The Scottish wildcat (*Felis silvestris*)

*A review of genetic information and its implications for management*

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*This review forms a case study for the guidance on genetic aspects of managing hybridising species developed by the Conservation Genetic Knowledge Exchange project. For more information visit [www.geneticsinconservation.org](http://www.geneticsinconservation.org)*
Summary

Background

The Scottish wildcat is thought to be under threat from hybridisation with domestic cats. Management of this problem is hampered by the lack of genetic characters, which clearly define the wildcat and distinguish it from domestic cats and their hybrids. In this report we review and summarise the findings of genetic analyses tackling this issue, and discuss these in the context of morphological criteria for defining wildcats. We also discuss how the results of the genetic analyses may be used to inform management decisions in hybridising species. We finish by outlining outstanding knowledge gaps/future research requirements.

Main findings

• Wildcats represent a distinct genetic lineage from domestic cats and can be distinguished (to some extent) using microsatellite and mitochondrial DNA markers.

• Scottish wild-living cats appear to consist of a mix of individuals with varying degrees of hybridity on a continuum between domestic cats and wildcats. A lack of baseline data precludes the determination of whether these wildcats are free of introgression from domestic cats.

• At an individual level, there is imperfect concordance between methods (i.e. morphological and genetic) used to define Scottish wildcats. There is general agreement between methods at the extremes; but there are also individuals which look genetically like wildcats, but with morphological characters consistent with introgression. Likewise, some individuals which appear to be hybrid based on genetic data have the morphological appearance of ‘pure’ wildcats.

• The ‘strict’ pelage classification (Kitchener et al. 2005) of wildcats typically identifies cats that are genetically at the ‘wildcat’ end of the spectrum, but this only represents a subset of the individuals that appear genetically wildcat. This suggests that this criterion may be overly restrictive, but may also be due to low resolution in the available genetic markers.

• A crucial issue for wildcat conservation and the application of genetic data is the clarification of desired outcomes as this will influence management strategies and data requirements. It is important to be clear whether the primary management aim is to maintain formally defined genetic or morphological purity, or to preserve populations of wild-living cats for their cultural and/or ecosystem value (e.g. they generally look or behave like wildcats).

• There are several outstanding areas where further research is required; these include increasing the number of genetic markers available and sampling of wild-living cats particularly in remote areas.
Introduction

The Scottish wildcat, *Felis silvestris*, is Britain’s only surviving native felid. Once widespread across Britain, it is believed to have become restricted (and near to extinction) in the northwest Highlands by the early 20th century. Harris *et al.* (1995) estimated there to be 3500 cats of general wildcat appearance in Scotland (although this undoubtedly included some hybrids). Extrapolating from this figure Yamaguchi *et al.* (2004) suggested there may be as few as 400 wildcats, as only about 12% of wild-living cats collected in Scotland possessed the more formally described wildcat pelage (Daniels *et al.* 1998). A recent questionnaire survey (2006-2008) inferred the majority of populations are in the north and east of Scotland, with some localised populations in the west (Davis and Gray 2010).

Globally, the wildcat is classified as Least Concern according to the International Union for the Conservation of Nature [IUCN] Red List 2011, due to its broad distribution. However, in Scotland the estimate of 400 individuals with classic wildcat pelage has led to suggestions that it be considered Critically Endangered (Kitchener *et al.* 2005; Yamaguchi *et al.* 2004). In the UK, the wildcat was originally protected under Schedules 5 and 6 of the Wildlife and Countryside Act 1981 (as amended in 1988). However, the Conservation (Natural Habitats, &c.) Amendment (Scotland) Regulations 2007 removed the wildcat and other European protected species of animal from Schedule 5 of the Wildlife and Countryside Act, 1981, so that its full legal protection is through the Conservation (Natural Habitats, &c.) Regulations, 1994 (amended in Scotland in 2004, 2007 and 2008), where it is listed on Schedule 2 as a “European protected species of animal” (Kitchener, 2012). It is listed as a UK BAP priority species and is included on the Scottish Biodiversity List (SNH 2007). The wildcat is also listed under Annex IV of the European Directive 92/43/EEC on the conservation of natural habitats of wild fauna and flora, which was transposed into UK law through the 1994 Act. In addition it is protected under Appendix II of the Bern Convention 2002, and Appendix II of the Convention on International Trade in Endangered Species [CITES] 1973.

The primary threats to the Scottish wildcat are habitat loss, persecution and hybridisation and disease transfer from feral domestic cats, *Felis catus*. Despite legislation to protect the wildcat, its conservation and protection have been hampered by considerable debate over what features distinguish wildcats from domestic cats and their hybrids. A lack of clearly defined characteristics creates two problems for conservation: 1. The distribution of wildcats in natural populations and the extent of hybridisation with domestic cats are difficult to assess. 2. Conservation and protection measures are difficult to enforce where differentiation between taxa is uncertain.

Aims:

In this report we review and summarise the genetic information available on wildcats, focussing on Scottish wild-living cats. The aims of this report are to;

1. Assess the composition of Scottish wild-living cats and the extent of hybridisation
2. Compare the results of genetic information with non-genetic methods for defining wildcats and assessing hybridisation
3. Discuss the potential for genetic information to inform management decisions for the Scottish wildcat.
Wildcats

Broader Taxonomic Context

Currently up to five subspecies are recognised on the basis of genetic, geographical and morphological data, the African wildcat (F. s. lybica), the Southern African wildcat (F. s. cafra), the Asian wildcat (F. s. ornata), the Chinese mountain cat (F. s. bieti) and the European wildcat (F. s. silvestris) (Driscoll and Nowell 2010). The Scottish wildcat belongs to the European wildcat subspecies. It should be noted, however, that there is little consensus over the taxonomic relationships of wildcat subspecies (Kitchener and Rees 2009). The domestic cat is typically recognised as F. catus despite being only recently derived from African wildcats (Figure 1; Driscoll et al. 2007; MacDonald et al. 2010). DNA sequence data suggest the European wildcat and African/domestic cat lineages have been separated for over 200,000 years ago (Driscoll et al. 2007).

Figure 1: Relationships between groups of wildcats and domestic cats based on mtDNA sequences (from Driscoll et al. 2007, see Annex B for methods). The size and shape of the shaded area reflects the number and level divergence of variants within the lineage. The genetic lineage containing the domestic cat is shown in blue and the Scottish/European wildcat is in purple. The European wildcat variants are shown in more detail to show the relationship between Scottish (S) and Iberian (Ib) variants.
Scottish wildcats

A large proportion of the research on European wildcats has focused on Scottish populations. In this section we review and summarise the information which has been obtained to describe the composition of Scottish wild-living cats, focusing on genetic data.

Information on Scottish populations of wild-living cats has been obtained principally from samples collected by Balharry and Daniels (1998; Figure 2) and analysed using multiple ecological, morphological and genetic approaches (see Annex A). To maximise comparability among datasets, we have compiled available data into a single matrix, and re-run some of the analyses (see Annex B for details). The genetic data presented here is based on microsatellite data (Beaumont et al. 2001; Kilshaw et al. 2010) and (where available) mitochondrial DNA (mtDNA; Driscoll et al. 2007).

Genetic diversity

Populations of Scottish wild-living cats possess two lineages of mtDNA: (1) variants of the domestic/African cat lineage and (2) a single mtDNA variant belonging to the European wildcat lineage (Figure 1). The single European wildcat mtDNA variant found appears to be restricted to Scotland and reflects the isolation of the British wildcat population from continental Europe by rising sea levels approximately 7000-9000 years ago (Yalden 1999). There is a similar level of divergence between variants across European populations as there is between the Scottish and European wildcat variants (Figure 1 and Annex B).
The low levels of wildcat mtDNA diversity in Scotland suggest wild Scottish cat populations have, or have had, a small effective female population size. This is not surprising given the isolation of the population from other European populations and that the population is believed to have been close to extinction during the early part of last century. Levels of genetic diversity screened by nuclear microsatellites are also lower in Scottish populations of putative wildcats wild-living cats compared with European wildcats (Annex B).

Hybridisation

Domestic cats and wildcats are thought to have co-existed and have been potentially hybridising in Britain for the past 2000-3000 years. At its simplest level the current population of Scottish wild-living cats may contain

1. cats that are entirely domestic in origin and the ancestral wildcat is extinct
2. two distinct groups (namely the ancestral wildcat (or a historically introgressed form of this) and domestic cat) and hybridisation is rare
3. individuals showing mixed ancestry forming a continuum between two groups/extremes (ancestral wildcat and domestic cat)

The presence of mtDNA variants from two lineages (wildcat and domestic/African wildcat) indicates cats of both wildcat and domestic origin exist in Scotland. However, mtDNA only represents a small component of an individual’s genetic make-up, and is insufficient to determine the extent of hybridisation between lineages (i.e. distinguish between options 2 and 3 above).

The most informative genetic data available on the levels of hybridisation in wild-living cats is microsatellite data. This is based on a total of nine different microsatellite markers. These data were analysed (by Beaumont et al. 2001; Kilshaw et al. 2010 and Annex B) to search for groupings, and the most strongly supported ‘split’ in the data is into two genetic groups (Figure 3). However, although two genetic groups can be distinguished, hybridisation is extensive and the groups represent ends of a continuum (Figure 4). One of these microsatellite groups is genetically similar to domestic cats, indicating a feral domestic population of cats (Figure 4). The interpretation of the second group is less certain. This group of cats shows little evidence of recent domestic cat ancestry and hence represents a putative wildcat population. This group also contains individuals with wildcat mtDNA. However, it is not possible to establish the extent to which this group represents the ancestral wildcat with no introgression of domestic genes, due to a lack of baseline data for wildcats. Even amongst individuals with a high assignment to the ‘wildcat’ microsatellite genetic group (i.e. >0.80 on the Y axis in Figure 3), the presence of domestic mtDNA variants indicates a domestic contribution to this group. This suggests introgressive hybridisation is extensive, although it should be noted that even old hybridisation events may leave a long lasting genetic footprint in mtDNA, as it is inherited differently to the rest of the genome (maternally and without recombination). An individual may possess largely wildcat nuclear genes and wildcat morphology but nevertheless, possess the mtDNA of a domestic cat.
Figure 3: Summary plot showing a. individual assignment (Q) to genetic groups based on microsatellite data; b. microsatellite groups based on a threshold value of 0.80 and c. the mtDNA lineage (where available) for sampled Scottish wild living cats. Each individual is represented by a single vertical line (in a. The line is broken into 2 coloured segments proportional to likelihood an individual belongs to a given group), domestic (blue), wildcat/non domestic (purple) or hybrid (light purple).
Figure 4: Plot of principal component analysis of genetic similarity (pairwise allele sharing) of wild-living cats (blue diamonds) and domestic cats sampled from domestic settings (orange triangles). Overlap between known domestic cats and wild-living cats suggests feral domestic cats within the population, but there are also wild-living cats that are clearly genetically divergent from domestic cats.
Comparisons between morphological and genetic data

As outlined in Table 1 and Annex A multiple morphological methods, including skull morphology, bone/intestine (BI) measurements and pelage characteristics, have been used to define wildcats and distinguish them from domestic cats and their hybrids. As with the mtDNA data, not all individuals that have been genotyped for microsatellites have been scored for morphological characters.

Figure 5 illustrates the morphological classifications mapped onto the framework from the microsatellite data. The samples are allocated to ‘domestic’, ‘hybrid’ or ‘wildcat’ microsatellite groups depending on their assignment score (>80% required to be ‘domestic’ or ‘wildcat’, intermediates with lower than 80% assignment to either group are classed as hybrids). Samples have then secondarily been arranged to maximise the grouping of individuals based on morphological methods (see Annex B for comparisons between groups arranged along the genetic continuum from domestic cat to wildcat). This shows that the wildcat microsatellite group (based on a threshold of 0.80) contains, but is not restricted to, individuals possessing features typically associated with wildcats (i.e. wildcat pelage and BI index, wildcat mtDNA). There are two (non-mutually exclusive) explanations for the heterogeneity in the microsatellite-defined wildcat group. Firstly, the morphological features used to describe wildcats may be overly restrictive and not fully represent the natural morphological variation present within wildcats. Secondly, the ‘wildcat’ genetic group includes individuals with some degree of introgression from domestic cats. This latter case may occur, if the microsatellite markers lack adequate discriminatory power and the genetic groupings are erroneous (they contain true wildcats, and some other individuals which are similar at these loci, but not the rest of their genome). It may also occur if they are genetically mainly wildcat, but a low level of introgression has resulted in a few genes being transferred with substantial morphological effects, giving the appearance of domestic cats.

The current data do not allow these scenarios to be easily distinguished.
Figure 5: Summary plot showing classification based on morphological criteria - a. Intestine/bone measurements (where available); b. Relaxed 7PS; c. Strict 7PS; and genetic data – d. microsatellite genetic groups (threshold 0.80); e. mtDNA lineage (where available). Only individuals where genetic and pelage data are shown. Each individual is represented by a single vertical line. Domestic (blue), wildcat/non domestic (purple) or hybrid (light purple)
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Classification</th>
<th>Measurements</th>
<th>Outcome</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelage</td>
<td>Traditional classification</td>
<td>Classification used by the National Museums of Scotland describing wildcat appearance (pelage and shape). Extent to which cats match this classification separation of domestic, hybrid, wildcat (also wild/hybrid; domestic/hybrid)</td>
<td>Balharry and Daniels 1998</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total pelage score (TPS)</td>
<td>20 variables</td>
<td>Variables are scored as domestic, wildcat or intermediate. The total score separates cats into three groups (domestic, hybrid and wildcat) although some overlap is apparent</td>
<td>Kitchener et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Strict 7PS</td>
<td>7 (of the TPS) variables</td>
<td>As TPS – but score based on seven most differentiated characters</td>
<td>Kitchener et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Relaxed 7PS</td>
<td>15 (of the TPS) variables</td>
<td>As 7PS – but score for ‘wildcat’ is reduced provided no domestic traits are apparent at eight additional traits</td>
<td>Kitchener et al. 2005</td>
</tr>
<tr>
<td>Skull</td>
<td>Total skull score</td>
<td>5 characters</td>
<td>Variables are scored as domestic, wildcat or intermediate. The total score separates cats into three groups (domestic, hybrid and wildcat)</td>
<td>Yamaguchi et al. 2004</td>
</tr>
<tr>
<td></td>
<td>Cranial index</td>
<td></td>
<td>Separation of domestic cats from wildcats (and some hybrids)</td>
<td>Schauenberg 1977</td>
</tr>
<tr>
<td>Small intestine/ body size</td>
<td>Intestine/bone size</td>
<td>Standardised intestine and bone measurements</td>
<td>Demonstrated bimodal variation presumably representing wildcat and domestic cats, although these groups represent ends of continuum</td>
<td>Daniels et al. 1998</td>
</tr>
</tbody>
</table>
Geographical and temporal variation in inferred hybridisation

The extent of inferred hybridisation between European wildcats and domestic cats varies throughout their distribution (Table 2). The Scottish sample exhibits a comparatively high level of hybridisation between wildcats and domestic cats, although some variation among estimates is expected due to differences in the number and type of markers investigated, and also different thresholds used in some cases. No populations have been identified where there is no evidence of hybridisation. Within Scotland, Beaumont et al. (2001) found little evidence to suggest a correlation between location and hybridity, although there was weak evidence that cats at higher latitudes were ‘purer’ (more genetically different from domestic cats). There was also tentative evidence suggesting an increase in introgression over time, with cats collected prior to 1970 (n=8) exhibiting higher levels of ‘purity’ compared with cats collected post-1970 (n=20). These comparisons used the traditional (Table 1) classification of wildcats. The small sample sizes mean further work is needed to confirm these observations.

Table 2: Summary of microsatellite analyses showing the extent of genetic differentiation and hybridisation between European wildcats and domestic cats.

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of loci</th>
<th>Genetic differentiation (F_{ST})</th>
<th>Percentage of hybrid individuals*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>13</td>
<td>0.16</td>
<td>26(^\dag)</td>
<td>O'Brien et al. 2009</td>
</tr>
<tr>
<td>Germany</td>
<td>11</td>
<td>0.12</td>
<td>18.4(^1)</td>
<td>Hertwig et al. 2009</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.19</td>
<td>~2(^%)</td>
<td>Eckert et al. 2010(^^)</td>
</tr>
<tr>
<td>Hungary</td>
<td>27</td>
<td>0.04</td>
<td>25-31(^1)</td>
<td>Lecis et al. 2006</td>
</tr>
<tr>
<td>Iberia</td>
<td>12</td>
<td>0.20</td>
<td>6.9(^1)</td>
<td>Oliveira et al. 2008</td>
</tr>
<tr>
<td>Italy</td>
<td>27</td>
<td>0.14</td>
<td>8(^1)</td>
<td>Lecis et al. 2006</td>
</tr>
<tr>
<td>Portugal</td>
<td>12</td>
<td>0.11</td>
<td>~14(^1)</td>
<td>Oliveira et al. 2008</td>
</tr>
<tr>
<td>Scotland</td>
<td>9</td>
<td>0.04</td>
<td>41(^\dag)</td>
<td>Beaumont et al. 2001</td>
</tr>
</tbody>
</table>

*Hybrids were assignment where admixture was between \(^\dag\) 0.2-0.8 or \(^\dag\) 0.1-0.9. \(^^\) mtDNA and allozymes showed additional evidence of introgression.
Limitations of the available information

Genetic data

The existing genetic data can separate two groups of wild-living cats, suggestive of wildcats and domestic cats. However, ambiguities remain between these ends of the continuum. The presence of domestic cat mtDNA in individuals with a high probability of belonging to the ‘wildcat’ microsatellite genetic group indicates that nine loci are insufficient to identify individuals that may have low levels of introgression. This means the current data may underestimate the level of introgression in the population. Examination of a larger number of markers would increase the power of these analyses to distinguish ‘pure’ individuals from those with low levels of introgression.

Type specimen/ baseline data:

There is a lack of unequivocal baseline data known to represent the ancestral wildcat in Scotland, since no specimens predate the introduction of the domestic cat. This means it is very difficult to distinguish naturally occurring variation in wildcat populations from that resulting from hybridisation with domestic cats.

Sampling biases

Biases in the collection of samples may also lead to a bias in the estimation of the extent of hybridisation. The sample used in the genetic analyses contains a high proportion of cats collected from roads (>70%). The potential for this to lead to biases in the estimates of hybridisation is unclear, although fewer live animal captures (41%) classified genetically as ‘wildcat’ compared to carcasses (58%) suggests potential biases are limited. It is possible, however, that the composition of the available samples may create a bias towards domestic and hybrid individuals (and hence overestimates the extent of hybridisation) as suggested by Kilshaw et al. (2010). Certainly, a much higher proportion of museum specimens (skins) were typically considered genetically as wildcat (82%), but the collection data for these specimens also differs (i.e. largely pre-1970 compared with post-1990). More intensive sampling across the distributional range is required to determine the impact of potential biases and the potential for variation in ‘purity’ among Scottish populations.
Summary of research findings

- Wildcats represent a distinct evolutionary lineage from domestic cats.

- Scottish wild-living cats appear to consist of a mix of individuals with varying degrees of hybridity on a continuum between domestic cats and wildcats. A lack of baseline data precludes the determination of whether the wildcat end of spectrum is free of introgression from domestic cats.

- At an individual level, there is imperfect concordance between methods (i.e. morphological and genetic) used to define Scottish wildcats. There is general agreement between methods at the extremes; but there are also individuals that look genetically like wildcats, but with morphological characters consistent with introgression. Likewise, some individuals which appear to be hybrid based on genetic data have the morphological appearance of ‘pure’ wildcats.

- The ‘strict’ pelage classification (Kitchener et al. 2005) of wildcats typically identifies cats that are genetically at the ‘wildcat’ end of the spectrum, but this only represents a subset of the individuals that appear genetically wildcat. This suggests that this criterion may be overly restrictive, but may also be due to low resolution in the available genetic markers.
Management implications

Current data shows genetic differences exist between wildcats and domestic cats in Scotland. However, these data also indicates many domestic and hybrid individuals in the wild, supporting concerns that there is a threat to ‘purer’ wildcats from hybridisation.

A crucial point in establishing management strategies for hybridising populations is to clarify the desired outcomes as this will influence the types of data required to inform management actions and the types of intervention required. Broadly speaking the aim of conservation actions will be to preserve the purity of the population, or maintain the population because it is considered valuable regardless of purity.

Purity: The conservation of pure wildcats (or as pure as possible) will focus management on minimising hybridisation. This may involve extensive sterilisation/culling programmes and hence requires a clear definition of what constitutes a wildcat and an effective method of implementing this in the field. It also requires some direct or indirect contact with each individual.

Managing for genetic purity requires a threshold value for ‘purity’ for a given set of genetic markers. It is important that threshold values are informed by data on the level hybridisation in the population. This is because if the set value is lower than current levels of hybridisation purity will continue to erode, while if it is too high, population persistence may be compromised as only a few individuals will be retained. Certainly if mtDNA was included in the current data this would reject some individuals who appear to be wildcat based on other methods. The nuclear microsatellite data are better suited to this approach but it is currently uncertain whether the genetic groupings based on nine microsatellite loci will be robust to further sampling of the genome. Gathering data from more markers will increase confidence in group limits and individual assignment to these groups. A downside to the genetic approach is that there is currently no easy method for identification in the field and hence implementation requires a morphological definition. Current data suggests limited correlation between morphological and genetic criteria. With the addition of more genetic data, it may be possible to find groupings which are both genetically and morphologically definable.

An alternative option would be to focus on morphological purity (based on the formally described characters that most likely reflect the ancestral wildcat and as much as possible genetic purity). A key consideration will be whether the morphological definition could be too narrow, such that it involves managing a subset of the population and fails to encompass natural variation, particularly given the disconnect between morphological and genetic data. This could be argued be to case for the ‘strict’ pelage classification. However, it is unclear whether the current genetic groups will be robust to higher resolution genetic methods.

Value judgements: It could be argued that purity is a somewhat academic point, particularly given the difficulties associated with effectively defining wildcats and a more operational strategy would be to preserve populations for their cultural and/or ecosystem value (i.e. they generally look and behave like a wildcat and/or they live in the wild). Under this scenario a hybrid population (where there may be little distinction between groups) may retain a conservation value.
This approach typically requires little or no intervention or contact with individuals. However, in practice it may be desirable to stem/reduce the influx of domestic cats into the wild to reduce further loss of genetic integrity and allow established population to become adapted to the environment. If the value of the population is associated with specific morphological or behavioural traits, then more intensive management may be required to maintain those aspects and will be similar to managing for formerly described morphological purity, as described in the section above.

Regardless of whether populations are conserved based on formal definitions of molecular or morphological purity or because they are valued more generally, the reasons for a particularly decision should be clearly stated.

**Future research**

There are several areas where more information/work is needed:

- Generating more genetic data to clarify the robustness of the microsatellite genetic groupings
- Matching the new genetic data with morphological data to establish whether there are sets of samples of wildcats that can be defined using both approaches.
- Further surveys to assess the geographical distribution of hybridisation threat
- Development of rapid genetic assays to speed up surveys of the distribution of hybridisation threat

**Recommended actions to address these knowledge gaps:**

*Additional analysis using existing data/markers:*

Thirty-six microsatellite markers have been developed for wildcats, only nine of which have been examined in Scottish populations. A larger number of markers will improve the detection of hybrids, although more powerful genetic approaches may also be developed (see below). Comparison of data from nuclear (microsatellite) marker and mtDNA (which is maternally inherited) may be used to infer the presence of sex biases in hybridisation. Currently, mtDNA data is only available for a relatively small number of samples (50) and increasing this number is desirable. More detailed investigation of biases would benefit from the development of sex-linked, particularly Y-linked markers (see below).

*Develop additional genetic markers:*

The use of SNP (single nucleotide polymorphism) assays can increase the number of markers analysed from 10s to 100s, substantially increasing the power to detect hybrids and identify introgressed genes. The lack of baseline data may still limit the definition of a wildcat, but additional markers will increase the power to distinguish between the taxa and provide further information on
the dynamics of hybridisation, which may be used to refine management aiming to maintain genetic purity. Recently, diagnostic SNPs have been developed for wildcats (e.g. Nussberger et al., 2013), and a set of 50 diagnostic markers are being developed and assessed for morphological patterns of variation (Andrew Kitchener pers. com.).

**Targeted geographical sampling:**

Further sampling of the wild-living population will provide more information on the extent of hybridisation and the geographical distribution of hybrids, which may be used to further target management. Increasing the number of available samples generally is important, but a focus on poorly sampled areas is critical, as this will provide a greater understanding of the geographical distribution of hybrids and areas which may harbour more genetically pure populations. The use of non-invasive sampling techniques, such as hair or faecal samples, may facilitate sampling in more remote areas and has been widely employed to study elusive species (Waits and Paetkau, 2005). In addition to providing information on hybridisation, non-invasive sampling may be used to monitor populations and provide biological insights into the movements of individuals (e.g. home range), and estimates of local population sizes. There is also the potential to combine remote genetic sampling with camera traps to provide further information on the correlation between appearance and genetic data. Finally, further investigation of museum specimens and archaeological remains will provide information on changes in the frequency of hybridisation over time.

**Techniques to improve field identification:**

Current genetic methods, with the possible exception of a genetic assay to detect domestic ancestry (but not hybridity) via mtDNA (McEwing et al. 2012), cannot be completed within a suitable time frame for integration into sterilisation programmes. If managing for genetic purity is the main goal, then rapid assays which give genetic measures of hybridity may be beneficial. Further evaluation of the key morphological features, particularly pelage characteristics that reflect genetic purity, will also enhance the implementation of programmes aiming to manage genetic purity.

**Models to assess the risk and management options**

With improved understanding of dispersal distances and dynamics, hybridisation frequency, and the distribution of introgressed and pure individuals in the landscape, spatially explicit modelling approaches could be used to identify populations at greatest risk / greatest need of management intervention. In addition, the effectiveness and the impact of various management options/threshold values for purity on the population could also be assessed.
References


## Annex A: Summary of methods using to investigate/define wildcats (and domestic cats and their hybrids)

<table>
<thead>
<tr>
<th>Character</th>
<th>Method</th>
<th>(Number of) variable/markers</th>
<th>Description and outcome</th>
<th>Study/ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td>Albumin immunological distance</td>
<td></td>
<td>Unresolved evolutionary relationships among wildcat subspecies (incl. domestic cat)</td>
<td>Collier &amp; O'Brien 1985</td>
</tr>
<tr>
<td></td>
<td>Allozyme loci</td>
<td>54 loci</td>
<td>Domestic cats appeared most similar to <em>Felis lybica</em> but there was little differentiation between groups; morphological wildcats had less diversity than domestics</td>
<td>Randi &amp; Ragni 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 loci</td>
<td>Frequency differences but no fixed differences at any locus</td>
<td>Hubbard et al. 1992</td>
</tr>
<tr>
<td>Microsatellite</td>
<td>loci</td>
<td>9 loci (single population)</td>
<td>First use of microsatellite markers to distinguish groups of wild-living cats, but showed extensive hybridisation</td>
<td>Beaumont et al. 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36 loci</td>
<td>Identified five distinct genetic groups corresponding to mtDNA lineages (see below) with the species. The European wildcat formed a distinct group, while domestic cats were associated with the African group</td>
<td>Driscoll et al. 2011; Driscoll et al. 2007</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>DNA 12S rRNA</td>
<td></td>
<td>Unresolved evolutionary relationships among wildcat subspecies (incl. domestic cat)</td>
<td>Janczewski et al. 1995</td>
</tr>
<tr>
<td></td>
<td>Cytochrome b</td>
<td></td>
<td>Unresolved evolutionary relationships among wildcat subspecies (incl. domestic cat)</td>
<td>Masuda et al. 1996</td>
</tr>
<tr>
<td></td>
<td>16S RNA &amp; NADH-5</td>
<td></td>
<td>Unresolved evolutionary relationships among wildcat subspecies (incl. domestic cat)</td>
<td>Johnson &amp; O'Brien 1997</td>
</tr>
<tr>
<td></td>
<td>ND5 &amp; ND6</td>
<td></td>
<td>Identified five distinct mtDNA lineages, with the European wildcat forming a distinct group, while domestic cats were derived from the African group</td>
<td>Driscoll et al. 2011; Driscoll et al. 2007</td>
</tr>
<tr>
<td>Single Nucleotide Polymorphism</td>
<td>48 SNPs</td>
<td></td>
<td>Diagnostic set of markers for distinguishing wildcats from domestic cats and hybrids (wildcats defined</td>
<td>Nussberger et al. 2013</td>
</tr>
<tr>
<td>Technique</td>
<td>Description</td>
<td>Source</td>
<td></td>
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<tr>
<td>-----------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NDS/6 SNP</td>
<td>Technique for rapid identification of cats with domestic ancestry (based on Driscoll et al. 2011; Driscoll et al. 2007)</td>
<td>McEwing et al. 2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelage</td>
<td>Classification used by the National Museums of Scotland, describing wildcat appearance (pelage and shape). Extent to which cats match this classification separation of domestic, hybrid, wildcat (also wild/hybrid; domestic/hybrid)</td>
<td>Balharry and Daniels 1998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corbett, 1979</td>
<td>Description of wildcat pelage</td>
<td>Corbett 1979</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total pelage score (TPS)</td>
<td>Variables are scored as domestic, wildcat or intermediate. The total score separates cats into three groups (domestic, hybrid and wildcat) although some overlap is apparent</td>
<td>Kitchener et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strict 7PS</td>
<td>As TPS – but score based on seven most differentiated characters</td>
<td>Kitchener et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relaxed 7PS</td>
<td>As 7PS – but score for ‘wildcat’ is reduced providing no domestic traits are apparent at eight additional traits</td>
<td>Kilshaw et al. 2010; Kitchener et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>French et al. key 1988</td>
<td>Key/score to classify cats based on measurements taken from pre-determined cats (of various years). Concerns have been raised about the accuracy of this approach but see Balharry and Daniels (1998 SNH)</td>
<td>French et al. 1988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>Description</td>
<td>Yamaguchi et al. 2004</td>
<td></td>
<td></td>
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<tr>
<td>---------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Skull score</td>
<td>5 characters (of above 29) Variables (based on the above information) are scored as domestic, wildcat or intermediate. The total score separates cats into three groups (domestic, hybrid and wildcat)</td>
<td>Yamaguchi et al. 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Craniometric index</td>
<td>Variables (based on the above information) are scored as domestic, wildcat or intermediate. The total score separates cats into three groups (domestic, hybrid and wildcat)</td>
<td>Yamaguchi et al. 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine/bone size</td>
<td>Standardised intestine and bone measurements</td>
<td>Kilshaw et al. 2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphometrics and pelage</td>
<td>Suminiski purity score 14 pelage characters (of 27 used in Balharry and Daniels, 1998), 1 body measurement and 1 variable derived from 2 body measurements Characters are scored, with the total providing an estimate (%) of purity</td>
<td>Kilshaw et al. 2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecological</td>
<td>Home range</td>
<td>Daniels et al. 2001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat use and activity patterns</td>
<td>Radio-tracking</td>
<td>Daniels et al. 2001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social organisation</td>
<td>Radio tracking and genetic relatedness</td>
<td>Daniels et al. 2001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Annex B: Supplemental information

The data examined in this review have been collated from multiple sources (see Annex C). To maximise comparability among datasets, we have compiled the available data into a single matrix, and re-run some of the analyses. Descriptions of the available data and additional analyses (and the results) are described here.

Samples and data matrix:

Information on populations of Scottish wild-living cats has largely been obtained from samples collected by Balharry and Daniels (1998). We compiled the available information from samples where genetic data had been obtained into a single matrix (n=232). The matrix contained information on sample collection (e.g. sex, date and location of collection), genetic data (genotypes based on 9 microsatellite markers and mtDNA), pelage characteristics, skull measurements and body measurements. The matrix contained samples collected across Scotland, between 1960 and 1994 (although dates were not available for all specimens). The samples included data from 44 live animals, 17 museum specimens and 168 road traffic accidents (RTAs). (Comparisons between published sample sizes (Balharry and Daniels, 1998) and the available data matrix suggest that the 168 samples labelled as RTAs undoubtedly also contain animals shot by gamekeepers). It should be noted that sample sizes vary between datasets, as information is not available for all individuals. This is because only a subset of individuals have been used in the studies (e.g. mtDNA) or measurements could not been obtained (e.g. skull measurements are not available from live-caught-and-released animals and may also be absent due to broken bones from RTAs). Figure 5 (of the report) provides a summary of the extent of data available for each individual.

Genetic diversity

mtDNA:

Information on mtDNA variants for European and Scottish populations of wild-living cats was obtained from Driscoll et al. (2007). A simplified neighbour-joining tree was generated according to the methods described in Driscoll et al. (2007) using this data (Figure 1 of the report). The mean number of pairwise differences between variants was calculated, both within and between European wildcat and domestic cat lineages. The difference between the Scottish variant and other European variants was also calculated (this was also done for the Iberian variant for comparison).

The single ‘Scottish’ haplotype (Table 1) differed, on average, from continental populations by 6.63±2.0 bp. This value is similar to that found among variants within Europe and smaller than that observed between the mtDNA variant found in Iberia (11.90±2.6 bp). For comparison the difference between domestic and wildcat lineages is approximately 24.92±4.06 bp.
**Table 1**: Pie charts showing the relative number of mtDNA variants found in wildcat and domestic cat lineages in European (including Scotland) and Scottish populations of wild living cats. Sample sizes are shown below.

<table>
<thead>
<tr>
<th></th>
<th><em>Felis silvestris</em> mtDNA variants</th>
<th><em>Felis lybica/catus</em> mtDNA variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td><img src="image1.png" alt="Pie chart" /> n=103</td>
<td><img src="image2.png" alt="Pie chart" /> n=385</td>
</tr>
<tr>
<td>Scotland</td>
<td><img src="image3.png" alt="Pie chart" /> n=25</td>
<td><img src="image4.png" alt="Pie chart" /> n=39</td>
</tr>
</tbody>
</table>

**Microsatellites:**

To assess the level of genetic diversity at microsatellite loci, allelic diversity (A), observed heterozygosity ($H_o$) and expected heterozygosity ($H_e$) were calculated using FSTAT (Goudet 2000).

To compare across groups of wild-living cats, we separated samples into wildcat, hybrid and domestic based on:

1. Strict pelage classification (Kitchener et al. 2005)
2. Relaxed pelage classification (Kitchener et al. 2005)
3. Genetic microsatellite groups based on STRUCTURE analysis (see below). A threshold value of 0.80 was used to assign individuals to a group, with hybrids possessing mid-range values.

Although the sample sizes and levels of diversity vary depending on the criteria used to group samples, there is a general indication that Scottish ‘wildcats’ contain lower levels of genetic diversity compared with domestic cats. These levels of diversity are comparable with those of European populations, although at the lower end of the scale (it should be noted that variation in sample sizes and the number of loci examined in these studies may influence the levels of diversity).
Table 2: Summary of genetic diversity indices (expected \(H_E\) and observed \(H_O\) heterozygosity, number of alleles \(A\) and allelic richness) for wildcat and domestic cats in Scotland (9 loci; see methods), Portugal (12 loci; Oliveira et al. 2008), Germany (7 loci; Eckert et al. 2010) and France (3 loci; Say et al. 2012). Scottish wild living cats are separated according to various criteria, while other European populations are based on a general assessment of appearance. * Sample sizes for allelic richness are 1 for strict pelage, 14 for relaxed pelage and 24 for microsatellite groups.

<table>
<thead>
<tr>
<th>Location</th>
<th>Grouping</th>
<th>Sample size</th>
<th>(H_E) (SD)</th>
<th>(H_O) (SD)</th>
<th>Number of alleles ((A))</th>
<th>Allelic richness*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotland</td>
<td>Kitchener et al. 2005 strict pelage classification</td>
<td>Domestic</td>
<td>91</td>
<td>0.75 (0.11)</td>
<td>0.62 (0.06)</td>
<td>10.44 (2.70)</td>
</tr>
<tr>
<td></td>
<td>Hybrid</td>
<td>93</td>
<td>0.72 (0.11)</td>
<td>0.64 (0.06)</td>
<td>9.22 (3.15)</td>
<td>1.72 (0.11)</td>
</tr>
<tr>
<td></td>
<td>Wildcat</td>
<td>8</td>
<td>0.61 (0.16)</td>
<td>0.52 (0.11)</td>
<td>2.67 (0.87)</td>
<td>1.54 (0.24)</td>
</tr>
<tr>
<td></td>
<td>Kitchener et al. 2005 relaxed pelage classification</td>
<td>Domestic</td>
<td>91</td>
<td>0.75 (0.11)</td>
<td>0.62 (0.06)</td>
<td>10.56 (2.55)</td>
</tr>
<tr>
<td></td>
<td>Hybrid</td>
<td>75</td>
<td>0.72 (0.11)</td>
<td>0.66 (0.06)</td>
<td>9.22 (2.55)</td>
<td>6.23 (2.29)</td>
</tr>
<tr>
<td></td>
<td>Wildcat</td>
<td>26</td>
<td>0.67 (0.29)</td>
<td>0.58 (0.11)</td>
<td>5.33 (2.5)</td>
<td>4.17 (2.19)</td>
</tr>
<tr>
<td></td>
<td>Genetic microsatellite assignment (threshold value 0.80)</td>
<td>Domestic</td>
<td>58</td>
<td>0.75 (0.12)</td>
<td>0.63 (0.08)</td>
<td>10.11 (2.67)</td>
</tr>
<tr>
<td></td>
<td>Hybrid</td>
<td>58</td>
<td>0.73 (0.11)</td>
<td>0.67 (0.08)</td>
<td>7.61 (3.27)</td>
<td>7.38 (3.10)</td>
</tr>
<tr>
<td></td>
<td>Wildcat</td>
<td>76</td>
<td>0.65 (0.12)</td>
<td>0.60 (0.05)</td>
<td>6.56 (2.70)</td>
<td>5.50 (2.30)</td>
</tr>
<tr>
<td>Portugal</td>
<td>Domestic</td>
<td>64</td>
<td>0.75 (0.10)</td>
<td>0.69 (0.13)</td>
<td>10.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wild</td>
<td>28</td>
<td>0.76 (0.09)</td>
<td>0.77 (0.13)</td>
<td>8.41</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>Domestic</td>
<td>148</td>
<td>0.73 (0.08)</td>
<td>0.73 (0.08)</td>
<td>7.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wild</td>
<td>96</td>
<td>0.65 (0.09)</td>
<td>0.56 (0.09)</td>
<td>4.46</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>Wild</td>
<td>131</td>
<td>0.73</td>
<td>0.70</td>
<td>11.78</td>
<td></td>
</tr>
</tbody>
</table>
Hybridisation

Three approaches were used to assess the extent of hybridisation between ‘wildcat’ and domestic cats within the sample based on the microsatellite genotypes. Firstly, principal component analysis (PCA) was used to assess the degree of genetic similarity amongst individuals, based on allele frequencies, using GENALEX 6 (Peakall and Smouse 2006). In addition to the wild-living cat genotypes, we included known domestic cats for comparison (see Beaumont et al. 2001). Secondly, we utilised a Bayesian clustering method in STRUCTURE 2.3.1 (Pritchard et al. 2000). This method estimates the proportion (Q) of an individual’s genotype derived from a defined number of genetic clusters (K). Again, genotypes of known domestic cats were included in the sample for comparison (from Beaumont et al. 2001). The programme was run using the admixture model with K inferred from the data, allele frequencies uncorrelated and lambda set to 1.0. After a burn-in of 100 000, 1 000 000 iterations were performed. We tested the number of genetic groups (K) present using values of K between 1 and 5, with 5 replicates of each. The most strongly supported separation of the data was determined using delta log-likelihood (Evanno et al. 2005).

The results of the hybridisation analyses are discussed in the report.
### Figure 1: Summary plot showing classification based on morphological criteria

- **a. Total skull score**
- **b. Bone intestine ratio**
- **c. Wildcat ID kit**
- **d. Relaxed 7PS pelage score**
- **e. Strict 7PS pelage score**
- **f. mtDNA haplotype**
- **g. Microsatellite group (probability of assignment to domestic cat group)**

Individuals highlighted in red are samples collected prior to 1970. Only individuals where genetic and pelage data were available are shown. Each individual is represented by a single vertical line, with domestic attributes shown in blue, wildcat/non domestic in purple and hybrid attributes are light purple.
Comparisons with morphological data

Morphological data were obtained from Kilshaw et al. (2010) and Balharry and Daniels (1998). This includes all the methods summarised in Table 1 of the report.

Figure 1 illustrates the morphological classifications mapped onto the framework from the microsatellite data, with samples arranged along the genetic continuum from domestic cat to wildcat. This shows that although cats that possess ‘wildcat’ morphological features typically cluster at the wildcat end of the genetic spectrum, there are several morphologically intermediate or domestic cats that are also at the ‘wildcat’ end of the spectrum. At the other end of the spectrum, cats that are genetically domestic also typically appear domestic. The potential reasons for a lack of congruence between genetic and morphological datasets are discussed in the report. Samples collected prior to 1970 are typically at the wildcat end of the genetic and morphological spectrum, suggesting wildcat genetic and morphological purity are being eroded by introgression from domestic cats. It should be noted that individual MD095 appears to be an exception, but this individual’s genetic ID is based on a single locus and hence unreliable.

On a finer scale, the classification of individual morphological traits assessed in the 7PS has been mapped onto the genetic framework in Figure 2. This shows that individuals at the domestic end of the genetic spectrum typically possess multiple traits classified as domestic. In contrast, although cats at the wildcat end of the genetic spectrum possess traits classified as wildcat, they may also possess traits classified as hybrid or even domestic. The erratic distribution of individual characters with respect to each other illustrates the need for scoring multiple morphological characters and that there is no single character capable of defining a wildcat.

From a management perspective, the application of genetic techniques in the field is still limited and hence a visual method for wildcat identification is required. The relaxed 7PS has been suggested as a potential method of field identification. The genetic data suggest that, although imperfect, this method typically identifies cats at the wildcat end of the genetic spectrum. More recently, a more simplified pelage classification was developed and implemented as part of the Cairngorms wildcat field identification kit (Hetherington and Campbell, 2012). Under this classification a cat is assumed to be wildcat if it possesses tabby markings, a thick, ringed, blunt tail and no white paws or stripe down the tail. Using previously recorded information from Kilshaw et al. (2010) on coat colour, and pelage characteristics 4 (white on paw), 7 (extent of dorsal line), 8 (shape of tail tip), 10 (distinctiveness of tail bands) and 11 (alignment of tail bands), we classified cats as wildcat or not. This method identified a similar set of individuals to other methods based on pelage characters, with morphological wildcats typically at the wildcat end of the genetic spectrum (see Figure 1).

Additional genetic data and analyses of the correlation between the assignment to the wildcat genetic group and individual traits may allow the identification of a set of traits capable of defining the wildcat genetic group.
Figure 2: Summary plot showing individual assignment (%) to wildcat genetic group based on microsatellite data (horizontal black curve) overlayed on the 7 point pelage score relaxed classification (g.) separated into a. Stripes on shoulder; b. Stripes on nape; c. Spots on flanks and hindquarters; d. Broken stripes on flanks and hindquarters; e. Distinctiveness of tail bands; f. Shape of tail; g. Extent of dorsal stripe. Each individual is represented as a vertical line, with domestic attributes shown in blue, wildcat/non domestic in purple and hybrid attributes are light purple. Only individuals with a 7pt Pelage Score are shown.
References


