 0.05 and could have, therefore, been sampled by chance. One locus (Fca96) possessed an allele (214), which had a frequency of 0.07 in the domestic group. In addition there were a further four private alleles in the hybrid group at four separate loci, but none of these was at a frequency greater than 0.05 and could, therefore, not be considered significant.

Weir & Cockerham (1984) F-statistics were calculated across all groups and between the three groups under both the Strict and Relaxed IDs. Although overall there appears to be low genetic differentiation across the whole sample set (Table 7), pairwise F_{st} comparisons indicate that in general, some level of genetic differentiation is seen between the three groups. This appears to correlate with the phenotypic classifications, with the lowest level of gene flow indicated between the wildcat and domestic groups, and the highest between the domestic and hybrid groups. However, as expected, this difference is less obvious under the Relaxed ID.

Table 7: Overall and pairwise F_{st} values for the three groups as defined under the Strict and Relaxed IDs.

F-Statistics	Strict ID	Relaxed ID
F_{st} Overall	0.029	0.024
Pairwise F_{st}		
Domestic vs Hybrid	0.023	0.019
Domestic vs Wildcat	**0.092**	0.048
Hybrid vs Wildcat	0.040	0.005

Our results correspond to that of other studies where the average F_{st} = 0.11 (p < 0.001), indicating that wildcats and domestic cats are subdivided into distinct genetic pools in most European countries (Randi *et al.,* 2001; Pierpaoli *et al.,* 2003).

5.1.4.2 Bayesian cluster analysis – no prior population information

STRUCTURE was initially run without any *a priori* classifications to determine the number of genetically distinct clusters within the population and to establish that pre-defined populations roughly agreed with the genetic data (Pritchard *et al.*, 2000) before the model was run with *a priori* classification information.

Using STRUCTURE with a cut-off point of 80% or q_{ik} = >0.8 (e.g. 80% of an individual's genome is probabilistically assigned to the correct population); two separate genetic clusters were identified. These were clarified as a "wildcat" cluster and a "domestic" cat cluster because individuals that were presumed to be either wildcat (e.g. museum specimens from pre-1950s with a 7PS score of > 19 under the Strict ID: N = 8) or domestic (e.g. those whom were neutered or belonged to a specific breed such as Burmese; N = 5) fell into each relevant cluster with q_{ik} > 0.9. The remaining individuals (N = 179) fell into either category or a mixture of both if they were genetically hybrid. In general, without any prior population information, STRUCTURE gave results that generally corresponded to the phenotypic classifications with those classified as wildcats having a high membership co-efficient to the "wildcat" cluster and those classified as domestic largely associated with the "domestic" cluster, although there is some overlap (Table 8).

Table 8: Mean proportions of membership (q_{ik}) of the two inferred genetic clusters for the three pre-defined groups of wild-living cats in Scotland (presumed wildcat, presumed domestic cats and presumed hybrid) based on the admixture model without any a priori population information.

A priori group	Strict ID		Relaxed ID	
	"Domestic" cluster	**"Wildcat" cluster**	**"Domestic" cluster**	**"Wildcat" cluster**
Domestic	0.641	0.359	0.641	0.359
Hybrid	0.314	0.686	0.343	0.657
Wildcat	0.048	**0.952**	0.155	**0.845**

5.1.4.3 Bayesian cluster analysis – with prior population information

When STRUCTURE was run with k = 2 and prior population information was assigned for each individual (N = 192), the results correlated closely with the phenotypic classifications under the both the Strict and Relaxed IDs (Table 9).

Table 9: Mean proportions of membership (q_{ik}) of the two inferred genetic clusters for the three pre-defined groups of free living cats in Scotland (presumed wildcat, presumed domestic cats and presumed hybrid) based on a priori population information.

A priori group	Strict ID		Relaxed ID	
	"Domestic" cluster	**"Wildcat" cluster**	**"Domestic" cluster**	**"Wildcat" cluster**
Domestic	**0.873**	0.127	**0.843**	0.157
Hybrid	0.454	0.546	0.437	0.563
Wildcat	0.003	**0.997**	0.063	**0.937**

On average 73% of domestic cats had a membership coefficient $q_{ik} \geq 0.8$ for the domestic cluster and between 76.9% - 100% of wildcats had a $q_{ik \geq} 0.8$ for the wildcat cluster under the Strict and Relaxed ID. In addition, a large percentage of those identified as hybrids had $q_{ik} < 0.8$ for either cluster, confirming that these individuals were admixed (Table 10).

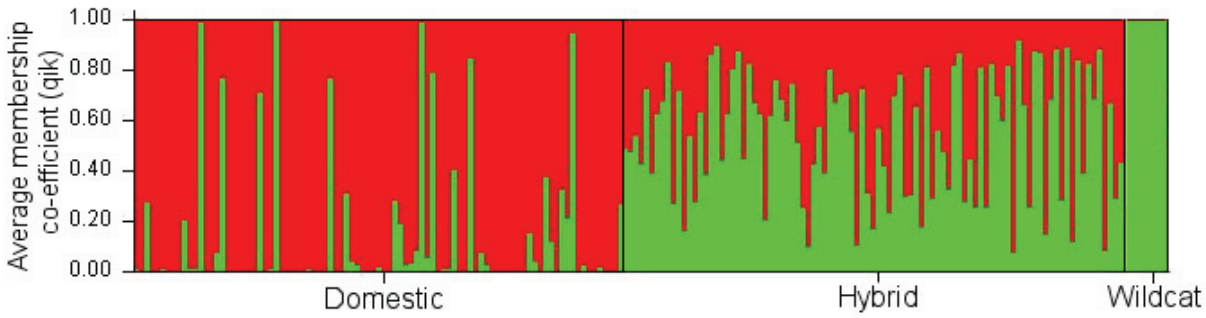
Table 10: Number of individual (N = 192) cats identified by their a priori phenotypic groups under the Strict and Relaxed IDs (wildcats, domestic cats, hybrids) separated into two genetic groups (wildcat and domestic clusters) based on having a value of q_{ik} > 0.9. The numbers of individuals with intermediate q_{ik} values are also shown.

		Strict ID		
A priori group	**q_{ik} > 0.8 for the "Wildcat" cluster**	**Intermediate q_{ik} values**	**q_{ik} > 0.8 for the "Domestic" cluster**	**n**
Domestic	4.5%	21.9%	73.6%	91
Hybrid	22.6%	67.8%	9.6%	93
Wildcat	100%	-	-	8
		Relaxed ID		
Domestic	6.6%	19.7%	73.7%	91
Hybrid	24%	64%	12%	75
Wildcat	76.9%	23.1%	-	26

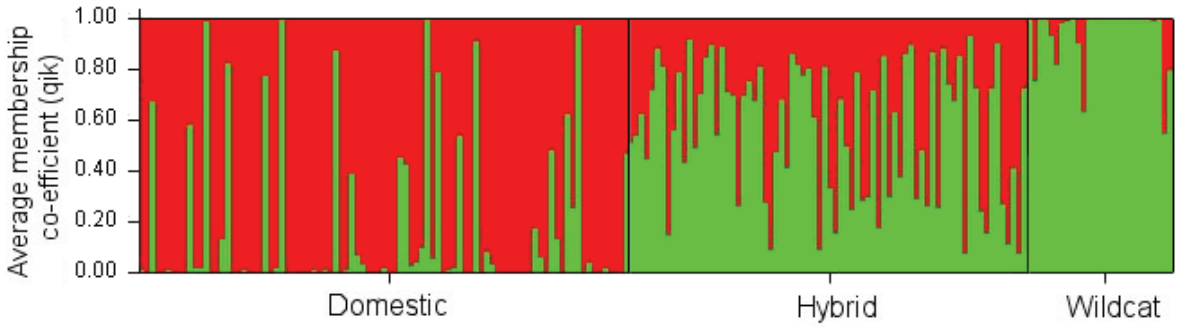
However the phenotypic classifications do not correspond 100% to the genetic clusters. Several cats identified as domestic or hybrid had membership in the wildcat cluster ($q_{ik} \geq 0.8$) and although the Relaxed ID picked up several individuals, which genetically belonged to the wildcat cluster that had not been classified as wildcats under the Strict ID, seven (out of 26) individuals classified phenotypically as wildcats were shown to have mixed genotypes with $q_{ik} < 0.8$ for either cluster and were instead more likely to be hybrids (Figure 12).

Figure 12: Summary plot of estimates of q a) Strict ID, b) Relaxed ID. Each individual is represented by a single vertical line broken into K coloured segments, with lengths proportional to each of the K inferred clusters. Predefined groups are shown along the horizontal axes.

a)



b)



Cases where individuals whose genotypes do not correspond to their phenotypical classification are described in more detail below:

1) Domestic phenotype with wildcat genotype

A total of six individuals had $q_{ik} \geq 0.8$ for the wildcat cluster. Of these, four individuals had classic tabby markings and two were black cats, both of whom had a score of two (hybrid) for their tail shape.

2) Domestic phenotype with mixed genotype

A total of 18 individuals classified as domestic had $q_{ik} < 0.8$ for either cluster and were therefore considered to be hybrids rather than domestics. Of these, eight had classic tabby pelage, eight were tabby cross (see 5.1.1.2.) and two were black, one of which scored two for its tail shape.

3) Hybrid phenotype with domestic genotype

Nine individuals were identified as having $q_{ik} \geq 0.8$ for the domestic cluster, with an average 7PS of 13. Further examination revealed that only four of these had classic tabby pelage, the remaining individuals were either tabby cross or ginger cats.

4) Hybrid phenotype with wildcat genotype

Under the Strict ID, 21 individuals classified as hybrids had $q_{ik} \geq 0.8$ for the wildcat cluster. Of these, seven were classified as wildcats under the Relaxed ID. The remaining 14 were all identified as having the classic tabby pelage and scoring a mean of 15 for their 7PS. Several individuals missed out on a wildcat classification on the basis of one of the 8PC characteristics scoring 1, such as having a white chin.

6) Wildcat phenotype with mixed genotype

As previously mentioned, under the Relaxed ID seven individuals with a wildcat phenotype were found to have $q_{ik} < 0.8$ for the wildcat cluster (0.07 – 0.77) and with some level of ancestry from the domestic cluster. This indicated that these individuals are hybrids. Table 11 shows these individuals have some ancestry in the domestic cluster but are probably not directly from the domestic cluster.

Table 11: The posterior probability (q_{ik}) that individuals identified as wildcats are correctly assigned to the wildcat cluster. Subsequent columns show the probabilities that the individual is from, or has ancestry (parent or grandparent) in the domestic cluster.

Individual ID	Wildcat cluster q_{ik}	Probability from domestic cluster	Probability parent from domestic cluster	Probability grandparent from domestic cluster
DB95	0.071	0.057	0.72	0.151
MD3	0.433	0.246	0.165	0.156
DB99	0.473	0.019	0.215	0.294
DB116	0.529	0.011	0.249	0.211
MD95	0.674	0.187	0.064	0.074
MD27	0.728	0.002	0.132	0.138
DB12	0.779	0.007	0.028	0.186

5.2 Skull assessment

There were a total of 208 complete or partially broken skulls. Of these, 140 skulls had some or all of the 30 skull measurements taken as described by Yamaguchi *et al.*, (2004) and TSCs were scored for 131 individuals, CIs for 130 and 128 skulls were digitised.

5.2.1 Skull Character assessment in relation to pelage classification

Of the 30 skull variables measured (see 5.1.2), 16 differed significantly between the three groups using the Strict ID (Kruskal Wallis Test $p < 0.05$; see Appendix 2). In particular individuals identified as domestic had, on average, significantly shorter or smaller skull measurements than those of both hybrids and wildcats for the variables listed in Table 12. Using the Strict ID, there was no significant difference between hybrids and wildcats (Mann Whitney U Test $p > 0.05$ for all 30 variables), although this may partly reflect a low sample size for wildcats (N = 7). A similar pattern was seen using the Relaxed ID, with domestic cat skulls significantly shorter/smaller than those of hybrids and wildcats for a number of variables (Table 12).

Table 12: A comparison of the mean measurements of the 30 skull variables from the three groups (Domestic = dom, hybrid = hybrid, wildcat = wild) using both Strict and Relaxed IDs and showing the total number of cats (N) in each category.

Measurement	Strict ID			Relaxed ID		
	Dom/Hyb N=46/61	Dom/Wild N=46/7	Hyb/Wild N=61/7	Dom/Hyb N=46/52	Dom/Wild N=46/16	Hyb/Wild N=52/16
	p - value					
Greatest length of skull	0.06	0.49	0.62	**0.04***	0.62	0.31
Condylobasal length	0.06	0.65	0.46	**0.04***	0.65	0.23
Facial length	0.28	0.63	0.74	0.17	0.99	0.23
Lateral length of snout	**0.01***	0.43	0.55	**0.01***	0.20	0.44
Length between $Pm^2$and M^1	0.78	0.27	0.14	0.66	0.41	0.15
Length between $Pm^2$and Pm^4	0.55	0.43	0.27	0.53	0.77	0.47
Greatest length of Pm^4	**	**0.02***	0.80	**	**0.01***	0.29
Greatest breadth of Pm^4	**	**0.02***	0.45	**	**0.01***	0.25
Anteroposterior diameter of the auditory bulla	**0.02***	0.97	0.32	**0.02***	0.63	0.21
Mastoid breadth	0.50	0.76	0.95	0.44	0.86	0.73
Greatest breadth of the occipital condyles	**	**0.03***	0.12	**	**0.03***	0.49
Greatest breadth of the foramen magnum	**	**0.01***	0.14	**	**	0.16
Greatest width of the brain case	**	**0.01***	0.42	**	0.06	0.29
Zygomatic breadth	0.10	0.31	0.98	0.06	0.65	0.31
Frontal breadth	0.71	0.88	1.00	0.59	0.87	0.49
Least breadth between the orbits	**0.01***	0.59	0.33	**	0.50	0.14
Greatest palatal breadth	**0.01***	0.39	0.57	**0.02***	0.06	0.71
Rostrum breadth: greatest breadth between the canine aveoli	**	0.64	0.32	**0.01***	0.20	0.59
Least breath of the postorbital constriction	**0.02***	**0.01***	0.11	**0.03***	**0.02***	0.42
Breadth between the infraorbital foramena	**	0.08	0.89	**	0.10	0.45
Minimum length of the nasals	0.44	0.63	0.34	0.62	0.59	0.79
Maximum length of the nasals	0.20	0.64	0.94	0.13	0.83	0.47
Width of cranial suture	**	**0.02***	0.42	**	**	0.30
Maximum distance between pongonion and coronoid process	0.17	0.66	0.78	0.11	0.87	0.30
Maximum distance between pongonion and angular process	**0.01***	0.32	0.71	**0.01***	0.32	0.45
Length between mandibular Pm^3 and M^1	**	0.20	0.91	**	0.08	0.73
Depth of the mandible behind M^1	**	**0.05***	0.64	**	**0.02***	0.98
Height of Ramus	**	0.09	0.76	**	0.06	0.86
Maximum width of mandibular condoyles (not shown)	0.45	0.23	0.46	0.39	0.51	1.00
Maximum width of mandibular Pm^4 (not shown)	**	0.07	0.43	**	**0.03***	0.79

** Significant at p≤ 0.05 Mann-Whitney U test*

*** Significant at p < 0.001 Mann-Whitney U test*

5.2.2 Total Skull Character Score

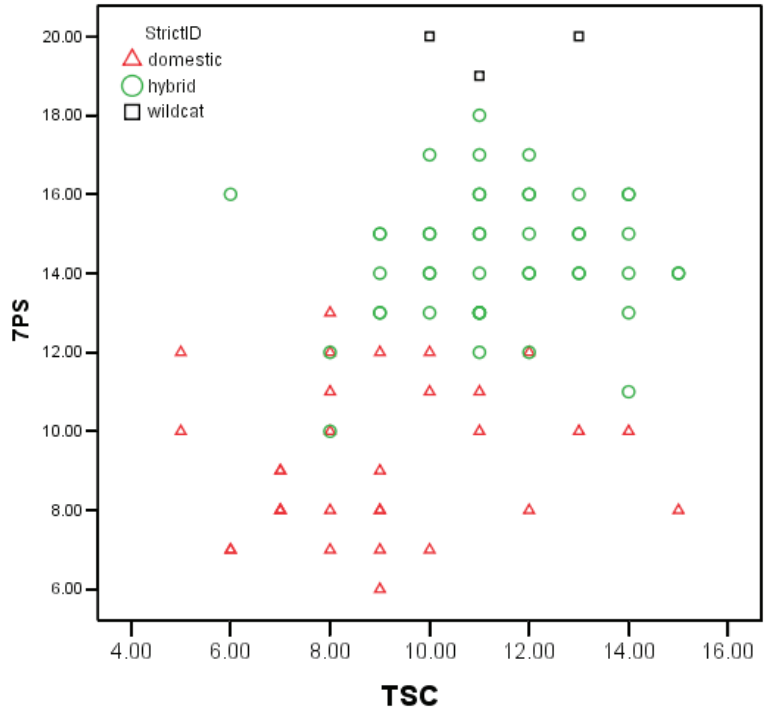
TSCs classified four skulls as domestic, five as wildcat and the remainder as hybrids (N=131).

5.2.3 TSC and pelage classification

Pelage assessments were available for 80 skulls with a TSC score. As can be seen from Figure 13, the relationship between TSC score and pelage score is not straightforward.

Overall there was a significant difference in mean TSC score between the three groups as classified under the Strict and Relaxed IDs (Kruskal Wallis Test χ^2_{strict} = 38.363, d.f. = 2, p < 0.001; $\chi^2_{relaxed}$ = 37.813, d.f. = 2, p < 0.001). Wildcats, whether using the Strict or Relaxed IDs, had a significantly higher mean TSC score than those of domestics (Mann Whitney U Test Z_{Strict} = -3.429, p = 0.01; $Z_{Relaxed}$ = -4.075, p < 0.001), but mean wildcat TSCs were not significantly different from those of hybrids (Z_{Stric} = - 1.047, p = 0.295; $Z_{Relaxed}$ = -0.867, p = 0.386). Mean TSC score was also significantly greater in hybrids than in domestics as indicated by their pelage scores (Z_{Stric} = -5.855, p <0.001; $Z_{Relaxed}$ = -5.742, p <0.001). However, two individuals identified as domestic had a wildcat TSC score, and one identified as having wildcat pelage had a hybrid TSC score (Table 13).

Figure 13: The relationship between TSC and 7PS scores for individuals assessed by the Strict ID as domestic, hybrid or wildcat.



There were also significant differences in the scores for the skull variables between each of the three groups as defined by their pelages; nasal curvature (χ^2_{strict} = 17.805, d.f. = 2, p < 0.001;

$\chi^2_{relaxed}$ = 18.220, d.f. = 2, p < 0.001), nasal pit (χ^2_{strict} = 13.290, d.f. = 2, p = 0.01; $\chi^2_{relaxed}$ = 13.300, d.f. = 2, p = 0.01), parietal suture (χ^2_{strict} = 20.948, d.f. = 2, p < 0.001; $\chi^2_{relaxed}$ = 18.282, d.f. = 2, p < 0.001) and mandible (χ^2_{strict} = 17.565, d.f. = 2, p < 0.001; $\chi^2_{relaxed}$ = 17.616, d.f. = 2, p < 0.001). There was a significant difference in nasal extension between the three groups using the Strict ID, but not using the Relaxed ID (χ^2_{strict} = 6.624, d.f. = 2, p = 0.036; $\chi^2_{relaxed}$ = 1.225, d.f. = 2, p = 0.542) (see Table 13). In general individuals with domestic pelage tended to score 1 or 2 for the five variables with the exception of nasal extension, where a higher percentage of individuals scored 2 or 3, and the mandible, where about equal numbers of individuals scored either 1 or 3. The majority of individuals identified as hybrids scored 2 for all variables, except mandible, where a larger percentage scored 3. The results for individuals identified as wildcat, using the Strict ID, were mixed; with wildcats scoring 2 or 3 for most of the variables but sometimes scoring 1 (see Table 13). Similar results were seen using the Relaxed ID. Therefore, although pelage classification generally correlated with overall TSC score, this relationship was not absolute.

Table 13: A comparison between the three groups (Domestic, hybrid, wildcat) as defined using the Strict ID and the percentage of individuals in each group having a score of 1 (domestic), 2 (hybrid) or 3 (wildcat) for each of the five variables used to calculate a TSC score.

	Nasal Curvature (%)			Nasal Pit (%)			Parietal Suture (%)			Nasal Extension (%)			Mandible (%)		
Score	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Domestic (N = 47)	59.2	25.5	12.7	21.3	31.9	10.6	48.3*	37.9*	13.8*	6.4	70.2	23.4	55.1	-	44.9
Hybrid (N = 61)	18	55.7	26.3	3.3	63.9	32.8	3.7*	66.6**	29.7**	4.9	67.2	27.9	16.4	-	83.6
Wildcat (N = 7)	28.6	71.4	0	0	71.4	28.6	0	28.6	71.4	0	28.6	71.4	14.3	-	85.7

* Some domestic cats had a fused parietal suture and were not given a score therefore N = 29

** Some hybrid cats had a fused parietal suture and were not given a score therefore N = 54

5.2.4 TSC and mtDNA

Only 13 skulls had data for both TSC score and mtDNA. Although individuals with wildcat mtDNA had a lower mean TSC score than those with the domestic mtDNA ($\bar{x}$ = 8.00, 10.67; S.D = 2.16, 2.45; N = 4, 9 for individuals with wildcat mtDNA and domestic mtDNA respectively), this difference was not significant (T = -1.869, d.f. = 11, p = 0.088) and all except one individual was given a hybrid classification from its TSC score. The remaining individual was classified as domestic. These results should be viewed with caution owing to the very small sample size.

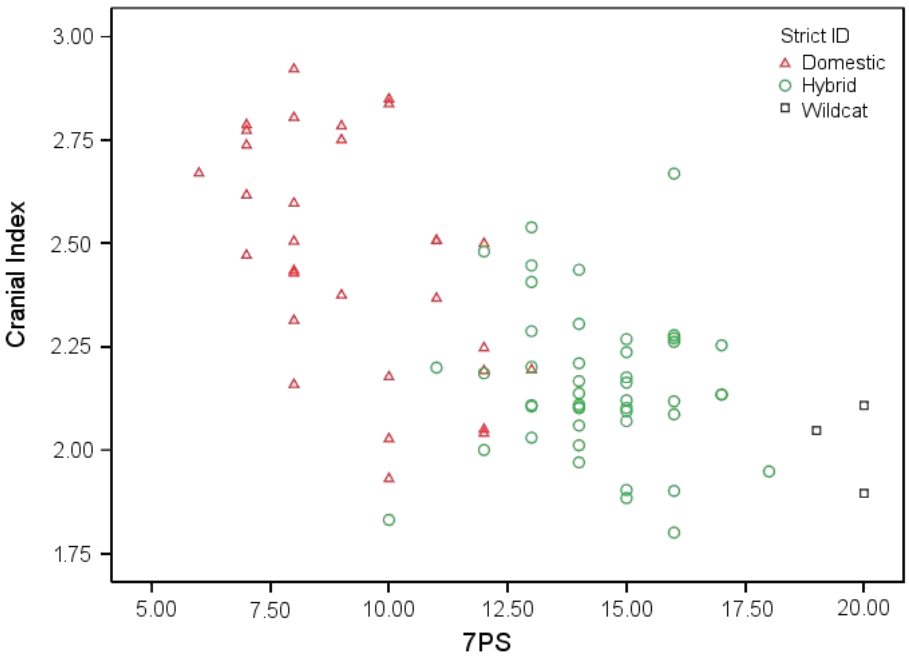
5.2.5 Cranial Index

CIs ranged from 1.77 to 3.53 ($\bar{x}$ = 2.29, S.D = 0.31, N = 130). Most individuals (90%) had a Cranial Index of <2.75 and would, therefore, be classified as wildcats according to Schauneberg (1969), with the remaining 10% as domestic cats.

5.2.6 Cranial Index and pelage classification

Pelage classification was inversely correlated with CI; individuals with higher 7PS scores had a lower CI. When individuals were classified into groups using the Strict and Relaxed IDs, this relationship was found to be significant (Anova: Strict ID $F_{2, 63}$ = 11.360, p < 0.001; Relaxed ID, $F_{2, 63}$ = 10.453, p < 0.001) (see Figure 14). Individuals classified as domestic using the Strict and Relaxed IDs were found to have a significantly greater mean CI than those of hybrids (T-Test, T_{Strict} = 4.382, d.f. = 61, p < 0.001; $T_{Relaxed}$ = 4.295, d.f. = 54, p < 0.001) and wildcats (T_{Strict} = 2.517, d.f. = 18, p = 0.022: $T_{Relaxed}$ = 2.856, d.f. = 25, p = 0.009). There was no significant difference in mean CI between hybrids and wildcats whether classified using the Strict or Relaxed IDs (T_{Strict} = 1.335, d.f. = 45, p = 0.188; $T_{Relaxed}$ = -0.019, d.f. = 47, p = 0.985). Although Schauneberg did not consider hybrids in his classification, our data using pelage classification shows a similar pattern to that of Ward and Kitchener (unpublished) who found that mean CI's for individuals identified by their gut length was lowest in wildcats ($\bar{x}$ CI = 2.28, S.D. = 0.18), followed by hybrids ($\bar{x}$ CI = 2.50, S.D. = 0.25) then domestic cats ($\bar{x}$ CI = 3.093, S.D. = 0.29). In this study, under the Strict ID, wildcats had the lowest mean CI ($\bar{x}$ = 2.02, S.D = 0.11, N = 3), and domestic cats the highest ($\bar{x}$ = 2.47, S.D = 0.28, N = 31) with hybrids falling in between ($\bar{x}_{strict}$ = 2.15, S.D = 0.16, N = 46).

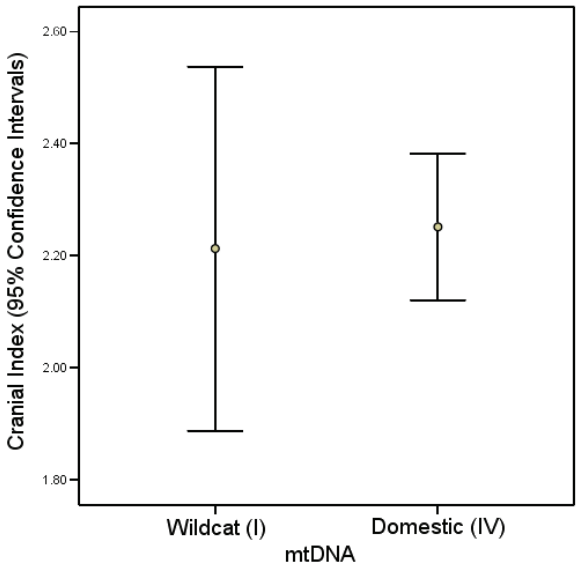
Figure 14: Relationship between 7PS and CI for individuals classified as domestic, hybrid or wildcat using the Strict ID



5.2.7 CI and mtDNA

Only 13 individuals had both a value for CI and mtDNA, consequently the following results should be treated with caution. Individuals with the domestic cat mtDNA (N = 9) had a slightly greater mean CI than those with wildcat mtDNA (N = 4) (Figure 15), although this difference was not significant (T = -0.358, d.f. = 11, p = 0.727).

Figure 15: Mean CIs of individuals with either the wildcat or domestic mtDNA.



5.2.8 Genetic variation in relation to skull classification

A total of 46 individuals with TSC scores had microsatellite data. Of these, 43 were classified as hybrids, two were domestic and one was a wildcat. Because of the low number of individuals who had domestic cat or wildcat TSC scores no microsatellite analysis was carried out because there were insufficient data to come to any firm conclusions on whether TSC classification corresponded to genetic classification.

5.2.9 Geometric Morphometric Analysis: variation in cranial size

A total of 128 skulls were digitised, of which 54 were male and 62 were female. Of the digitised skulls, only five had wildcat mtDNA and nine had domestic mtDNA. Owing to this low sample size and its low statistical power, results from the following analyses should be treated with caution.

For the limited sample of cats with mtDNA markers, cats with wildcat mtDNA had larger crania than those with domestic cat mtDNA ($F_{1,12}$ = 12.30, p < 0.005) (Figure 16). However this result needs to be confirmed with a larger set of data. It was not possible to investigate an interaction between sex and mtDNA, because all cats with wildcat mtDNA (included in this study of skull morphometrics) were male.

A General Linear Model (GLM) of sex, TSC score and Strict ID was developed without mtDNA as a factor, because of the limited sample size for this factor. There was no significant difference in cranial size among cat groups identified by TSC score ($F_{2,81}$ = 0.41, p < 0.667). However, males had bigger crania than females' ($F_{1,81}$ = 19.84, p < 0.001), while cats which were identified as domestic using the Strict ID had smaller crania than those identified as either hybrids or wildcats ($F_{2,81}$ = 5.15, p < 0.01). There was no significant interaction between sex and Strict ID ($F_{2,81}$ = 0.14, p = 0.872) (Figure 17). Similar results were seen using the Relaxed ID in the GLM.

Figure 16: A comparison of centroid size in cats with wildcat mtDNA (I) and domestic cat mtDNA (IV).

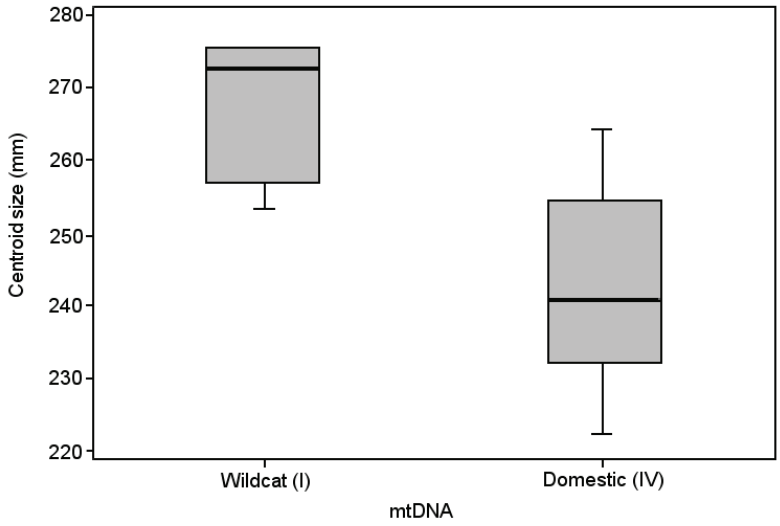
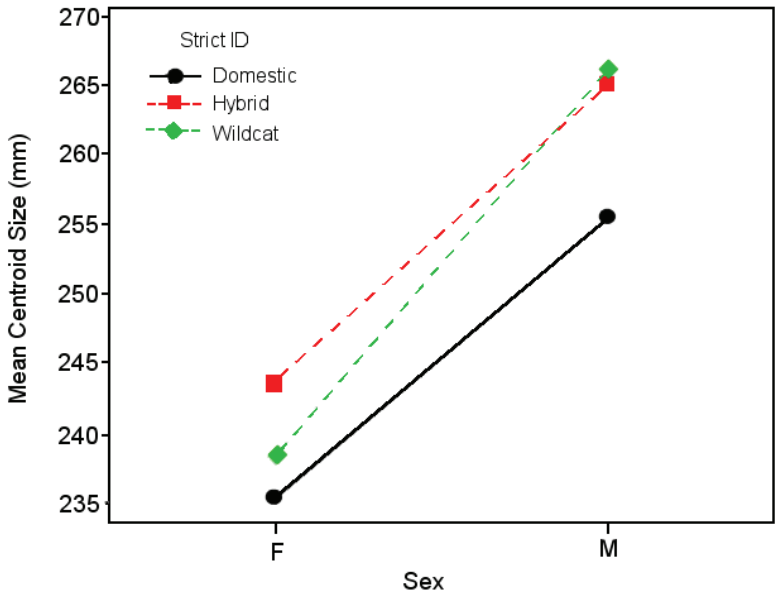


Figure 17: Male cats have larger crania than those of females, while cats with domestic pelage have significantly smaller crania than those of either wildcats or hybrids.



5.2.9.1 Principal Component 1

A principal components analysis (PCA) of the procrustes coordinates (relative warps analysis) was conducted to investigate the major axes of shape variation. The first principal component (PC1) captured 14.07% of the shape variation. As can be seen in Figure 18, cats at the positive end of PC1 have narrower cranial vaults and the sagittal crest is lower and more posteriorly extended. Cats at the negative end of PC1 have a wider neurocranium, and a higher and shorter sagittal crest.

A GLM of sex, Strict ID, TSC score and mtDNA (Figure 19) revealed that only TSC score ($F_{2,123}$ = 4.84, p < 0.01) was significantly associated with PC1, although it explained little of the variation (R-Sq = 7.3%). Cats categorized as hybrids by TSC score are found at the positive end of PC1, while those identified as wildcats and domestics scored negatively on PC1 (Figure 20). In a separate GLM, using characters that comprise the TSC score (Nasal Curvature, Nasal Pit, Nasal Extension, Parietal Suture and Mandible) only parietal suture shape (PSS) ($F_{2,112}$ = 7.38, p < 0.001) was significantly associated with PC1. However, PSS only explained 11.65% of the variance.

Figure 18: Skull shape variation (dorsal and lateral views) associated with the positive end of PC1. The grey circles show the average shape. The change from the grey circles and lines to the black circles and lines indicates the landmark shifts corresponding to the positive end of PC1.

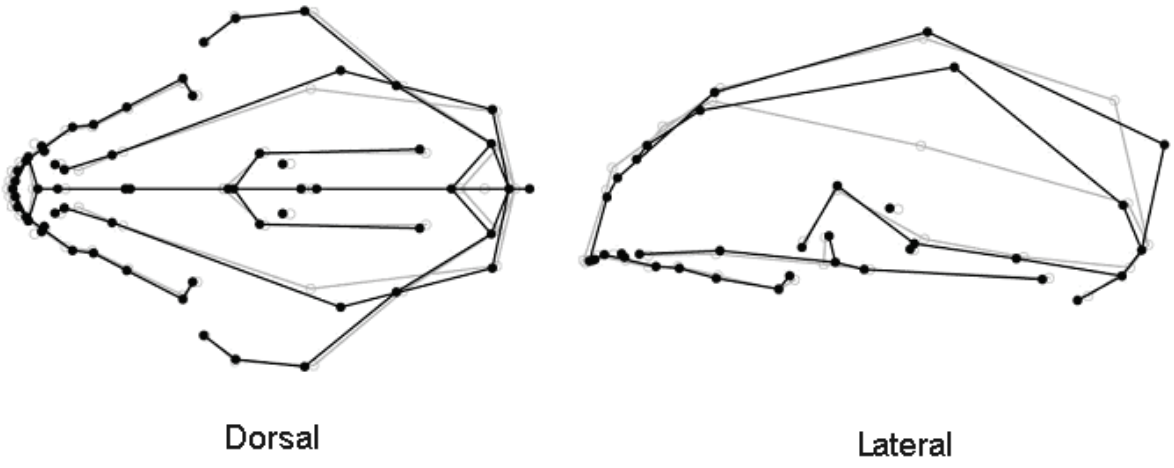
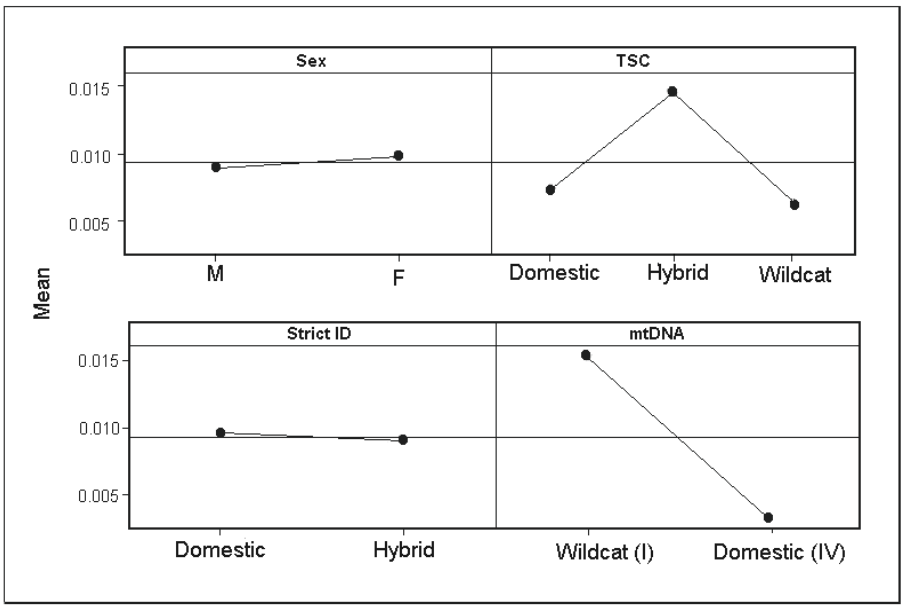
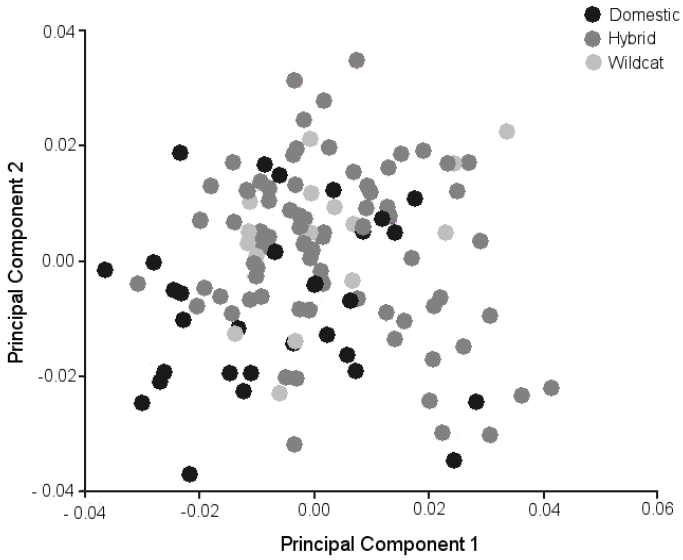


Figure 19: Plots of the main effects of the variation of PC1 with sex, TSC score, Strict ID and mtDNA marker.



The low variance explained by these factors indicates that there are other factors, which may explain the shape variation in PC1. Strict and Relaxed IDs are co-linear and so could not be included in the same GLM. A separate GLM of sex, Relaxed ID, TSC score and mtDNA gave similar results. Relaxed ID, sex and mtDNA were not significant. PC1 is not correlated with centroid size (R-Sq = 0%, p = 0.88) and is therefore non-allometric.

Figure 20: Plots of PCs 1 and 2, showing those cats which are categorized as wildcat (light grey), hybrid (dark grey) or domestic (black) by their TSC scores.



5.2.9.2 PC2

PC2 captured 12.17% of cranial shape variation in the dataset. Cats that score positively on PC2 have relatively wider and higher cranial vaults and zygomatic arches, while the cranial base is more ventral (Figure 21).

A GLM of sex, Strict ID, TSC score and mtDNA revealed that Strict ID ($F_{2,83}$ = 3.44, p < 0.037) and sex ($F_{1,83}$ = 14.87, p < 0.001) were significantly associated with PC2 (Figure 22).

There was no significant interaction between sex and Strict ID. The amount of variation captured by these two factors was low (R-sq = 25.98%), indicating that there other factors which explain the skull shape variation in PC2. Domestic and male cats scored negatively on PC2 while female cats and hybrids scored positively. Wildcats were close to zero on PC2. Strict and Relaxed IDs are co-linear and so could not be included in the same GLM. In a separate GLM of sex, Relaxed ID, TSC score and mtDNA, both Relaxed ID ($F_{2,83}$ = 3.42, p < 0.04) and sex ($F_{2,83}$ = 15.03, p < 0.001) were significant. There was no significant interaction between Relaxed ID and sex. The amount of variation explained by Sex and Relaxed ID is only 22.95%.

There is a very weak negative correlation between Centroid Size and PC2 (slope = -.0004, R-sq = 20.0%, p < 0.001). Larger animals tend to score negatively on PC2 and, therefore, the shape differences are partly allometric, but this mostly reflects average size differences between males and females. Although Strict and Relaxed IDs are significant, they capture relatively little variation in PC2 compared to sex-related shape differences.

Shape variation in PC2 seems to indicate that male cats and to lesser extent those with a domestic pelage have relatively smaller cranial vaults for their size than female cats and hybrids.

Figure 21: Skull shape variation (dorsal and lateral views) associated with the positive end of PC2. The grey circles show the average shape. The change from the grey circles and lines to the black circles and lines indicates the landmark shifts corresponding to the positive end of PC2.

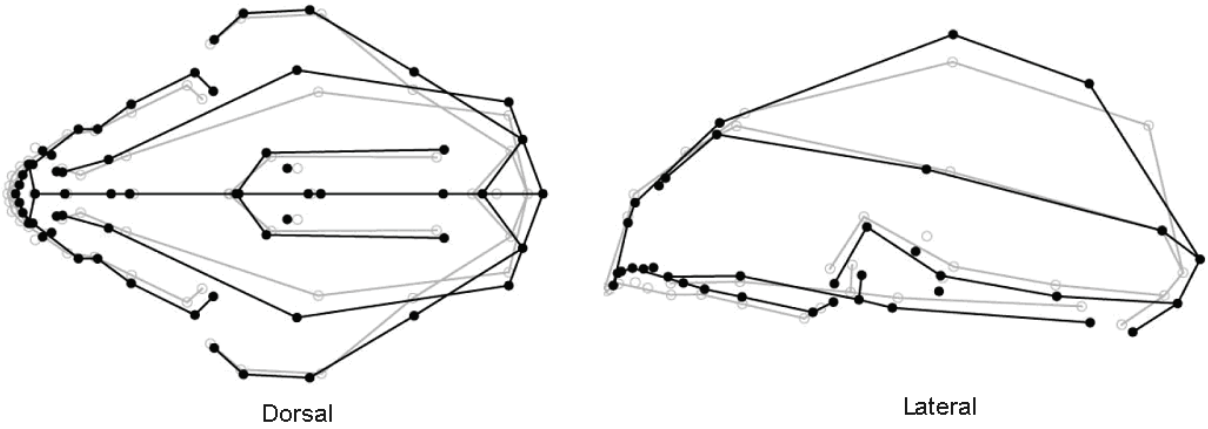
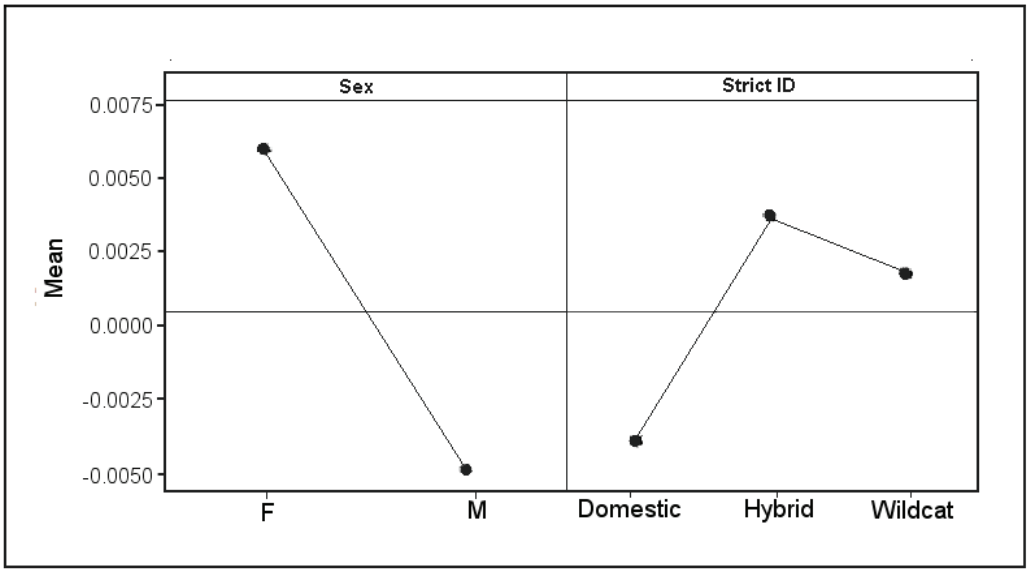


Figure 22: Sex and Strict ID predict some of the skull shape variation in PC2.



5.2.9.3 PC3

A further 8.7% of cranial shape variation was captured by PC3. Specimens with positive scores on PC3 have a wider, elongated and higher neurocranium, and a shortened and convex face (Figure 23).

A GLM of sex, Strict ID, TSC score and mtDNA revealed that Strict ID ($F_{2,81}$ = 6.60, p < 0.003) and sex ($F_{1,81}$ = 12.62, p < 0.002) were significantly associated with PC3. There was no significant interaction between sex and Strict ID ($F_{2,81}$ = 2.68, p < 0.074). Again the amount of variation captured by these two factors was low (R-sq = 25.53%), indicating that there are other factors which explain skull shape variation in PC3.

Strict and Relaxed IDs are colinear and so could not be included in the same GLM. In a separate GLM of sex, Relaxed ID, TSC score and mtDNA both Relaxed ID ($F_{2,81}$ = 5.59, p < 0.006) and sex ($F_{2,81}$ = 17.34, p < 0.001) were significant. There was also a significant interaction between Relaxed ID and sex ($F_{2,81}$ = 3.41, p < 0.04) (Figure 24). The amount of variation explained by sex and Relaxed ID is only 26.63%.

The sexual dimorphism in wildcat skulls is revealed in PC3 with female wildcats more similar in shape to the skulls of male and female domestic cats. A regression of centroid size on PC3 showed the shape variation captured by PC3 is only very slightly allometric (slope = -0.0005, R-sq = 39.3%, p < 0.001).

Figure 23: Skull shape variation (dorsal and lateral views) associated with the positive end of PC3. The grey circles show the average shape. The change from the grey circles and lines to the black circles and lines indicates the landmark shifts corresponding to the positive end of PC3.

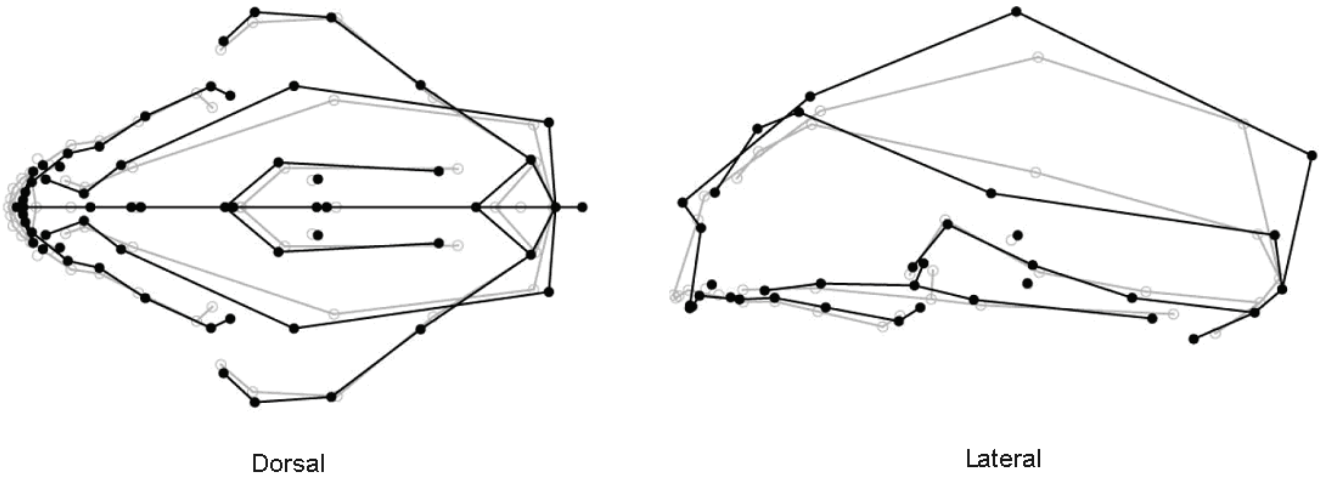
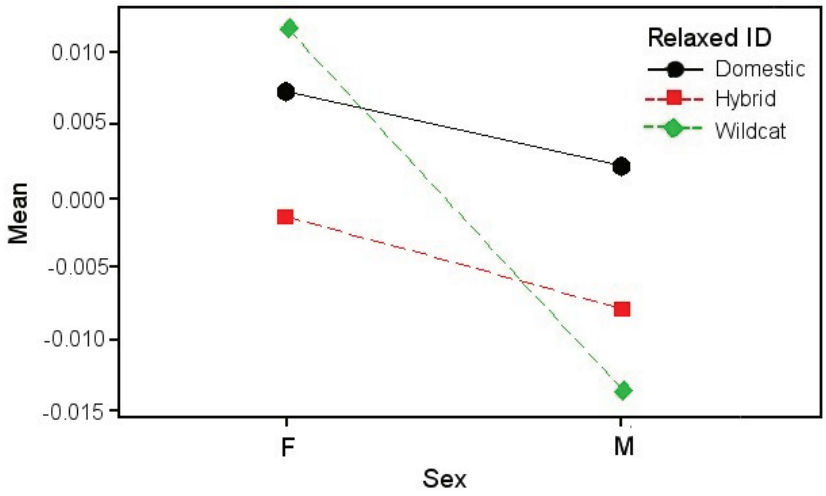


Figure 24: Interaction plot for PC3, fitted means. Sex and Relaxed ID as well as the interaction between them predict some of the skull shape variation in PC3.



5.2.9.4 Canonical Variates Analysis (CVA)

A CVA showed a clear separation between male and female specimens (Figure 25). This is confirmed by Mahalanobis distance permutation tests ($p < 0.0001$). Males have relatively narrower and shallower cranial vaults than those of females (Figure 26).

Figure 25: CVA of male and female cats.

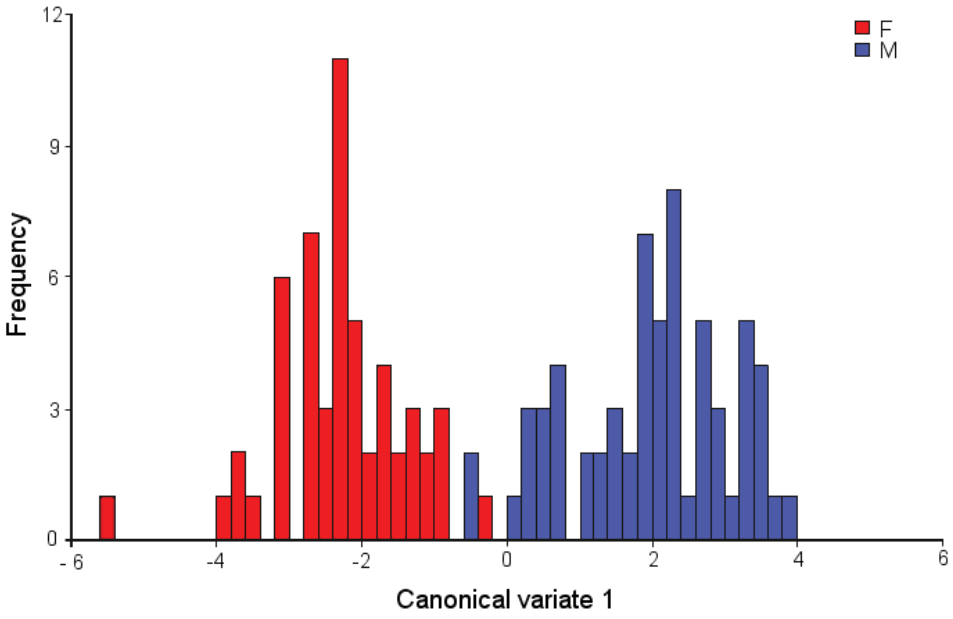
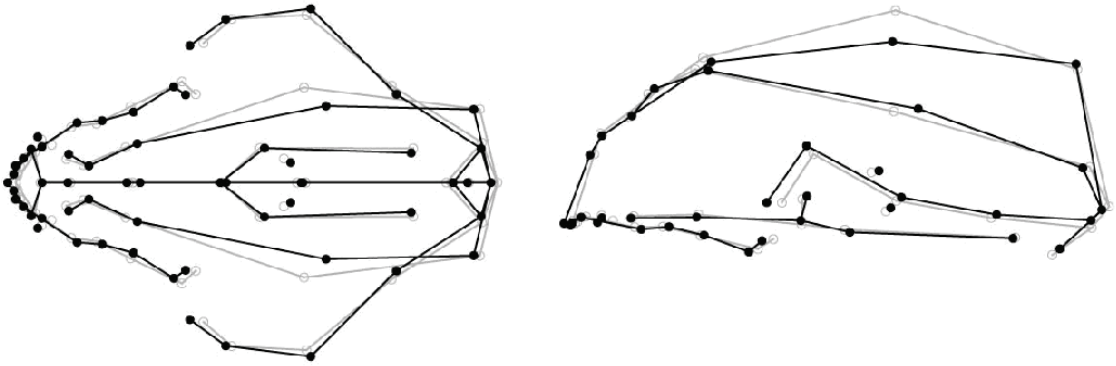


Figure 26: Shape variation associated with positive scores on the CV1 of the CVA by sex. The grey circles show the average shape. The change from the grey circles and lines to the black circles and lines indicates the landmark shifts corresponding to the positive end of CV1.



In a CVA of Relaxed ID the three classifications of domestic, hybrid and domestic were all separated with different cranial shapes (Mahalanobis distance test: $p < .0001$). Cats with wildcat pelage were separated from those with domestic and hybrid pelages on the first axis (Figure 27). Wildcats have wider zygomatic arches and posterior palates, and more convex posterior nasals. The basicranium is more ventral, making the neurocranium relatively larger in wildcats (Figure 28). Our results support those found by other studies using intestinal length and bone length (French *et al*., 1988; Daniels *et al*., 1998; Reig *et al*., 2001) and nominal identifications from museum labels (Yamaguchi *et al.*, 2004) to distinguish wildcats from domestic cats and hybrids.

Figure 27: A CVA of Relaxed ID.

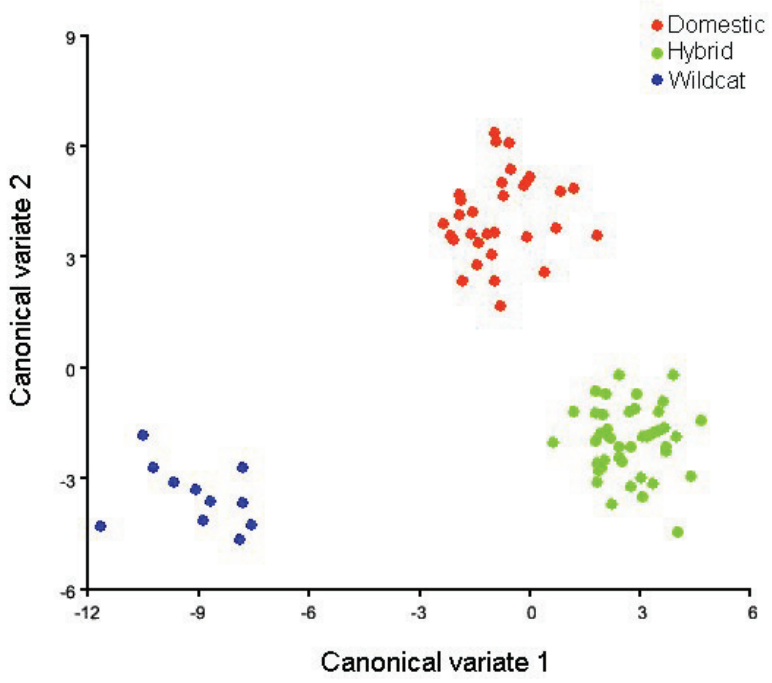
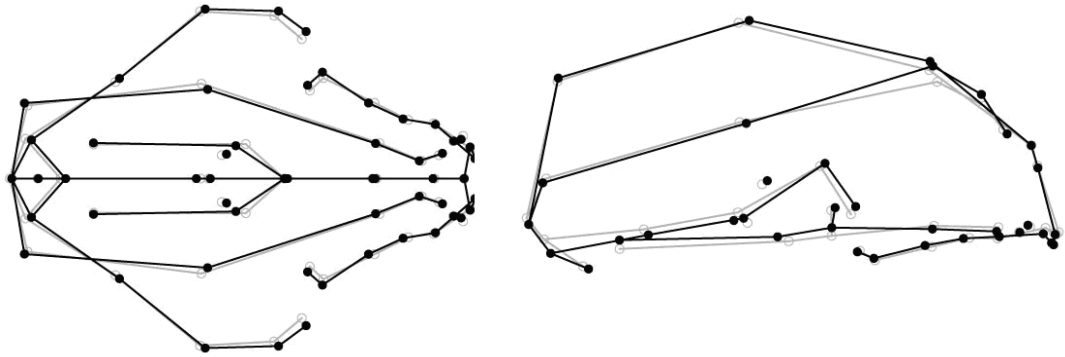


Figure 28: Shape variation associated with negative scores on CV1 of the CVA by Relaxed ID. The grey circles show the average shape. The change from the grey circles and lines to the black circles and lines indicates the landmark shifts corresponding to the positive end of CV1.



Using the Strict ID in a CVA also successfully separated the three pelage groups ($p < 0.0001$). However, these results should be treated with some caution, owing to the small wildcat sample size. In this CVA the wildcats, domestics and hybrids were all separated from each other along the first axis and the wildcats were distinguished from the other two groups on CV2 (Figure 29).

CV1 separates the wildcats and hybrids from the domestic cats. As can be seen in Figure 30, the wildcats and hybrids have relatively wider neurocrania, but their faces are more concave and the sagittal crest is flattened and more posterior.

Figure 29: Canonical variates analysis of Strict ID.

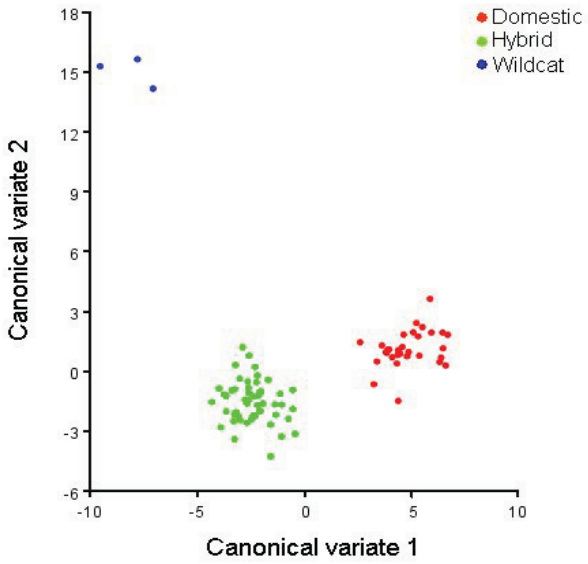
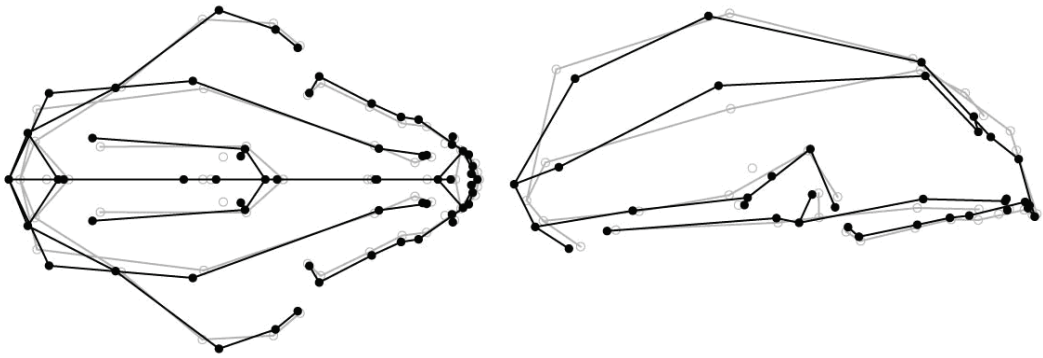


Figure 30: Shape variation associated with negative scores on CV1 of the CVA by Strict ID. The grey circles show the average shape. The change from the grey circles and lines

to the black circles and lines indicates the landmark shifts corresponding to the positive end of CV1.



5.2.10 **Summary of Geometric Morphometric Analysis**

Skull digitisation indicated that males had bigger crania than those of females and, in particular, domestic cats had smaller crania than individuals identified as either hybrids or wildcats using both the Strict and Relaxed IDs. A PCA, based on the procrustes coordinates, resulted in three PCs. PC1 was related to the width of the cranial vaults and the shape of the sagittal crest, PC2 was related to overall size of the cranial vault, width of the zygomatic arches and shape of cranial base and PC3 related to the size of the neurocranium and shape of the face. Overall the PCA revealed that female wildcats had a skull shape more similar to that of domestic cats and that male cats in general, and domestic cats, have smaller cranial vaults relative to their size than those of females or hybrids. In addition individuals identified as wildcats by both the Strict and Relaxed IDs have a wider neurocranium, and a higher and shorter sagittal crest than those of hybrids, which is supported by the CVA (see below). However, the PCA explained little of the overall variation, indicating that other factors may be involved.

The CVA showed a clear separation of male and female individuals, with males having a significantly narrower and shallower cranial vault than that of females. The CVA also showed that individuals classified as wildcats, using both the Relaxed and Strict IDs, had a different cranial shape to those of both hybrids and domestics. Wildcats and hybrids have relatively wider neurocrania, but their faces are more concave and the sagittal crest is flattened and more posterior than that of domestics. In addition individuals identified as wildcats have wider zygomatic arches and posterior palates, more convex posterior nasals and a larger neurocranium than those of hybrids or domestics.

6. DISCUSSION

The aim of this project was to re-evaluate the sample of wild-living cats collected by Balharry & Daniels (1998), using the methodology described by Kitchener *et al*., (2005), to determine whether there was any correlation between morphological and genetic classifications of the Scottish wildcat. In addition, in order to provide a more robust analysis of the relationship between the two identification techniques, we examined several other pelages and skulls, for which genetic data were also available.

6.1 Overview of the sample

6.1.1 Pelage classification

Although the majority of the sampled individuals exhibited the classic tabby coat colour more commonly associated with wildcats (Kitchener *et al.*, 2005), most were identified as either domestic or hybrid under both Strict and Relaxed IDs. The Relaxed ID accounted for the greatest number with 13.1% out of a total of 330 individuals classified as wildcats. This is perhaps not surprising when we consider that the specimens collected by Balharry & Daniels (1998) were predominantly road traffic accidents. More samples were, therefore, likely to be collected from areas with increased levels of traffic and, by association, increased levels of urbanisation (see Appendix 4) with a greater likelihood of domestic cats. Introgression is widespread in Scotland (Hubbard *et al.,* 1992; Beaumont *et al.,* 2001; Macdonald *et al.*, 2004) and on the basis of empirical evidence from Yamaguchi *et al.*, (2004), Macdonald *et al*. (2004) hypothesised that hybridisation between wildcats and domestic cats occurs to a greater extent around areas of increased human inhabitation, where a higher population density of domestic and feral cats exists. This is supported by studies in Europe which suggest that rates of hybridisation could increase locally in rural areas with a widespread and abundant presence of domestic cats, if wildcat populations decline strongly as a result of direct persecution and/or habitat loss (Pierpaoli *et al.,* 2003). Therefore, we would expect to see a large proportion of hybrids in the sample collected by Balharry & Daniels (1998).

6.1.2 Skull classification

In addition to pelage classification, individuals were classified according to their CI, following Schauenberg (1969), and their TSC score, following Yamaguchi *et al.*, (2004). The majority of individuals had a CI of <2.75 and would, therefore, be classified as wildcats (90%). Schauenberg did not consider hybrids, so these cannot be identified by this simple analysis. However, this method is useful for distinguishing domestic cats (Kitchener pers. comm.). Our results show that CI seems to correlate closely with pelage classification, with individuals having a lower CI and a higher 7PS score, which, therefore, would make them more likely to be classified as wildcats or hybrids based on their pelage scores. It should be noted that most individuals had a TSC score of between six and 14, and were, therefore, classified as hybrids. Although individuals with a higher TSC score were more likely to be classified as hybrids or wildcats based on their pelage, this was not always the case. However, two reasons could account for these results. The first is that these apparent discrepancies may reflect widespread introgressive hybridisation, which has occurred across Scotland, particularly in the north-east; the second is that TSC may not be as accurate in differentiating between wildcats and domestics as previously thought.

6.1.3 Skull morphometrics

Overall individuals identified as domestic by both Strict and Relaxed IDs have on average smaller and shorter skulls than those of hybrids and wildcats. The most highly significant differences between the three groups were between domestics and wildcats, and between domestics and hybrids. There were no significant differences between hybrids and wildcats in any of the 30 skull variables measured.

6.1.4 Geometric Morphometric Analysis

The Geometric 3D analysis comparing skull shape of individuals categorised by pelage under the Strict and Relaxed ID showed similar results to the previous study which classified individuals based on intestinal length and limb bone length. Individuals with the "wildcat" pelage showed a larger degree of sexual dimorphism than those identified as "hybrids" or "domestic" cats and had larger skulls (Macdonald *et al*., 2004). The lack of a posterior nasal pit and more robust skull identified in this study has been also been noted in previous morphological studies of wildcats (e.g. Yamaguchi *et al.,* 2004; Kitchener *et al.,* 2005).

The CVA showed a clear separation of male and female individuals, with males having a significantly narrower and shallower cranial vault than that of females. The CVA also showed that individuals classified as wildcats, using both the Relaxed and Strict IDs, had a different cranial shape to those of both hybrids and domestics.

6.2 Genetic Analysis

6.2.1 mtDNA and morphological data

Data for mtDNA, for which there were also skins and/or skulls, were few and, as a result, analyses had low statistical power. However, skull digitisation indicated that individuals with wildcat mtDNA (N = 5) had larger crania than those with domestic mtDNA (N = 11). Although other data suggested that individuals with wildcat mtDNA tended to have domestic cat pelages, a higher CI and lower TSC score, individuals with domestic cat mtDNA often had hybrid or wildcat pelage characteristics, lower CIs and higher TSC scores. However, these observations should be treated with caution owing to the very small sample size. In particular, these data may be explained by the disruption of some characteristics seen in F1 and F2 hybrids where wild-type characters may mask domestic ones (e.g. Carr *et al*., 1986; Gaubert *et al*., 2005; Homyack *et al*., 2008)) and vice versa. In addition, because mtDNA is carried through the maternal side, it only represents a small proportion of an individual's DNA. Therefore, although an individual may have wildcat mtDNA, if it is exhibiting pelage and skull characteristics of a domestic or hybrid, then it is probable that this individual is a hybrid. Theoretically, it is possible that coat colour mutations could occur in wildcats (c.1.3% in Slovakia; Sladek, 1976), but to be certain of this, we would expect a concordance of all other genetic and cranial characters and measurements to confirm this.

Studies have shown that during the breeding season, male European wildcats shift their home ranges to cover the home ranges of female farm cats (Szemethy, 1993). If similar behaviour occurs in the Scottish wildcat population, then the higher numbers of individuals with domestic cat mtDNA and exhibiting wildcat or hybrid morphological characteristics could be explained by this directional hybridisation. For example, a study of hybridisation between white-tailed deer *(Odocoileus virginianus*) and mule deer (*O. hemionus*) concluded that the observed sharing of a

common mtDNA genotype between sympatric populations is potentially a result of hybridisation between white-tailed does and mule deer bucks, with a preferred absorption of hybrid offspring into the mule deer gene pool. The F1 hybrid offspring have equal proportions of white-tailed and mule deer nuclear alleles, but have mtDNA from white-tailed deer. If an F1 female then mates again with a mule deer buck, the B1 (backcross) offspring will have a lower proportion of white-tailed deer nuclear alleles, but will still have white-tailed deer mtDNA (Carr *et al*., 1986), until eventually a deer could arise resemblling a mule deer, but with white tailed deer mtDNA and a high proportion of mule deer nuclear DNA. Therefore, it is possible that this pattern of hybridisation has occurred in Scotland over a period of several years with F1 hybrids backcrossing into the wildcat population resulting in individuals with wildcat phenotypes, but with domestic cat mtDNA (e.g. Randi *et al*., 2001). It would be interesting to consider whether these apparent hybrids should be categorised as wildcats rather than hybrids, given that mtDNA does not determine the phenotype of an animal.

6.2.2 Microsatellite data and morphological data

6.2.2.1 Genetic diversity

Overall the number of alleles varied per locus and between the three groups as defined by their phenotypes. There were no alleles at frequencies greater than 5% in the wildcat group as defined under the Strict and Relaxed IDs that are not found in the domestic or hybrid populations. There is only one such allele, which is found in the "domestic" cat population that is not in the "wildcat" population, suggesting that these two groups are genetically differentiated from each other to some degree. This is further supported by the presence of some degree of structuring of sub-populations within the overall sample as confirmed by Weir & Cockerham F-Statistics (F_{st} = 0.024 – 0.029 overall for Relaxed and Strict IDs, respectively) (Weir and Cockerham, 1984). However this value is less than that obtained by other studies between the European wildcat and domestic cat where the average F_{st} = 0.11 ($p < 0.001$), indicating that wildcats and domestic cats are subdivided into distinct genetic pools in most European countries (Randi *et al.,* 2001; Pierpaoli *et al.,* 2003). Our lower results are likely to be the result of higher levels of hybridisation that have occurred between Scottish wildcats and domestics than that found across Europe (Beaumont *et al*., 2001; Pierpaoli *et al.,* 2003) although the current Scottish sample may be highly biased owing to the way in which most of the sample was made, i.e. road casualties. Pairwise analysis of the groups did show a significantly higher degree of sub-structuring between individuals classified as wildcats and domestics, indicating minimal gene flow between these two groups, with hybrids falling in between. Although this effect is reduced using the Relaxed ID, it does suggest that phenotypic classification reflects genetic differences among cats. It is also encouraging that despite apparently high levels of hybrids within the sample, wildcats have retained their distinctive morphological and genetic integrity.

Two groups (domestic and hybrid) using the Strict ID and all three groups (domestic, hybrid and wildcat) using the Relaxed ID had a significantly lower levels of heterozygosity (e.g. homozygote excess) than expected and were therefore not in Hardy-Weinberg equilibrium. This is probably the result of population mixing (e.g. hybridisation) and the positive F*IS* indicate that these two groups are possibly inbred.

6.2.2.2 Bayesian cluster analysis

STRUCTURE clearly identified two genetic clusters within the dataset before any *a priori* information on cat phenotype was incorporated into the model. Known wildcats fell into one cluster and known domestic cats fell into another cluster. When *a priori* information was

incorporated into the model, 100% of individuals classified as wildcat under the Strict ID and 76% of those classified as wildcats under the Relaxed ID were assigned to the "wildcat" genotype cluster, and 73% of individuals classified as domestic fell into the domestic cluster with hybrids falling in between the two. These data confirm the effectiveness of the phenotypic classifications by Kitchener *et al.,* (2005) in particular the Strict ID, in allocating most individuals to the correct genetic cluster, although the degree of hybridisation in this sample of Scottish wild-living cats may obscure the discriminatory potential of morphological classification somewhat. For example, although the Relaxed ID lowers the concordance between the two identification methods slightly, it conversely also picks up individuals that have a $q_{ik} > 0.9$ for the wildcat cluster that would not have been identified as wildcats under the Strict ID. Therefore, it is suggested that if some hybrids have most of the pelage characteristics of a wildcat with the exception of one or two, then genetic identification should also be used to confirm whether the cat should be considered a wildcat or a hybrid. Studies of the European wildcat and domestic cat have shown similar results, although most cases show a clearer distinction between the two genetic groups based on their phenotypical assessment, probably because of the lower degree of hybridisation that has occurred between these species in many other parts of Europe (Randi *et al.,* 2001; Pierpaoli *et al.,* 2003; Lecis *et al.,* 2006; Oliviera *et al.*, 2008; Randi 2008; O'Brien *et al.*, submitted). For example, across Europe the percentage of phenotypically good wildcats assigned to a "wildcat" genetic cluster varied depending on the local level of hybridisation (Italy - 98%, Randi *et al.* (2001); Sardinia - 100%, Randi *et al.* (2001); Bulgaria - 83%, Randi (2008); Belgium - 95%, Randi (2008); Portugal - 86%, Oliviera *et al.* (2008)). In Hungary, where hybridisation is at a similar level to that found in Scotland, only 43% of individuals with a wildcat phenotype were found to have a wildcat genotype and the remainder were found to be hybrids (Pierpaoli *et al.*, 2003). These data compare to this study where under the Relaxed and Strict ID between 76-100% of phenotypically identified wildcats fell into the wildcat genetic cluster. However, we should also note that different studies may be using different criteria for assessing wildcat phenotype and we cannot be certain that the morphological results from these studies are comparable to this one.

Some specimens classified as domestic or hybrids, based on their phenotypes, were classified as wildcats or admixed based on their genotypes. Most of these individuals that fall into the wildcat genetic cluster were seen to have the classic tabby coat pattern with the exception of two domestic cats which were black and both scored 2 for their tail shape. Several hybrids also fell into the wildcat genetic cluster and further examination showed that all these individuals had the classic tabby pelage, and a high 7PS, but missed out on being classified as a wildcat on one or more of the 8PC characteristics. This supports the value of these further characters in detecting evidence for hybridisation. In addition, using the Relaxed ID, seven individuals considered phenotypical wildcats had admixed genotypes, and were therefore considered hybrids. Three had the domestic cat mtDNA, further supporting the hypothesis that these individuals had ancestors in the domestic cat genetic cluster, although as previously described, this may result from backcrossing of F1 and B1 hybrids into the wildcat population, leading to apparent wildcats with domestic mtDNA and more or less mixed nuclear DNA.

Phenotypical classification, therefore, does largely correspond to genetic clusters. However, this effect may be diluted by high levels of introgression in wild-living cats across Scotland (Beaumont *et al.*, 2001), because other studies on European wildcats have shown that the concordance between genetic and morphological identifications is greater where fewer hybrids are recorded in a wild-living cat population. Currently it is not known how many wildcats actually remain in Scotland and to what extent hybridisation occurs in this wildcat population. A recent study on the European wildcat indicated that hybrids themselves may play a role in hybridisation by behaving as wildcats and by sharing at least a part of their range with both wildcats and domestic cats, increasing the likelihood of production of offspring with admixed genes (Germain

et al., 2008). However, it remains important to test whether some hybrids may provide a source of important wildcat genes for sustaining Scottish wildcat populations or whether their inclusion in the population will result in continued erosion of the genetic integrity of the Scottish wildcat. Therefore, we propose population modeling based on data from an up-to-date wildcat survey would help to determine whether the inclusion of hybrids with >80% wildcat genotype in a wildcat population will continue to erode the genetic integrity of the wildcat or contribute towards its continued survival. In the meantime, the Strict ID is suitable for confidently identifying cats with wildcat pelage that also fall into the wildcat genetic cluster (e.g. most likely to be wildcats), and the Relaxed ID is suitable for use if the inclusion of hybrids with a high percentage of wildcat genes is considered to be an important factor in wildcat conservation.

6.3 Summary

Most of the sample was identified as domestic or hybrid based on morphological and genetic results. In addition, analysis of microsatellite data indicates that two genetic clusters exist, which largely accord with morphological characteristics proposed by Kitchener *et al.* (2005) for distinguishing wildcats from domestic cats and hybrids. Wildcats and domestic cats have been sympatric, and hence potentially hybridising, since before the wildcat was first described scientifically by Schreber in 1777 (Beaumont *et al*., 2001) and therefore it may be, difficult to reliably identify wildcats from their nuclear DNA because of the lack of reference wildcats. However, wildcats in the NMS collection from between 1915 and 1950 are likely to more closely resemble wildcats than the cats from the more recent Daniel & Balharry dataset and these, along with known domestic cats (as previously described) were used to confirm that the two genetic clusters identified represented “wildcat” and “domestic” cats. Our results accord with those from other studies on the Scottish wildcat that suggest that there is strong evidence for a group of individuals that may not necessarily be pure “wildcats”, but which are genetically and morphologically different from domestic cats (e.g. Beaumont *et al*., 2001) and that the Strict ID proposed by Kitchener *et al*. (2005) is a suitable methodology to identify these individuals. However, inherent collecting biases for the 1990s sample may have resulted in much higher proportions of hybrids and domestics being collected than are actually reflected in the wild-living cat population in Scotland today. The results of the current wildcat survey may provide further evidence for the status of the Scottish wildcat.

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Appendix1: Description of the 20 pelage characters assessed

Where 1 = DOMESTIC CAT, 2 = INTERMEDIATE, 3 = WILDCAT (Taken from Kitchener *et al.*, 2005).

	Score		
Character	1	2	3
(1) White on chin	White extensive on muzzle	White on chin	Buff or off-white on chin
(2) Stripes on cheek	No dark stripes	Indistinct stripes	3 clear stripes (2 fused)
(3) Dark spots underside	Absent	Indistinct	Distinct
(4) White on paw	White extensive on paw	White tuft on paw	No white on paw
(5) White on flank	Present	–	Absent
(6) White on back	Present	–	Absent
(7) Extent of dorsal line	Absent/covers entire tail	Continues onto tail	Stops at base of tail
(8) Shape of tail tip	Tapered to a point	Intermediate	Blunt
(9) Colour of tail tip	Neither black nor dark	Dark	Black
(10) Distinctness of tail bands	Absent/joined by dorsal line	Indistinct or fused	Distinct
(11) Alignment of tail bands	Absent/not aligned	Disjointed	Aligned
(12) Stripes on hind leg	<4 or >7 stripes	–	4–7 stripes
(13) Bands encircling foreleg	<2 or >3 bands	–	2 or 3 bands
(14) Tabby coat patterns	Absent/not predominant	–	Predominant pattern
(15) Broken stripes on flanks & hindquarters	>50% broken/no marking	25–50% broken	<25% broken
(16) Stripes on body	<7 or >11 unbroken stripes	–	7–11 unbroken stripes
(17) Spots on flanks & hindquarters	Many/no marking	Some	None
(18) Stripes on nape	Thin/no stripes	Intermediate	4 thick stripes
(19) Stripes on shoulder	Indistinct/no stripes	Intermediate	2 thick stripes
(20) Colour of the back of ear	Same colour as head	Weak ochre/reddish	Ochre/reddish

Appendix 2: Comparison of mean (mm) ± SD (N sampled) of each skull variable for the three groups

AS DEFINED BY STRICT ID USING ANOVA. *P* VALUES IN BOLD ARE SIGNIFICANT AT $P < 0.05$.

No.	Skull Variable Measure	Strict ID				
		Domestic	Hybrid	Wildcat	F	*p*
1	Greatest length of skull	79.74 ± 5.88 (35)	82.33 ± 5.39 (55)	81.58 ± 4.3 (7)	2.37	0.10
2	Condylobasal length	89.15±6.10 (34)	91.91±5.32 (55)	90.82±4.32 (7)	2.58	0.08
3	Facial length	34.56±4.29 (46)	35.04±3.72 (60)	35.36±1.80 (7)	0.26	0.77
4	Lateral length of snout	36.86±2.61 (58)	38.11±2.14 (66)	37.67±1.26 (7)	4.44	**0.01**
5	Length between $Pm^2$and M^1	20.37±1.65 (60)	20.37±1.13 (70)	19.67±0.89 (7)	0.86	0.43
6	Length between $Pm^2$and Pm^4	18.94±1.43 (60)	19.08±1.14 (70)	18.46±1.10 (7)	0.82	0.44
7	Greatest length of Pm^4	10.54±1.37 (61)	11.18±1.36 (71)	11.16±0.51 (7)	3.88	**0.02**
8	Greatest breadth of Pm^4	5.14±0.60 (62)	5.59±0.45 (72)	5.71±0.38 (7)	13.57	**<0.001**
9	Anteroposterior diameter of the auditory bulla	20.75±1.86 (56)	21.51±1.63 (65)	20.88±1.63 (7)	3.00	**0.05**
10	Mastoid breadth	36.69±2.83 (40)	36.93±1.89 (56)	36.72±2.07 (7)	0.14	0.87
11	Greatest breadth of the occipital condyles	23.43±4.03 (50)	23.74±1.26 (62)	24.52±1.98 (7)	0.52	0.60
12	Greatest breadth of the foramen magnum	14.28±0.86 (48)	15.17±1.02 (62)	15.82±1.52 (7)	14.69	**<0.001**
13	Greatest width of the brain case	42.23±5.11 (43)	44.85±2.00 (55)	56.49±1.65 (7)	7.15	**<0.001**
14	Zygomatic breadth	61.98±4.43 (40)	63.64±4.27 (56)	64.35±3.79 (7)	2.09	0.13
15	Frontal breadth	48.42±3.65 (50)	48.63±3.47 (63)	48.34±3.47 (6)	0.06	0.94
16	Least breadth between the orbits	17.99±1.82 (52)	19.06±2.40 (64)	18.36±1.21 (7)	3.65	**0.03**
17	Greatest palatal breadth	37.04±2.48 (48)	38.57±2.06 (61)	38.11±1.54 (7)	6.46	**<0.001**
18	Rostrum breadth: greatest breadth between the canine alveoli	20.50±2.05 (48)	21.71±1.78 (62)	21.01±1.20 (7)	5.65	**<0.001**
19	Least breath of the postorbital constriction	32.21±1.73 (52)	33.51±4.91 (65)	33.97±0.92 (7)	2.01	0.14
20	Breath between the infraorbital foramena	25.65±2.49 (49)	27.32±2.69 (62)	27.66±1.83 (7)	6.39	**<0.001**
21	Minimum length of the nasals	21.81±2.51 (46)	21.23±2.03 (61)	21.96±1.19 (7)	1.05	0.35
22	Maximum length of the nasals	24.78±2.79 (46)	25.04±3.42 (62)	25.30±1.45 (7)	0.14	0.87
23	Width of cranial suture	13.29±4.54 (47)	16.31±4.44 (65)	17.78±3.80 (7)	7.55	**<0.001**
24	Maximum distance between pongonion and coronoid process	62.11±4.63 (55)	62.29±4.38 (66)	61.99±3.89 (7)	0.86	0.42

Appendix 2: (Cont.)

No.	Skull Variable Measure	Strict ID				
		Domestic	Hybrid	Wildcat	F	*p*
25	Maximum distance between pongonion and angular process	60.76±5.24 (55)	63.37±4.87 (70)	62.98±3.87 (7)	4.29	**0.02**
26	Length between mandibular Pm^3 and M^1	19.62±1.26 (66)	20.38±1.10 (73)	20.33±1.37 (7)	7.30	**<0.001**
27	Depth of the mandible behind M^1	10.63±1.22 (67)	11.37±1.07 (74)	11.65±1.15 (7)	8.39	**<0.001**
28	Height of Ramus	26.74±3.12 (66)	28.80±3.43 (74)	29.00±2.67 (7)	7.43	**<0.001**
29	Maximum width of mandibular condoyles (not shown)	13.49±1.58 (63)	13.77±1.41 (71)	14.32±1.39 (7)	1.27	0.28
30	Maximum width of mandibular Pm^4 (not shown)	3.32±0.35 (65)	3.49±0.26 (74)	3.61±0.32 (7)	6.41	**<0.001**

Appendix 3: Anatomical description of digitized landmark points

1. Midline point on the premaxilla at the inferior tip of the bony septum between upper central incisors.
2. Nasal, anterior tip, left side.
3. Nasal, anterior tip, right side.
4. Premaxillary-maxillary suture, anterior, left side.
5. Premaxillary-maxillary suture, anterior, right side.
6. Nasale, nasal, anterior, midline.
7. Premaxillary-maxillary suture, posterior, left side.
8. Premaxillary-maxillary suture, anterior, right side.
9. Frontal-maxillary-nasal suture, left side.
10. Frontal-maxillary-nasal suture, right side.
11. Nasion, nasal-frontal suture, midline.
12. Posterior projection of the maxilla, left side.
13. Posterior projection of the maxilla, right side.
14. Frontal-parietal-sphenoid suture, left side.
15. Bregma, frontal-parietal suture, midline.
16. Frontal-parietal-sphenoid suture, right side.
17. Lambda, parietal-occipital suture, midline.
18. Asterion, posterior at occipital-parietal-temporal suture, left side.
19. Asterion, posterior at occipital-parietal-temporal suture, right side.
20. Opsithion, dorsal lip of foramen magnum, midline.
21. Occipital Condyle – widest point of foramen magnum, left side.
22. Occipital Condyle – widest point of foramen magnum, right side.
23. Basion, ventral lip of foramen magnum, midline.
24. Zygo-maxillare inferior, left side.
25. Squasmosal-jugal suture, anterior projection of zygomatic process of temporal bone, left side.
26. Optic Canal – ventral lip, left side.
27. Squasmosal-jugal suture, posterior projection of jugal, ventral, left side.
28. Auditory Canal, left side
29. Zygo-maxillare inferior, right side.
30. Squasmosal-jugal suture, anterior projection of zygomatic process of temporal bone, right side.
31. Optic Canal – ventral lip, right side.

32. Squasmosal-jugal suture, posterior projection of jugal, ventral, right side.
33. Auditory Canal, right side.
34. Premaxillary-maxillary suture, posterior, left side.
35. Premaxillary-maxillary suture, posterior, right side.
36. Premaxillary-maxillary suture, posterior at midline.
37. Maxillary-palatine suture, anterior at midline.
38. Palatine, posterior at midline.
39. Palatine-presphenoid suture, midline.
40. Palatine-pterygoid suture posterior, left side.
41. Palatine-pterygoid suture posterior, right side.
42. Presphenoid-basisphenoid suture, midline.
43. Tympanooccipital fissure, anterior lip, left side.
44. Tympanooccipital fissure, anterior lip, right side.
45. Incisor 1 – posterior buccal corner, left side.
46. Incisor 2 – posterior buccal corner, left side
47. Incisor 3 – posterior buccal corner, left side
48. Canine 1 – posterior buccal corner, left side
49. Premolar 2 – posterior buccal corner, right side.
50. Premolar 3 – posterior buccal corner, right side.
51. Premolar 4 – posterior buccal corner, left side.
52. Molar 1- posterior buccal corner, left side.
53. Incisor 1 – posterior buccal corner, right side.
54. Incisor 2 – posterior buccal corner, right side
55. Incisor 3 – posterior buccal corner, right side
56. Canine 1 – posterior buccal corner, right side
57. Premolar 2 – posterior buccal corner, right side.
58. Premolar 3 – posterior buccal corner, right side.
59. Premolar 4 – posterior buccal corner, right side.
60. Molar 1 – posterior buccal corner, right side

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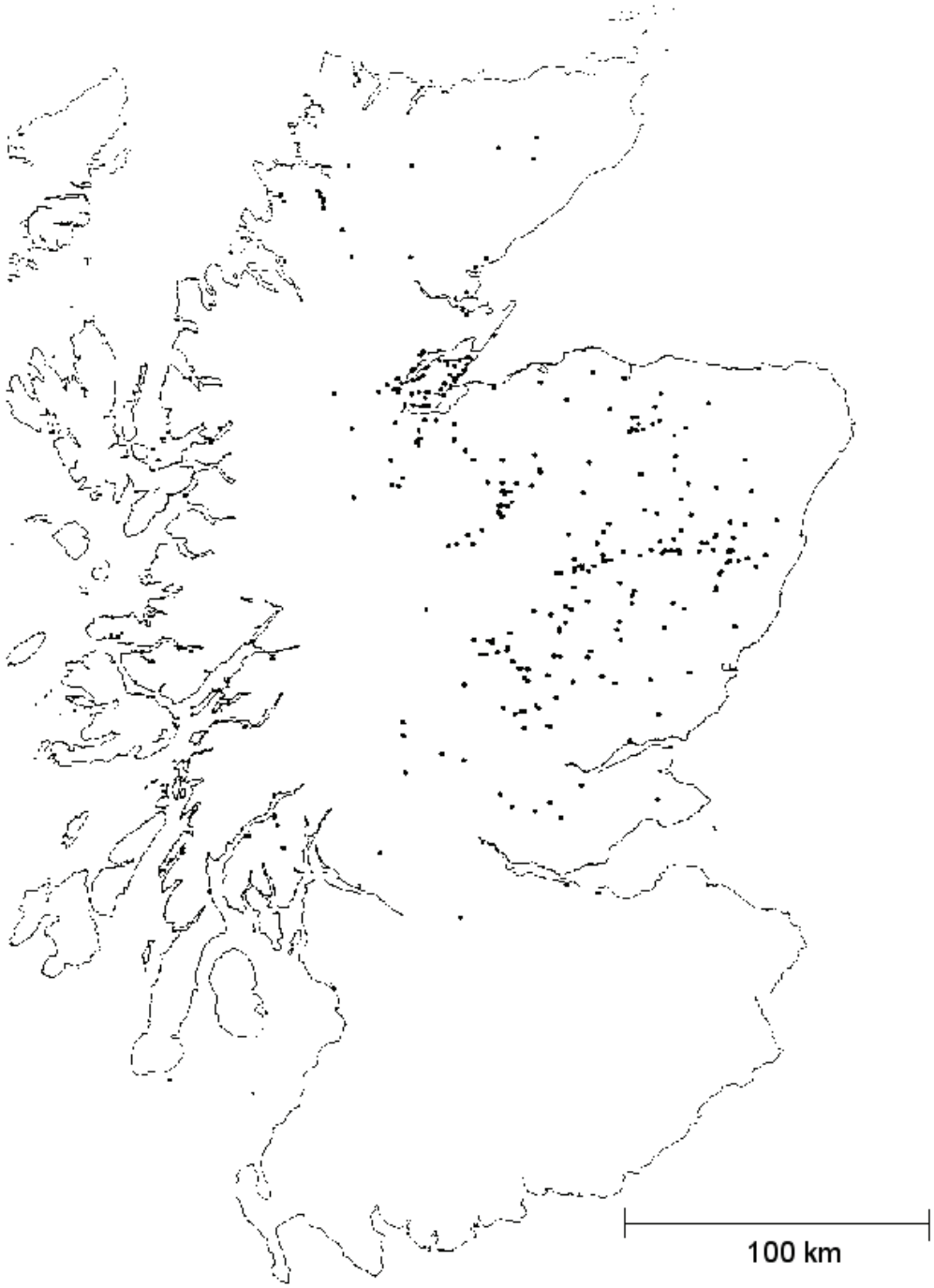
Appendix 4: Distribution of wild-living cats collected by Balharry & Daniels

(from Balharry & Daniels, 1998)



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Statement of principles:

Scottish Natural Heritage – the government body that looks after all of Scotland's nature and landscapes, across all of Scotland, for everyone. Our 5 strategic priorities are:

- Caring for Scotland's nature and landscapes
- Helping to address climate change
- Delivering health and well being
- Supporting the Scottish economy
- Delivering a high quality public service

Find out more at **www.snh.gov.uk**

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