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Hybridisation in British Mammals

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Summary

1. The introduction presents hybridisation as a natural part of evolutionary processes, but describes circumstances where it becomes a conservation issue.

2. Species definitions are discussed, with particular reference to biological species concepts and the conservation viewpoint.

3. Techniques available for examining differences between populations and species of animals are reviewed. The most recent techniques using nuclear or mitochondrial DNA have been effectively used in hybridisation studies of mammal species.

4. The occurrence of hybridisation between four pairs of British mammals are examined in detail.

4.1 Hybridisation between mountain hare and brown hare is very rare under natural conditions and hybrids in Britain would appear to represent only novelty value.

4.2 The genetic mix of red deer and sika deer in Britain is complex. Morphometric studies have suggested that hybridisation between these two species is widespread in some areas. Current investigations using molecular and genetic techniques hope to further evaluate the real extent of hybridisation and introgression. Current evidence suggests that introgression of sika into the red deer populations will increase rather than decrease.

4.3 Whether wildcats and domestic cats can be considered to be subspecies or separate species is unresolved. Past European studies, mainly skull morphometrics, suggested that hybridisation between the two types was widespread. The proportion of hybrids within a population has yet to be objectively measured. Current research in Scotland is using DNA techniques, sampling living and historic cats across Scotland.

4.4 British ferrets are probably domesticated directly from European polecats. The recent spread of polecats from Wales into the English Midlands may lead to the introgression of domestic genes into wild polecats. However, it is not known to what extent feral ferrets survive in mainland Britain, and as yet hybridisation is not perceived as a substantial threat to the species.

5. Examples of hybridisation in British birds and fish are summarised. Many bird species hybridise and the reasons for this are discussed. Conservation issues concerning ruddy ducks, crossbills and goshawks are briefly outlined.

6. In conclusion, the two mammals at greatest risk from hybridisation and subsequent loss of native type, are the wildcat and red deer. The importance of maintaining native habitat for native species is also stressed. It is recommended that studies initiated using modern molecular techniques be followed through, and that hypotheses based on these findings, about the ecological/behavioural reasons for hybridisation be investigated. Even though the best methods available are used, it should be recognised that descriptions of a species for legal purposes will contain a degree of subjectivity.
1. Introduction

A hybrid is defined as an offspring of two plants or animals of different species or varieties. A hybrid zone is a region in which genetically 'distinct' populations meet, mate and produce hybrids (Barton & Hewitt 1989). Naturally occurring hybrid zones have been found in all major taxa of higher organisms, and are attracting much research on the nature and effects of differences among incipient species (Barton & Hewitt, 1989).

Reticulate evolution (natural hybridisation between different evolutionary lineages) is suggested as playing a significant role in evolution (Heiser 1973). A review of recent studies, incorporating molecular and ecological analyses, goes further by suggesting a prominent evolutionary role for hybridisation in numerous species complexes (Arnold 1992).

For example, introgressed forms can expand into a novel habitat (Lewontin & Birch 1966) and parasites may be preferentially attracted to hybrids, thus limiting the ability to adapt to parent individuals (Whitham 1989). Hybridisation has also been shown to have led to the formation of a new species of cyprinid (Del Marais et al. 1992), and hybridisation, therefore plays a role as a natural process.

Hybridisation becomes a conservation issue when one species 'threatens' to overwhelm or fully introgress into another (e.g. Greig 1980; Gillespie 1985; Stromberg & Boyce 1986; Herrero, Schroeder & Scott-Brown 1986; Ankney, Dennis & Bailey 1987; Lehman et al. 1991), or when a domesticated or farmed form of the species 'threatens' the genetic integrity of a wild population (e.g. Blanco, Reig & de la Cuesta 1992; Boitani 1992; Tulgat & Schaller 1992). Appreciation of the 'threat' relies on the assumption that there is genetic difference inherent in the two hybridising populations which is worthy of conservation, and which will be lost if introgression continues. Maintenance of biodiversity is a concept which has gained universal acceptance, but there are as yet no satisfactory criteria for the units of measurement of such diversity. Likely candidates such as species, sub-species or race are unsatisfactory because conventional taxonomy has largely been based on comparative morphology, rather than phylogeny (Thorpe, 1987; O'Brien & Mayr 1991; Geist 1992). The relative genetic distances among and between such taxa are thus largely unquantified.

Nevertheless existing taxa become included in conservation laws in order to protect sub-species or species perceived to be threatened and hybridisation then becomes a legal issue (O'Brien & Mayr 1991; Geist 1992). These issues become centred around the definition of sub-species and species, and it has become apparent that existing taxonomy is inadequate for this purpose. Traditional taxonomic designations are currently the basis of management and eligibility for protection, but when these designations are questioned in a court of law, or the endangered listing encourages an investigation into molecular genetics and field ecology, they can be found wanting. For example, the inability to distinguish a protected sub-species, the Tibetan argali, from a conspecific form which is not protected under the US Endangered Species Act, led to an international incident, a prolonged legal hearing and blackmail of scientists to change their testimony (Geist 1992). Without rigorous taxonomy for endangered species, the Convention on International Trade in Endangered Species (CITES) is unworkable. In Britain the only test case of the protection of the wildcat (Felis silvestris) (Stonehaven Sheriff Court 1988), founded when the prosecution was unable to prove beyond any reasonable doubt that the cat shot was a true wildcat and not a hybrid with domestic cat. Interpretation of the hybrid policy under the US Endangered Species Act has led to arguments over the genetic integrity and, therefore, the protection afforded to red wolves (Canis rufus), plains bison (Bison bison) and the Florida panther (Felis concolor corgi), amongst others (O'Brien & Mayr 1991; Gittleman & Pimm 1991; Geist 1992).

This review has 4 objectives.

1. To examine current species concepts with the aim of identifying the most suitable definition for conservation purposes, and particularly how these concepts relate to hybridisation.
2. To discuss the perceived 'threat', in terms of introductions of exotic sub-species and in terms of introgression between domesticated/feral forms of the native species.
3. To review the techniques that have been used to evaluate hybridisation.
4. To review hybridisation in four important 'pairs' of British mammals and summarise the occurrence of hybridisation in other British vertebrates.
2. Species Concepts and Definitions

The Biological Species Concept (BSC) (Mayr 1942, 1969) is generally supported by zoologists concerned with processes operating at the microgeographic level of demes and populations (e.g. Dobzhansky 1970; Lewontin 1974; Endler 1977; Futuyma 1986; Nei 1987) and criticised by other biologists, generally those working with higher taxonomic differences or with plants (e.g. Ehrlich 1961; Sokal & Crovello 1970; Donoghue 1985; Cracraft in press; McKitrick & Zink 1988). Some authors not only criticise Mayr's definition, but also the relevance of a species concept to evolutionary biology, and consider species to be simply arbitrary groups, without biological significance (Sokal & Crovello 1970; Mishler & Donoghue 1982). Following Mayr's definition, other definitions arose, which can be broadly classed under two headings: biological and phylogenetic (Cracraft 1989). Cracraft (1989) gives four examples from mammal and bird taxa where debated species/sub-species groups would be classed differently under the two (biological or phylogenetic) concepts. In these examples, the species/sub-species which were found to hybridise in a contact zone were thought to be less closely related to each other by descent than they were to other populations with which they did not interbreed.

A species concept can only be evaluated in terms of a particular goal or purpose (Templeton 1989). Cracraft (1989) points out that biologists have used the word 'species' for two distinct purposes: first to describe and catalogue biotic diversity and to represent the historical relationships of that diversity in a hierarchical manner, and second, to represent the basic entities of evolutionary theory, species are the entity that speciates. Some recently suggested species concepts are given in more detail below and then discussed in relation to conservation problems.

2.1 Biological Species Concepts

Three biological species concepts have been defined in the recent literature: 'recognition' (Paterson 1985), 'cohesion' (Templeton 1989), and the original BSC renamed to the 'isolate species concept' (ISC) (Paterson 1985; Templeton 1989).

2.1.1 Isolation

The BSC or ISC defines a species as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups" (Mayr 1963). Isolating mechanisms are given the prominent role for speciation events to take place. Recently, following debates on hybridisation, O'Brien & Mayr (1991) added, "Hybrids or hybrid zones do not disintegrate the genetic integrity of the species, while hybridisation between sub-species produces gene flow and genetic mixing". To classify sub-species they offer "Members of a sub-species share a unique geographic range or habitat, a group of phylogenetically concordant phenotypic characters, and a unique natural history relative to other subdivisions of the species". They also suggest that sub-species are "normally allopathic and show time-dependent accumulation of genetic difference in the absence of gene flow". Paterson (1985) criticises the emphasis on the maintenance of the isolating mechanisms, rather than the processes of speciation, and proposed the 'recognition species concept' (see below). Barton & Hewitt (1989) point to the fact that taxa that remain distinct either side of a naturally occurring hybrid zone (of which there are now many examples) have been classified, even by supporters of the BSC, as separate species. With the BSC, the emphasis is on reproductive incompatibility, either through pre-mating mechanisms or fertility. Although Mayr (1970) argues that most species have distinct ecological niches, and that this ecological distinctiveness is the "keystone of evolution", he argues that adaptive transitions and natural selection generally play no direct role in speciation and contribute to defining a species only as an "incidental by-product of isolating mechanisms" (Templeton 1989). Thus not only do isolation species concepts ignore asexual taxa, but they also ignore factors other than gene flow, by which hybrid zones occur, and by which speciation occurs.

2.1.2 Recognition
The recognition species concept emphasises the importance of mechanisms which assist reproduction. Rather than thinking of courtship rituals as isolating mechanisms which prevent interspecies matings, these and the other functions of premating behaviour (such as suppression of escape of courted animal, synchronisation of mating activities, and the co-ordination in time and space of the whole mating procedure (Tinbergen 1953) are viewed as having the active role in the speciation process. Paterson (1985) defines a species as the "most inclusive population of individual biparental organisms which share a common fertilisation system". Although this approach emphasises the positive side of reproductive mechanisms in speciation, it is subject to the same criticisms as the isolating concept.

2.1.3 Cohesion

The cohesion species concept was proposed by Templeton (1989) to incorporate the key parts of previous biological and evolutionary species definitions, without their restrictions. The mechanisms of cohesion that maintain a group as an evolutionary lineage are used to define the species, recognising the cohesive forces of 'genetic exchangeability' (biological concepts) and 'demographic exchangeability' (phylogenetic concepts). Genetic exchangeability is the ability of individuals to exchange genes during sexual reproduction. Demographic exchangeability is where the individuals share the same fundamental niche, defined by the genetic tolerances of the individuals to various environments, and where, by the forces of genetic drift or natural selection, every individuals is potentially a common ancestor to the entire population at some point in the future. Templeton (1989) points out that the isolation and recognition species concepts not only ignore non-sexual taxa but also cannot explain the cohesion of a species which is self-mating, or has a closed mating systems, or where outbreeding is at a very low level. Genetic drift and natural selection are microevolutionary forces applicable to all organisms, not just outcrossing sexual species. The phylogenetic species concept on the other hand (see 2.2) is not a mechanistic definition and so provides little guidance on which characters are important in defining the species. Individuals can be dissimilar in certain aspects and still be part of a demographically exchangeable population.

A species may be defined as "the most inclusive group of organisms having the potential for genetic and/or demographic exchangeability" (Templeton 1989). A list of cohesion mechanisms is described in Templeton (1989). Demographic exchangeability is weakened as individuals vary in their abilities or their tolerances to the local environment. Balancing the tendency for microevolution is the cohesion caused by gene flow. Templeton's species definition allows for the fact that both gene flow and demographic exchangeability are operating on most taxa, with asexual taxa at one extreme where only demographic exchangeability occurs, and syngameons at the other extreme.

2.2 Phylogenetic Species Concept

Two recent descriptions of a phylogenetic species are: "an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent" (Cracraft 1987); or "the smallest aggregation of populations or lineages diagnosable by a unique combination of character states in comparable individuals" (Nixon & Wheeler 1990). Supporters for this species concept tend to be evolutionary biologists who view species as the basic evolutionary units. They dislike the biological species concept with its emphasis on reproduction, when not all newly evolved diagnostic characters will necessarily affect reproductive isolation, when sub-species and polytypic species have to be called in to explain various evolutionary stages, and the fact that biological species definitions do not constrain a species to be strictly monophyletic. Proponents of the phylogenetic approach can use cladistic analysis to generate species groups based on the best estimate of the historical speciation events (see examples in Cracraft 1989). This approach makes the assumption that once speciated complete introgression between two species does not occur. Cracraft (1989 page 41) assumes
that two species of quail thrush which hybridize sporadically in a zone of overlap and are separated by habitat would not in fact introgress if climatic change altered the habitats. He assumes this because cladistic analysis suggests that the two species are not sister species but are separated by several specification events. Ironically, as reproductive isolation is not longer a requirement of a species under this definition, then, in theory at least, is it not plausible that complete introgression occurs and populations may be paraphyletic? The advantage of the phylogenetic species concept is that it attempts to separate out the most newly differentiated populations (sister species) from those populations which are separated by many speciation events. However, if species can be separated by phylogeny and the evolution of diagnostic differences in for example vocalisations and external morphology alone, then what about human beings?

2.3 Conservation, Species and Subspecies

At one end of the spectrum is a possible plethora of species under the phylogenetic concept. Isolation or recognition concepts ignore the process of hybridisation, but the cohesion concept is perhaps too broad, accepting all groups potentially linked by hybrid forms (such as sika (*Cervus nippon*) and red deer (*Cervus elaphus*) coyotes and wolves etc). In practice, most conservationists accept the essence of the original biological species concept, and are thus faced with a dilemma over hybridisation and hybrid zones or clines in geographic variation and the need to see them results in argument over the use of terms such as 'sub-species' 'semi-species' polytypic species' etc. The over-emphasis on isolating mechanisms, rather than demographic exchangeability is unfortunate because the former rules out speciation or subspeciation between groups which are apparently capable of interbreeding, but which either do not (perhaps because of social organisation) or do, but the resulting progeny, hybrids between groups, are always disadvantaged because of the widely different selection pressures acting on each group. Isolation concepts also imply that the longer a species or subspecies has been separate for, the more different it will become.

However, as isolation alone may not have caused major differences to arise between populations, the length of time over-which populations are physically separated may be irrelevant. Similarity and stability of the environment may be much more important factors. This is particularly relevant to concerns of interbreeding between domestic/farmed strains and wild ones. The time scale of separation is possibly not so important as the type of selection pressures during domestication.

From their study of hybrid zones, Barton & Hewitt (1989) suggest that 'there is a clash between two views of a species; one based on the pattern of gene flow, and the other on the maintenance of a cluster of phenotypes that is stable to invasion by foreign genes'. The definition of a species as 'a cluster of phenotypes that is stable to invasion by foreign genes' (given an adequate population size and within the organisms natural environment) is possibly a very useful one. It asks the question, why is the population stable, i.e. what mechanisms, ecological, behavioural, morphological, biochemical etc. are important in differentiating two populations? Where hybridisation and introgression is occurring over a significant part of the species range, the question then becomes, why have the phenotypes become unstable?

There are very few definitions for subspecies, other than that of O'Brien & Mayr (1991) (see 2.1.1). However, the presence of transition zones between groups of characters (characters can be colour patterns, scale counts, body measurements, biochemical alleles etc) within species (Thorpe 1985; Barton & Hewitt 1989; Thorpe et al. 1991), reflecting past isolation (such as isolation in pleistocene refugia from which the populations have subsequently expanded and met) or due to current ecology (such as an altitude which may favour particular lineages with particular genes) suggests that patterns of geographic variation amongst certain characters should be similar in subspecies. This suggests a definition for subspecies as 'groups of phenotypes separated from one another by variation in certain characters, the groups remaining stable alongside the inclusion of more character variables'.
3. Techniques for investigating differences in species and subspecies and therefore in investigating hybridisation

3.1 Morphological Characteristics

Taxonomy was, and sometimes still is, based on a degree of measurable difference in only colour and external morphology, with little regard to the variation inherent within most natural populations. For example twenty three endemic British subspecies of mammals have been described, but most were subsequently revoked (Dadd 1970). The description of an endemic Scottish polecat was based on four specimens, (Tetley 1939), and these were probably ferrets or hybrids (Dadd 1970). Corbett (1979) suggested that given the variation in Felis silvestris throughout Europe, subspecific status for Scottish cats based on size and colour (Felis silvestris grampia Miller 1907) was not justified.

Geist (1992) suggests that "comparative morphometrics of crania or skeletons of free-living populations can no more be used to measure taxonomic (genetic) differences than a rubber band can be used to measure distance". However, this ignores the powerful possibilities of multivariate analysis.

Geographical variation in morphology is almost universal and often complex. Conventional approaches to naming subspecies do not usually consider variation in several characters simultaneously and therefore ignore the fact that different characters may have different patterns of geographic variation (Thorpe 1981). Using multivariate techniques, the variation in a wide range of morphological variables can be simultaneously analysed to reveal patterns of geographic variation (Thorpe 1987; Thorpe et al. 1991). The eleven subspecies of European grass snake (Natrix natrix) were revised by using many character variables, such as scale counts, colour patterns, internal and external body measurements etc (Thorpe 1979).

Many studies of mammals have used cranium morphology to distinguish between species, races and domestic compared to wild animals (e.g. Scheuenbert 1969; Lowe & Gardiner 1974; Newsome, Corbett & Carpenter 1980; Youngman 1982) and therefore studies of hybridisation have concentrated on finding characters or combinations of measurements which can distinguish an intermediate, or hybrid, skull. Although hybridisation can undoubtedly be confirmed by the occurrence of intermediate-type skulls, associated with other intermediate types of characters, most studies find considerable overlap between putative hybrids and the wild types. On an individual level cranium morphometrics were found to be unsatisfactory in distinguishing between red deer and hybrids with sika. Although sika and red deer have clearly separable skulls; only the F1 generation could to any extent be determined (Bartos 1991).

However, morphometrics can suggest that substantial hybridisation and subsequent introgression has taken place within deer populations (Lowe & Gardiner 1975 Zima, Kozena & Mubalek, 1990). Domestic animals often show similar tendencies in the morphological change in skulls, such as a smaller size of auditory bullae (Heptner & Mayushkin 1972; Newsome & Corbett 1982). However, some variables change as a function of captive breeding rather than genotype (Newsome & Corbett 1982) and the degree of reversion to 'wild type' among feral animals is not known. Studies are rarely supported by known hybrid material, other than for the F1 generation. Cranial measurements alone may limit the scope of morphological investigations. Multivariate character techniques (e.g. Fleischer & Johnston 1982; Thorpe 1987) using a few selected, non-linked, skull characters may be profitable lines of investigation.

Multivariate character techniques can include all characters such as ones outlined in the techniques below, as well as morphological characters.

3.2 Karyotype

Species often differ in diploid number and chromosome morphology, with some lines of evolution having a very stable karyotype and others a more variable one. For instance, in the Cervidae, Cervus elaphus subspecies have an almost stable karyotype, whereas it varies for Cervus nippon subspecies (Bartos 1991). For some closely related species such as trout and salmon
there is a large difference in chromosome number, although hybrids may be fertile. Thus karyotype is not a good indicator of phylogeny although it can be used to support other data. However, from studies of sika deer, red deer and sika/red hybrids (Harrington & Power 1985; Herzog 1987; Herzog & Harrington 1991; Bartos 1991) hybridisation was shown to be a major factor in karyotype polymorphism, which, whilst not suitable for determining individuals as hybrids, could be used for labelling the population as a whole (Bartos 1991). A hybrid between a steppe polecat and domestic ferret was also found to have an intermediate karyotype (Blandford 1987).

3.3 Sperm structure

Frient (1936) found it was possible to recognise any species of British murid rodent on sperm alone. Van der Horst et al (1991) suggested that, although there is virtually no information on the structural differences between sperm of closely related mammals, from their studies on polecats, sperm might be a fairly sensitive taxonomic indicator and useful in hybrid studies.

3.4 Protein/enzyme techniques

Protein or enzyme techniques are based on the fact that small differences between molecules of the same protein are abundant and that these differences are heritable, reflecting minor changes in the nuclear DNA (Hoelzel & Dover 1991). These variations of the same protein are called alleles and can be separated and 'visualised' using gel electrophoresis.

Variation in alleles and allele frequency was originally understood to be selectively neutral (Nei 1972), giving rise to the theories of a molecular clock and calculations of genetic distance, by which a measure of divergence between populations could be roughly estimated (e.g. 5% variation for a subspecies). However, these assumptions only ever gave a rough guide and were not a basis for taxonomy, as considerable variation between and amongst taxa is apparent. It was widely accepted that polymorphisms were selectively neutral, and despite electrophoretic studies of 1,111 (by 1988) species, very few studies had addressed this particular question (Pemberton et al. 1988). Several studies have shown that these mutations are not selectively neutral, for example, juvenile survival in red deer has been linked to the presence of certain alleles, and that females heterozygous for some alleles also survived better than the homozygotes (Pemberton et al. 1988). Isozyme variation in Colias butterflies has been shown to be related to the ability to sustain flight at sub-optimal temperature (Watt, Cassin & Swann 1983). Protein electrophoresis may, in fact, be a useful technique for studying natural selection (Amos & Hoelzel 1992).

The use of protein electrophoresis in hybrid studies has been limited because closely related populations or species tend to differ in allele frequency rather than presence or absence of 'marker' alleles (Buth 1984, Swofford & Berlocher 1987). Also of the 20 to 30 proteins commonly surveyed, only a few are polymorphic (Wayne, Lehman & Jenks 1992). However, allozyme markers have been developed for some taxa which are present in one or another subspecies/species but which only occur together in a hybrid (Dratch 1986; O'Brien et al. 1987).

3.5 DNA analysis

DNA can be cut up in a predictable way using restriction endonucleases. The enzymes recognise very precise arrangements of nucleotides (generally four to six). Thus mutations in this DNA cause changes in the lengths of the fragments. This general methodology is called restriction fragment length polymorphism (RFLP). Probes are then found which target or hybridise to particular sequences or repetitive units and the targeted fragments are separated on a gel. At present, there are well over 100 known restriction enzymes (Amos & Hoelzel 1992). Only about 5% of the genome is represented by regions which encode for proteins, the rest appearing to be non-coding or repeats of the genes, and mutations accumulate at various rates depending on a particular loci in the genome (Amos & Hoelzel 1992).

3.5.1 Nuclear DNA
The most commonly investigated parts of nuclear DNA are the non-coding hypervariable regions found throughout the genome. These regions are called mini or micro-satellite sequences and are arrays of a repeated core sequence. The length variation of these repeats is assayed using restriction enzymes with recognition sites outside the arrays, which therefore cut the DNA into fragments according to the length of the repeats (Wayne, Lehman & Jenks 1992). The name mini or micro depends on the number of base pairs in the core sequence. The mini satellite sequences first described by Jeffreys, Wilson & Thein (1985), are unique to an individual and have been used for DNA 'fingerprinting', and for testing parentage. Satellite sequences can also be used at the population level. Low variation between individuals of isolated island species of the Californian Channel Island fox (Urocyon littoralis) has been used to demonstrate the effect of genetic bottlenecks and island size (Gilbert et al 1990). The degree of dissimilarity in sequences between populations reflects their degree of isolation from one another. For example, populations of killer whales were surveyed for their degree of mini satellite band sharing (Hoelzel & Dover 1991). The results which supported both mtDNA analysis (see 3.5.2), and the observed morphological dissimilarities, suggested that there were five distinct populations from which the rate of dispersal from local communities was very low, and largely independent of whether the populations compared were sympatric in the Eastern North Pacific or allopatric, from Iceland or Argentina. Other regions in nuclear DNA tend to be conserved within reproductive populations e.g. the rDNA group (the gene family coding for ribosomal RNA). These regions are a potentially rich source of information on the degree of reproductive isolation shown by two populations (Amos & Hoelzel 1992).

3.5.2 Mitochondrial DNA (mtDNA)

Mitochondria are inherited from the maternal side (for discussion see Hoelzel & Dover 1991), and they evolve independently of nuclear DNA. When combined with other analysis they can be used to investigate sex-biased dispersal. The mitochondrial genome mutates considerably faster than its nuclear counterpart, different parts change at different rates, and rates are not necessarily the same between taxa (Hoelzel & Dover 1991).

However, the mean rate of divergence over a wide range of taxa was found to be 2% per million years (Wilson et al. 1985). Calculated times since reproductive isolation between species groups of cichlid fishes in the East African lakes, Victoria, Malawi and Tanganyika using mtDNA, were consistent with the estimated age of lakes and major lake level fluctuations (Strumbauer & Meyer 1992). However, levels of genetic diversity were unrelated to the wide range of morphological diversity between species groups.

Although mtDNA is assumed to be selectively neutral, especially in the non transcribed D-loop region, other mtDNA contains genes whose products are crucial in energy production, and so mutations here must be highly visible to selection (Avise et al. 1987). Molecular clock hypotheses, and measures of genetic 'distance' between populations have always to be treated with caution. Even in non-coding regions, the assumption that the dominant force generating sequence change is the gradual and constant accumulation of neutral mutations can be violated (Dover 1982).

Because mtDNA is maternal and non-recombining (an individual inherits all the mtDNA from one great great grandparent but only 1/16 of nuclear DNA from that ancestor) studies in mitochondrial DNA are a bridge between systematics and population dynamics (Avise et al. 1987). They suggest that many species show a deep and geographically structured mtDNA phylogenetic history, which they called intraspecific phylogeography. Mitochondrial DNA analysis is therefore an ideal technique for investigating subspecies to species differences.

3.5.3 PCR (Polymerase chain reaction)

This is a means by which minute quantities of a specific DNA sequence can be enzymatically amplified (Amos & Hoelzel 1992). PCR-based techniques can be used on extremely degraded remains (Wayne et
al. 1992), or museum or archaeological samples of skin & bone (Ellegren 1991; Wayne & Jenks 1991).

Shed skin and sloughed cetacean skin have been used as a non-invasive source of DNA for population studies (Amos et al. 1992)

3.5.4 The application of DNA analysis techniques to studies of hybridisation

Mitochondrial DNA techniques have been successfully applied to hybridisation studies, and can be particularly powerful when used in conjunction with other techniques. Native (unhybridised) populations should contain unique mtDNA genotypes and unique microsatellite alleles; these unique microsatellites should be most common in populations with unique mtDNA genotypes. By using PCR, historic samples can be compared to modern ones. A higher proportion of unique mtDNA and satellites in the historic or archaeological sample provides evidence of recent hybridisation. Patterns of variation in hybridisation occurrences can be used to test hypotheses, for example that hybridisation occurs predominately in disturbed areas or recently colonised areas. Loss of genetic variability can also be examined. If historical populations have higher levels of heterozygosity, then perhaps hybridisation has occurred because numbers of individuals became low.

Relatively little mtDNA variation was found between populations of coyotes (Canis latrans) and grey wolves (Canis lupus) over their whole North American range (Lehman & Wayne 1991), which was not consistent with the recognised subspecies groups. They suggested that the high mobility of these canids caused high rates of gene flow and genetic mixing on a continental scale. Recent range expansion in coyotes has coincided with a decline in range of grey wolves and habitat conversion from woodland to agriculture (Nowak 1979). In these areas of changing population numbers, individuals that were phenotypically identified as grey wolves had genotypes identical to or phylogenetically grouped with coyotes (Lehman et al. 1991). As no coyotes with grey wolf mtDNA were found, it was suggested that only matings between male grey wolves and female coyotes occurred, or were successful (Wayne et al. in press). hybrids were not generally found where the two species were in sympathy, but only in areas into which coyotes had spread during the last 100 years. In addition, farming practices had changed the habitat and caused an increase in predator control, both factors reducing wolf numbers (Wayne et al. in press).

Early studies on the red wolf, using multivariate analysis of morphology suggested that it was an intermediate between grey wolves and coyotes. Lack of genetic difference, using protein electrophoresis (Ferrell et al 1980) also questioned the status of the red wolf as a species. Neither of these two approaches gave compelling evidence of hybrid status, however. Examining both the historic red wolf pelts and the remaining red wolves in captivity. Wayne & Jenks (1991) found that red wolf mtDNA was always very similar to coyote, and no types were found that were unique to red wolves. Although there is no longer debate as to whether hybridisation has occurred in red wolves, there is debate as to whether they should be conserved (Gittleman & Pimm 1991; O'Brien & Mayr 1991).
4. Four British Mammals

Recognition of hybridisation relies on the ability to distinguish between the two hybridising forms, and concern over hybridisation is related to the degrees of expected difference (phylogenetic, behavioural, or ecological) which can be inferred from the animals' history and distribution. The background information on the four species is therefore presented as follows:

i. Systematics and origins of the non-native, and its taxonomic relationship to the native form.

ii. History and current distribution of the two forms in Britain, and the timescale over which hybridisation may have occurred.

iii. Differences between the two forms and recognition of hybrids.

The current research is then described, and hybridisation in these, or closely related taxa, in Europe is outlined. Finally, protection for the threatened form is discussed with respect to mitigating hybridisation.

4.1 Hares: (Mountain Hare *Lepus timidus*)/Brown Hare (*Lepus europaeus*)

4.1.1 Taxonomy and distribution

Within the genus *Lepus*, the mountain hares (also called arctic, varying or blue hares) are a reasonably distinctive group, including *timidus* in Eurasia, *othus* in Alaska and *arcticus* in North America and Greenland. Whether these are two or three species is debatable (Corbet 1986). The brown hare, *Lepus europaeus* (also called European or common hare) occupies the lowlands of Europe from Britain across Russia, and as far east as Lake Baikal. It is replaced by *L. capensis* in Africa and SW Asia. Species differentiation between *L. europaeus* and *castroviejoi* (in NW Spain) and *L. grananensis* (in Iberia) is less certain (Corbet 1986). Brown hare and mountain hare distributions overlap in the northern-most part of the range of the brown hare, for example in Britain, Southern Sweden and Southern Finland.

4.1.2 History and distribution in Britain

Mountain hares may have been present in Britain through the last glacial stage (70,000 years b.p.) and certainly from the end of the Devonian (10,000 b.p.) (Yalden 1982). There was a possible land-bridge to Ireland about 8,000 b.p. (Yalden 1982, but after this period Irish hares (*Lepus timidus hibernicus*) would have been separated from the rest of the British Isles.

There is only one pre-Roman record of brown hare (from Star Carr, 9,500 b.p.) but this may well in fact be *L. timidus* (Yalden 1982; Corbet 1986). Brown hares were, therefore, probably not native to the British Isles after the last glaciation, and are most likely to have been introduced during Roman times (Corbet 1986).

There are notable differences in habitat utilisation in Britain where the two species co-exist. Mountain hares occur in upland areas, particularly heather-dominated moorland, and are replaced by brown hares on lower ground with grassland (including farmland) in Scotland (Watson & Hewson 1973), the Peak District (Yalden 1971) and the Isle of Man (Fargher 1977). By contrast, in Ireland, where brown hares are virtually absent except for several small, highly localised, populations that result from introductions in the late 19th Century (Barrett-Hamilton 1898), Irish hares are found in all the habitats described above including farmland where they are abundant (Whelan 1985).

4.1.3 Recognition of hybridisation: a description of difference between brown hares and mountain hares

The mountain hare is distinguishable from the brown hare by its smaller size, shorter ears, absence of black on the top of the tail, and the position of the root of the upper incisor in the skull (Hewson 1991). The length of gestation in mountain hare, from research in north-east Scotland, is 47-54 days (Flux 1970), and for the brown hare, in The Netherlands from 41-44 days (Broekhuizen & Martinet 1979). Unlike most mountain hare populations, brown hares never moult to white in the winter. Winter pelage of brown hares is a slightly redder colour than the summer coat, with whiter fur around the face and ears and a grey area on the rump (Tapper 1992). In autumn/winter the Scottish mountain hare
moults to white or grey white, and in spring/summer moults from white to brown. The degree of complete transition to white is genetically determined and is related to air temperature and snowless lie (Jackes & Watson 1975). Irish hares, however, usually partially moult or may not whiten at all (Hewson 1991). Mountain hares have been found to have high proportions of birch in their winter diet compared to brown hares, and differing abilities to detoxify secondary plant compounds have been demonstrated in the two species (Iason & Palo 1991).

4.1.4 Hybridisation

Hybridisation between *Lepus timidus* subspecies and *Lepus europaeus* has been reported captivity, both spontaneously and by artificial insemination (Gray 1971). The fertility of the hybrids however, was either unknown or they were infertile. In Finland a hybrid was bred in captivity and an antiserum marker found. Morphology and serum immunoglobulins were examined for 42 wild hares, but there was no evidence of hybridisation (Schroeder et al. 1987). No other published information has been found on the occurrence of hybrid in wild populations in Britain or Europe, and so the following authors who have worked on hares in areas where both species occur, were consulted: R Hewson, I Hulbert, G Iason (Scotland), D Yalden (Peak District), and A. Person (Sweden - Grimso Wildlife Research Station). In Sweden, hares are bred in captivity to boost numbers for hunting, hybrids are sometimes bred and also released, the offspring being fertile (A. Person pers. comm.). Some captively bred hybrid hares were exported to the USA for medical studies (A. Person pers. comm.). Hybrids show mixed characters for example in ear length, eye colour, tail and footprints. However, in wild populations, Person had seen only five hares which showed around four typically mixed characters. In Britain only one possible case of hybridisation has been observed in Deeside, based on measurements throughout many years of field work, by R. Hewson (pers. comm.). Other workers have not observed any individuals with mixed characters. Occasionally, Irish hares have been wrongly labelled as hybrid hares. Irish hares have been introduced into South-west Scotland, and a small population appears to be maintaining Irish characteristics distinct from a neighbouring population of Scottish mountain hares (R. Hewson pers. comm.). A putative collection of hybrid hares, skins and some skulls, was presented to Edinburgh museum (A. Kitchener pers. comm.). Several of the specimens were collected from Ayrshire in the 1930’s and 15 to 20 specimens were collected from Garvald (Lammermuir hills near Edinburgh) between 1958 and 1964. It has not been established whether these samples are in fact hybrids.

4.1.5 Current research

Wild hybrids between mountain hares and brown hares occur rarely in Britain, if at all, and so would represent only novelty value. A hybrid zone is not found between the two species in continental Europe where their native ranges overlap, so the two species must be almost completely reproductively isolated under natural conditions. The apparent lack of introgression between Irish and Scottish mountain hare populations under natural conditions is worthy of further investigation. If reproductive isolation is found, the two races could be considered as separate species.

4.2 Deer: Red deer (*Cervus elaphus*), Sika (*Cervus nippon*)

4.2.1 Taxa, species and subspecies

The taxonomy of sika deer (*Cervus nippon*) has long been debated. Ellerman & Morrison-Scott (1951) recognised seven subspecies, Whitehead (1964) gives 13, and Lowe & Gardiner (1974) suggested only two. Lowe and Gardiner (1974) also argued that the Asiatic mainland subspecies may be a hybrid of great antiquity, resulting from interbreeding between Chinese wapiti (*Cervus elaphus xanthopygus*) and Japanese sika. The obvious differences in size, colour and antler growth between three types commonly used to described sika subspecies. 'Formosan', 'Manchurian' and 'Japanese', do not in themselves justify subspecific status (Ratcliffe 1987). Recent papers support the split between the mainland and Japanese island sika deer, but also recognise the deer from Hokkaido,
Japan, as both separate from the mainland deer, and also from other island deer (Omura, Fukomoto & Ohtaki 1983; Bartos 1991; Ratcliffe et al. 1991). Immunological evidence (Harrington 1985), epigenetic (Zima, Kozena & Mubalek 1990), and karyotype (Herzog 1987, Bartos 1991) all lend support to the hypothesis that the Asiatic mainland sika are of hybrid origin. The mainland sika are now grouped under the name *C. n. hortulorum*.

The red deer native to Britain, *Cervus elaphus scoticus*, is not clearly distinguishable on the basis of skull shape from continental deer (Lowe & Gardiner 1974), red deer from four European subspecies, including *C. e. scoticus* were examined for allozyme variability (Gyllensten et al. 1983), although differences between populations were small overall, there was a dichotomy between the British and Norwegian deer in one group and the Swedish and continental deer in the other.

### 4.2.2 History of red deer and sika deer and hybrids in Britain, and their current distribution

**Sika deer.** Sika deer were first introduced to England and Ireland in 1860 (Lever 1977). From then until the 1930's some thirty estates and parks were stocked with sika, many from the original herd at Powerscourt in Co. Wicklow (Lever 1977). The details of these introductions to Britain and Ireland, and subsequent deliberate releases or escapes are reviewed by Ratcliffe (1987), and summarised below. Although many populations are referred to as Japanese sika, most releases followed the keeping of sika alongside red deer in parks, with possible natural or deliberate attempts at interbreeding. Therefore, in the following lists, Japanese does not necessarily mean pure Japanese sika. Only those deer which came directly from Japan, those in Peebles-shire and north Devon (although the latter may be extinct) can be considered to be 'pure' sika (Ratcliffe 1987). In Kent there is a localised population of sika from China, Japan and Manchuria, also containing purposely bred hybrids between Japanese and Manchurian deer. In Sussex, Hampshire and Wiltshire there are pockets of sika of unknown origins. Also in Hampshire, following escapes and deliberate releases from Windsor, there is a well established population of unknown genetic origin (but see Ratcliffe et al. 1991). In Dorset and Oxfordshire and possibly in Norfolk and Devon, there are small numbers of Japanese sika. In Yorkshire there is a well established population of sika of unknown genotype in the Bowland-Gisburn area, and a mainly hybrid population centred on Cartmel Fell further north. In Peebles-shire these are established population of pure Japanese origin. In Fife there is an established but localised population. In Angus there are possibly some Japanese sika. In Argyll there is an extensive high density population in Knapdale and Achagliachgach forests which is now spread throughout the Kintyre peninsula, north to Loch Etive and south to Cowal. Also in Argyll, small local populations exist near Morar. In Inverness-shire two introductions occurred at Glenmazeran and at Aldourie. These two populations may now have coalesced and are spreading from the Moray Firth, along both sides of Loch Ness and into the Forest Augustus area, to Ratagan on the coast, north towards Culloden, and south east into Speyside. In Ross-shire there are sika of unknown genetic origin in Strathbran and to the north and west. In Sutherland there is an extensive high density population in and around Rosehall, the area of original introduction, but this has now spread out in all directions from there, and has coalesced with another established population further south in Strathcarron, Glencalvie. In Caithness there is a localised population of unknown genotype around Langwell.

By comparing the sika distribution in 1972 with that in 1984 (Ratcliffe 1987) it is apparent that sika populations in southern England are increasing, and that the populations in north and west Scotland have expanded their range dramatically. There have been recent sightings of sika stags well outside the 'normal' areas, for example, one on Skye, one on Ballachulish, one shot in the Borders and one just north of Loch Rannoch (A Chadwick pers. comm.).

**Red deer.** Red deer are native to Britain. However, both the American sub-species,
C. elaphus canadensis (wapiti) and continental races have been introduced to various parks and estates in Britain to 'improve' stock (Whitehead 1964). The history of introductions, and subsequent escapes of park deer is largely undocumented. The original British race is thought, or suspected, to have been largely replaced by a genetic mix in the English populations, other than those in north-west England (Lowe & Gardiner 1975). Also, park deer have been introduced to most, if not all, areas of the red deer range in Scotland. The genetic discreteness of the Scottish population is, therefore, in some doubt. There has been a decline in body size since the last ice age (Ritchie 1920; Lowe 1961), firstly through the influence of climate change on the habitat and then, in historic times, by the razing of forests. From being distributed throughout Britain, red deer became extinct in much of England, Wales and the Scottish lowlands by the end of the 18th Century. Following subsequent re-introductions and population expansions, the main populations are now in the Scottish Highlands and many of the islands, south-west Scotland, Exmoor, north-west England and East Anglia (Staines 1991).

Hybrids. The earliest record of a feral red/sika hybrid was made in 1940, at Rigmaden, Westmorland (Lever 1977). Although the sika from Rigmaden deer park has been dispersed of by 1924, some had escaped. Many hybrids have since been noted, particularly in recent years, centred on Cartmel and Middleton Fell (Lowe & Gardiner 1974; Ratcliffe 1987). Lowe & Gardiner (1974) suggested that complete introgression had occurred between red deer and sika on these fells, resulting in an entirely hybrid population. This hybrid population can potentially spread north into the only native red deer population in England. The main northern English sika population, in the Bowland-Gisburn area of Yorkshire, lies further south.

Increasing numbers of hybrids have been seen and shot in Knapdale (Argyll) since the early 1970s where native red deer and introduced sika are no sympatric (Ratcliffe 1987). Hybrids have also been recorded in other areas in Argyll and in Sutherland (Ratcliffe 1987). Preliminary results from protein electrophoresis suggest that about 22% of deer culled in forestry commission plantations on the Kintyre and Cowal peninsulas in Argyll could be hybrid (Abermethy 1992).

4.2.3 Difference between the two species: identification and measurement of hybridisation

Pelage, antler shape and general morphology. Harrington (1973) and Bartos & Zirovnicky (1981) used colour of coat, rump patch, tail, ear and metatarsal gland, tail length and antler profile to determine hybrids, but concluded that although many first generation hybrids (F1's) often have an obvious mix of external characteristics, this was not always the case, and for subsequent backcrosses (F2's) there were no reliable characters.

Cranial morphology. Morphometric analysis has been used to determine hybridisation in north west England (Lowe & Gardiner 1975) and Eastern Europe (Bartos, Hyaneck & Zirovnicky 1981). Hybrid 'type' skulls can be distinguished, provided that individuals of the same sex, the same age category and from within the same population are compared (Lowe & Gardiner 1975). However, this method is only useful for F1's (and it is not known what proportion of F1's) and cannot be used as an independent assessment for subsequent generations (Bartos 1991). Non-metric characters, i.e. discrete differences, cannot be used to distinguish a skull to species level or determine hybridisation (Zima, Kozena & Mubalek 1990).

Behaviour and ecology. The life history and social structure of sika and red deer is similar, their rutting periods approximately coincide and both are polygamous (Ratcliffe 1987). Sika are usually associated with dense cover within woodland. Thicket stage commercial forestry (15-25 years) thus provides suitable habitat (Ratcliffe 1987). The expansion of sika populations have always taken place through commercial forest block: sika make forays into open habitat but are not thought to stay long from cover. This is supported by the recent recording
(Ratcliffe 1987) of a small population of sika, centred on oak woods which are surrounded by open hill habitat, near Moray, Argyll. This population appears to have been stable since its introduction there, around 1910, and sika appear neither to have dispersed from there or introgressed with the resident red deer.

However, there have been reports in north west Scotland of sika on open hill areas devoid of tree cover (A Chadwick pers. comm.).

Flexible mating systems, depending on habitat type, have been described for both species (Carranza, Alvares & Redondo 1990; Bartos, Zeeb & Mikes 1991). Both species have been observed in close proximity to each other, but interspecific agonstic behaviour has not been reported. Observations in the Forestry Commission Knapdale forest showed that the two species used the same feeding areas, occurred in roughly equal densities in plantation to thicket stage forestry, but were not generally found in mixed groups (K Abernethy pers. comm.). Sika deer in Argyll, however, have a much higher conception rate than the sympatric red deer (Chadwick & Ratcliffe in prep), and a higher fat content in winter carcasses of culled animals (observations by Rangers in Abernethy 1992), suggesting that sika may have the potential for maintaining a higher population growth rate than red deer.

No studies have yet been done on the behaviour and ecology of hybrids, although the current study by Abernethy will allow comparisons between genotype, digestive efficiency, and diet between sympatric populations of the two species.

Karyotype. Karyotype (chromosome number) is very variable in sika populations. Although the Cervidae karyotype is generally rather uniform (usually 2n=68, as for nearly all the Cervus elaphus sub-species) (Zima & Grafodatskij 1990), sika deer karyotypes range from 2n=62-68. From studies of sika, red and sika/red hybrids (Harrington & Power 1985; Herzog 1987; Bartos 1991 Herzog & Harrington 1991, hybridisation is accepted as a major factor in karyotype polymorphism. The karyotype is not suitable for identifying individuals as hybrids, only for analysing the population as a whole (Bartos 1991).

Molecular. Herzog & Harrington (1991) used enzyme electrophoresis to type animals from both Harrington's (1973) breeding experiments and from different populations in Germany. Polymorphism was found for one enzyme marker in the hybrid population. Three alleles were found in New Zealand sika at three different loci which did not occur in the red deer, and known F1 hybrids were all found to be polymorphic, containing one allele from each species (Tate 1985). The greater the number of markers used, the greater is the chance of picking out all the hybrid animals, and so workers are moving to nuclear and mitochondrial DNA methods to increase the number of enzyme markers available. As sika may be of hybrid origin in the first place, some markers may give confusing results. However, differential selection has been demonstrated to be operating on the enzyme systems of cervids (Pemberton et al. 1988), and therefore differential selection for particular types of hybrids can be examined.

4.2.4 Factors contributing to hybridisation

Early studies and observations suggested that once a strain had hybridised, subsequent hybridisation would more readily follow. They suggested that sympatric wild populations of native red deer and Japanese sika would not hybridise, whereas when either mainland Asiatic sika or park animals containing hybrids co-occurred with red deer, then hybridisation followed more readily (Harrington 1973, 1982; Lowe & Gardiner 1974). This hypothesis was used to explain the complete introgression of sika and red deer in Wicklow, Ireland (Harrington 1982) and Cartmel Fell, (Lowe & Gardiner 1974), but not in other areas of Britain. However more recent information suggests that hybridisation is occurring between native red deer and Japanese sika (Ratcliffe 1987, Ratcliffe et al. 1991) although the only known viable population of sika deer introduced directly from Japan, into the wild having been first held in parks with red deer first occurs in Peebles-shire, where there are no red deer (Ratcliffe et al. 1991).
The wide size dimorphism between red deer stags and sika deer hinds has led to suggestions that only young red stags are compatible with female sika (Harrington 1973, 1982), but this has not been rigorously tested. Putative hybrids have been recorded in areas where sika are colonising predominately red deer areas and where red deer are colonising predominately sika areas, leading to the suggestion that hybridisation is more likely as colonising stags move into the range of the other species (Ratcliffe 1987). Sightings of sika well outside their normal range in recent times have all been stags (A Chadwick pers. comm.) and colonising males of either species have been recorded up to ten years before the following hinds in some areas (P. Ratcliffe pers. comm.).

Hypotheses based on preliminary analysis of the data from a current PhD study (Abemethy, 1992) suggest that, in dense commercial forestry plantations, sika genotypes will predominate. Introgression occurs in an advance wave where the two species meet, but as the sika spread, in areas behind their advance, animals become more sika-like rather than remain evenly introgressed. However, in more open habitats such as the Great Glen area, populations would be less likely to introgress. As the study continues these hypotheses may be supported or rejected.

4.2.5 Current research

Observations and cull records from Forestry Commission rangers will continue to give an indication of the extent of hybridisation, although this is obviously not one of the specific purposes behind counts and records. A three year study looking at population biology, feeding ecology and hybridisation of red and sika in Argyll is ongoing (Abernethy, 1992) and the preliminary results have been outlined above. Genotypes based on protein electrophoresis will be compared with those found using mitochondrial and nuclear DNA techniques, enabling animals to be typed more accurately and, given the highly polygamous mating systems, enable sex differences in dispersal to be examined. The purity of red deer populations in Britain is currently being examined by Loudon & Benford (Institute of Zoology, London) using mitochondrial and nuclear DNA.

4.2.6 European and world perspective

The Cervus genus as a whole hybridises readily in captivity, and therefore many species have been mixed in parks and farms throughout the world to 'improve' stock (Lever 1977). Because of their importance in meat production, and the endangered status of several taxa, there is now an extensive library of genetic information, mostly biochemical, on the Cervidae (Dratch & Pemberton 1992).

Imported sika deer have been found to hybridise in the wild with red deer in Czechoslovakia (Bartos, Hyanek & Zirovnichy 1981) and in New Zealand (Davidson 1973). Red deer and wapiti (Cervus canadensis) have both been introduced to New Zealand, and where populations met and mixed in Fiordland, Dratch (1986) found that 95% of animals were now hybrid. Natural matings between mule deer (Odocoileus hemionus) and white-tailed (O. virginianus) deer have been well documented in a zone of introgression from Alberta to Texas, (e.g. Carr et al. 1986; Cronin, Vyse & Cameron 1988). Displacement of mule deer by white-tailed deer is occurring in Texas, and the preferential absorption of hybrid offspring into the mule deer gene pool was thought to be involved in this displacement (Carr et al. 1986). Mule deer and black-tailed deer are thought to be subspecies of Odocoileus hemionus. However, the divergence in mtDNA between the two was found to be amongst the highest intraspecific values in Cervidae so far reported (Cronin, Vyce & Cameron 1988). Behaviour is sometimes important in categorising a species. The escape gait of mule deer and white-tailed is one of the differentiating characters between the species; mule deer stott and white-tails gallop. Hybrids had a slower gallop speed than white-tails, when alarmed, and the specialised mule deer stott is largely not apparent (Lingle 1992).

4.2.7 Protection of native deer

Introgression of genes from sika deer to red deer seems likely to increase in occurrence rather than decrease. If sika-like hybrids
are better competitors in dense woodland sika-like deer may completely replace red deer in such habitats. Eradication of sika throughout Britain is thought to be impractical and the general spread of sika inevitable. However management of sika through management of habitat is a possibility (Ratcliffe 1987), such that forestry plantations are not planted where they will act as corridors for the spread of sika into areas where 'pure' populations of native red deer exist. A zoned approach to the control of sika (suggested by Ratcliffe 1987) could also be practised. In areas of high sika density, often in dense plantations where control is ineffective in sika would not be specifically targeted. In surrounding areas sika could be selectively culled in preference to red deer, and in areas in which there are early signs of colonisations eradication attempts could be made. In addition, island populations of red deer offer a means of retaining the red deer genotype. Under the Deer (Scotland) Act, (1959, amended 1984), sika and sika/red hybrids are subject to a closed season. However, given the potential introgression of sika into red deer, this should be reviewed.

4.3 Cats: Wildcat (*Felis silvestris*)//Domestic cat (*Felis catus*)

4.3.1 Origin of the domestic cat, systematics and the domestication process

The process of domestication of cats is thought to have occurred about 4000 years ago possibly in Egypt (Bradshaw 1992), but it is not until about 1600 BC that there is conclusive evidence of domestic status (Serpell 1988; Bradshaw 1992). The most likely precursor of the commonest British domestic cat (*Felis catus*) is the African wildcat (*Felis lybica*) (Kratochvil & Kratochvil 1976; Todd 1978; Bradshaw 1992).

Opinions differ as to whether the African wildcat is a different species to the European wildcat, and whether, therefore, the domestic cat is also a separate species. Resolution of this argument would to some extent resolve the problem of the degree of difference to be expected between domestic cats and wildcats in Europe. The domestic cat is commonly called *Felis catus*, although if it originates primarily from one wildcat or the other there is a strong argument for naming it a subspecies of its precursor. The lack of genetic differentiation between Italian representatives of African, domestic and European wildcats (Randi & Ragni 1991) led these authors to suggest sub-species status of *Felis silvestris* for all three forms. Pocock (1951) gave the two wildcats separate species status based on subtle differences in pelage, whereas Haltenoth (1953) considered them to be conspecific, showing clinal geographic variation in pelage. Some follow Pocock (Hapold 1987; Skinner & Smithers 1991) but others consider them to be conspecifics, using *Felis silvestris silvestris* for the European wildcat, and *F.s. lybica*, the African (Corbet & Hill 1991; Kitchener 1991; Nowak 1991).

Unlike other domestic animals, the relationship between cats and man is largely commensal. Todd (1978) suggests that the domestication process of *F. catus* was essentially initiated by cats to exploit the opportunities afforded by human settlements. Even if cats received positive encouragement from humans as a means of rodent control (Bradshaw 1992), the lack of morphological distinction between wild and domestic forms (other than coat colour) suggests a lack of human selection pressure for a particular, 'useful', cat type.

4.3.2 History of wildcats and domestic cats in Britain

Wildcats were present in post-glacial Britain prior to the rise in sea level about 9000 b.p. which isolated Britain from the rest of Europe (Yalden 1982). From the Middle Ages the wildcat population has declined as a result of persecution and habitat changes. They probably disappeared from southern England around the 16th Century and from northern England and Wales by 1880 (Langley & Yalden 1977). From 1800 to 1850, wildcats disappeared from southern and eastern counties of Scotland, and around 1915 were rare or extinct anywhere south of the Caledonian Canal (Langley & Yalden 1977). Ritchie (1920) supposed that it still survived in Western Ross, Sutherland, Inverness and Argyll, but in view of the rapid decline, was not optimistic about its future. The wildcat,
however, made a rapid comeback, summarised in Jenkins (1962) and Easterbee, Hepburn & Jefferies (1991). The 1983-1987 survey (Easterbee, Hepburn & Jefferies 1991) based on sightings and observations of road casualties, found that wildcats were distributed throughout mainland Scotland, in most areas north of the Central Belt between Glasgow and Edinburgh.

Domestic cats were brought to Europe by the Romans, and were present in the British Isles by the last few centuries BC (Maltby 1979; Noddle 1987). They were probably not common in Roman Britain, but following the Norman invasion (11th Century) cat populations increased (O'Connor 1992). Feral cats are now found throughout mainland Britain in rural and urban areas, but the extent to which wild and feral (or semi-domestic) cats overlap (or have overlapped) in habitat is unknown (Macdonald 1991). Colonies of feral cats survive on some uninhabited islands (Corbett 1979), but they are generally found in association with man, in areas of high food density such as rubbish tips, farms, dockyards and hospitals. The area considered to have held the relict population of wildcats in Scotland is generally described as wild and remote. The human population density there was relatively low, and therefore the domestic cat population is also assumed to have been low. However, cats of domestic/feral appearance have been trapped in north west Scotland several miles from the nearest house (M Daniels pers. comm.), and human populations were considerably higher in these areas before 1945. There were probably feral domestic cats on Arran by 1774, as Pennant (1774) notes that “wildcats” were present, but supposedly true wildcats never reached Arran. Domestic cats were recorded on Bute (Ross 1880), and interbreeding of wild and domestic cats is noted by Colquhoun (1840).

Hybridisation or introgression between wild and domestic cats may have increased as wildcats recolonised less remote and wild areas after a population low around 1915 (Corbett 1979; French, Corbett & Easterbee 1988). However, introgression could have been occurring for 900 years as feral cats increased in number and wildcats declined, or could have occurred when wildcat numbers were low and their distribution fragmented, around or before 1915.

4.3.3 Recognition of hybridisation: a description of difference between wild and domestic cats

There are a few examples of F. silvestris and catus inter-breeding in captivity or in the wild (Pitt 1939; Gray 1971). It is commonly believed that they are interfertile because of the occurrence of feral cats with pelage colour and markings which appear to be hybrids (e.g. Corbett 1979; French, Corbett & Easterbee, 1988; Easterbee, Hepburn & Jefferies, 1991). Hybridisation is not just considered a recent phenomena. For example Hamilton (1987) suggested that all wildcats in Scotland were descended primarily from feral domestic cats of African origin and that the original wildcats, as found in the middle ages, were extinct.

The two features said to characterise wild and domestic forms of the cat are pelage and behaviour. Variation in two internal characters, gut length and skull morphology have also been identified (Schauenberg 1969). Recent investigations into biochemical methods of differentiation suggest a sub-specific level of difference (Hubbard et al. 1992; Randi & Ragni 1991).

Pelage. Wildcats generally have a homogeneity of pelage phenotype with only subtle variation (Haltenorth 1953; Crobett 1979), although melanism may occur naturally in wildcats from the west Carpathians (Sladek 1976). Variability in pelage in Scotland similar to that found in continental Europe led Corbett (1979) to reject the suggestion of a Scottish sub-species F. s. grampia (Miller 1901). In domestic cats there are extensive polymorphisms for coat colour, pattern and texture.

Identification of hybrid animals by pelage is subjective, leading to obvious problems in monitoring the recovery of wildcat populations by reported sightings or carcases (Easterbee, Hepburn & Jefferies, 1991). The rings on the tail and tail shape are commonly used as a method of hybrid
assessment (Macdonald 1991) but opinion is swayed in the light of other characters.

Pelage colour in *F. catus* does not necessarily originate from human selection. Coat colour frequencies of feral cats in urban areas do not reflect human choice (Dyte, 1974, Clark 1975). Coat colour frequencies vary significantly from urban to rural areas in Scotland, suggesting either a lack of gene flow between populations of urban cats and rural cats, or significant differences in natural selection pressures (Clark 1976). Similar differences were observed between Reykjavik and rural Iceland (Todd, Gloss & McLure, 1974). An increase of wild-type alleles in less populated areas (Clark 1976) may reflect a degree of hybridisation with wildcats; it may equally or partly be a consequence of natural selection pressure within the domestic cat lineage (Todd 1978). The 'blotched tabby' allele probably originated in Britain before the 17th Century and has since spread into Europe where it becomes progressively less common (Todd 1978). It seems to be gradually replacing the wild-type 'striped tabby' allele found in feral and domestic populations, although the advantage it confers has not been identified (Bradshaw 1992). An important differentiation between 'pure' wildcat and hybrid in Britain has been the occurrence of any form of tabby blotches as opposed to stripes in the coat. Stripey hybrids would more likely go unnoticed and an increase in occurrence of blotched tabby types in wildcats could suggest introgression of a new gene type rather than increase in the real level of hybridisation.

Gut length. Schaunberg (1977) found that both gut length, and gut length divided by head and body lengths, was distinctly larger for domestic cats than it was for wildcats. Crobett (1979) also found a significant difference in the gut length of domestic and wildcats in Scotland. However, this method has not been useful in distinguishing hybrids (Kitchener & Easterbee 1992). It is also not known to what extent diet determines gut length.

Cranial morphology. Suminski (1962), using coat pelage and external body measurements, concluded that there were no pure populations of wildcat left in Europe, but Kratochvil & Kratochvil (1976) disagreed. Cranial volume of supposed wildcats was found always to be relatively greater than that of domestic cats (Schauenberg 1969) but this method does not distinguish hybrids from wildcats (Kitchener & Easterbee 1992). In an attempt to quantify the degree of hybridisation in wildcat populations, French, Corbett & Easterbee (1988) measured 50 variables of skull morphology and analysed skulls using three types of statistical analysis. In order to avoid the assumption of pure wildcat material, museum skulls were included, to give three groups of wildcat: modern, recent and old. Skull morphometrics were unable to distinguish to 99% certainty between the designated 'hybrid' cats and recent or modern wildcats. The authors suggested that old wildcats (from 1901-1941) are significantly purer than recent or modern wildcats. Only small samples were available, however, and all but two of the modern and recent wildcat sample came from recently recolonised areas of Scotland. Their results may reflect a zone of increased hybridisation during range expansion, or zones of hybridisation close to large human populations, rather than the current status of the wildcat population as a whole.

Comparison of domestic cats and hybrids with a time sequence of wildcat skulls successfully gets around the problem of having to designate some skulls as 'pure wildcat' based on other criteria, a problem inherent in most morphometric comparisons. The assumptions are then that the old wildcat sample was morphometrically different to recent and modern wildcats because of phylogeny i.e. hybridisation, rather than other factors such as geographical variation, inherent change in variation of skull shape through time, or the possibility of selection or bias for a particular cat type in museum samples. The morphometric data does not support the categories defined by subjective assessment ('designated hybrids'). Given that morphometric techniques have not yet been established to distinguish between wildcats and wildcat/domestic cat hybrids (French, Corbett & Easterbee, 1988), the use of a key to distinguish black wild-living, 'Kellas' cats, as introgressed hybrids of
wild and domestic cats (Kitchener & Easterbee 1992) requires further support.

**Behaviour and ecology.** There have been few studies on the ecology and behaviour of wildcats, but they have been found to be solitary and intra-sexually territorial (Corbett 1979, R. Scott pers. comm.). Feral cats are generally found to be social (Liberg 1983; Macdonald *et al.* 1987; Kerby & Macdonald 1988). Groups of matrilines of successive generations of daughters have been described for farm cats (Macdonald *et al.* 1987). Colonies can develop where food is particularly abundant and clumped. The effect of hybridisation on behaviour, and the extent to which either types of cat could be flexible in this respect is not known. The ability of feral cats to live in resource-poor areas where they would be competing for resources with territorial wildcats is also not known. Varying resistance to disease and parasites may also be an important differentiation between the two. The survival of hybrids may be favoured due to a flexibility in behaviour and ecology, or diminished due to inappropriate responses to conspecifics or prey.

**Molecular.** Two studies to date have used a biochemical approach to investigate genetic differences between populations of wild and domestic cats (Ragni & Randi 1991; Hubbard *et al.* 1992). There have been several studies on the biochemical genetics of domestic cats (O'Brien 1980; Allan, Putt & Fisher 1981; Nozawa, Fukui & Furukawa, 1985). In Scotland, Hubbard *et al.* (1992) found genetic difference from domestic cats in only 8 out of 42 putative wildcats and concluded that extensive hybridisation had occurred. However, these authors assumed that they were looking for genetic difference at the species level. If, as is likely, *F. lybica* and *F. silvestris* are allopatric conspecifics, sub-specific difference could also be expected between *F. catus* and *silvestris*, and the conclusion that extensive hybridisation had occurred is mitigated or valid.

Allozyme electrophoresis was used to investigate the genetic variability and phylogenetic relationship of domestic cats, European wildcats and African wildcats in Italy (Ragni & Randi 1991). They suggested a genetic distance between *F. lybica* and *F. silvestris* representing 20,000 years of divergence or separation. Their data supported the hypothesis that domestic cats came from the African wildcat lineage, but that the genetic difference between the three lineages was small (no fixed allelic differences were found between wild and domestic cats, although certain alleles were unique to domestic cats). They suggested conspecific status for the three forms. Furthermore, they found low genetic variability in their *F.s.silvestris* sample.

**4.3.4 Current research in Britain**

Scottish Natural Heritage are collecting cat samples from across Scotland (both wild and feral/domestic) from road casualties, trapped animals and historic samples from museums and collections. Microsatellite DNA, and mitochondrial DNA analysis is being carried out Wayne Institute of Zoology, London. Two hypotheses are being explored: that native wildcats do remain in Scotland, and that hybridisation is more prevalent in certain areas. Comparisons will be made with samples from other European countries. By looking at pelage and morphology in association with the degree to which unique mtDNA and unique microsatellite alleles occur, a correlation between genotype and phenotype can be explored. A re-analysis of morphological characters used to distinguish between wildcats, domestic cats and hybrids, is also being undertaken (Kitchener, Royal Museum of Scotland).

**4.3.5 European perspective**

The European wildcat has disappeared from a substantial part of its former range in Western Europe, although the status of many populations has improved over the last fifty years (Stahl 1992). In many countries of Eastern Europe and the former USSR, the wildcat is listed as vulnerable or endangered (Remanowski 1992). The question of hybridisation with feral cats, and the difficulty of distinguishing hybrids is currently being addressed in Italy, Hungary and Portugal as well as Britain (Council of Europe...
At one time Scottish populations of wildcat were thought to be relatively 'pure' because domestic cats were spread through the European continent before they reached Britain. Suminski (1962) suggested that his sample of 35 Scottish wildcats were 66% pure, compared to 44.73% from populations in continental Europe. Pelage and cranial characters were used in the comparison. However assessment of 'typical' wildcat characters (given that there appears to be exceptions to all 'distinguishing' characters) is subjective. Corbett (1979) notes that "the problem is that standard or genuine wildcat material is not available for comparisons against modern wildcats and suspected hybrids". It is important that studies on the degree of difference between catus, lybic and silvestris cats use European wide as well as local samples, or the results will not be applicable in the European context, and will not allow comparisons on the degree of hybridisation throughout Europe and Africa. Particularly where re-introduction programmes are being undertaken, an understanding of the degree of difference found throughout Europe would be very useful. Populations of cats with the least apparent hybridisation with domestic animals would be worthy of protection at a European as well as national level. Ragni and Randi (1991) found little genetic difference between F. catus, lybica and silvestris in Italy. Vigne (1992) questioned the 'purity' of lybica in Italy.

Likewise, because of the very fragmented nature of wildcat populations in many European countries, we may be looking for markers to hybridisation in an already hybrid population, and conclusions over markers and degrees of hybridisation will not necessarily be useful or applicable in other regions.

4.3.6 Protection of the wildcat

The wildcat is fully protected under Schedule 5 of the Wildlife and Countryside Act, 1981 (amended 1988). It is only described by name, Felis silvestris. This is inadequate given that domestic cats and hybrids between the two forms can also be considered to be Felis silvestris. There are two suggested alternative ways to protect a form of wildcat. Firstly, a cat could be described on pelage characteristics, which is consistent with the description of 'type specimen' (see Corbett 1979) but which allows for a subjective degree of variation. This does not protect the genotype, and some domestic cats would inevitably be protected or wildcats killed. A cat could also be described based on the relationship between genotype and pelage, if any are found through the current research. Alternatively, if domestic/feral cats and wildcats differ in their behaviour and ecology, but overlap in their phenotypes, cats could be protected based on geographical area, and habitats which favour the survival of wild-type genes. Suitable areas may be identified following the results of the genetic study, or following studies on the behavioural and ecological differences between feral and wildcats. Zones of hybridisation, following the genetics study, may be identified where selective control is desirable. In other areas blanket protection or control could be decided by habitat type and land use strategy. Cats are currently controlled by keepers in all areas, particularly in association with pheasants. Legal protection of wildcat in these areas is not currently practical since there is doubt over the genetic integrity of a dead cat, let alone one which is alive and running. Control of cats by live capture is currently practised in some areas (D. Balharry, M. Daniels pers. comms.) which would allow selective culling if an agreement of the description of the protected cat can be reached. Protection of hybrids in areas where cat control is practised may be counter productive, as it may be publicly perceived as too much protection. However, control of feral cats will tend to mitigate against hybridisation. In areas where all cats are protected, neutering of domestic animals and publicity against the encouragement of feral cats could be advocated, and sympathy for the protection of wildcats sought from other land users.

The industrial belt in central Scotland was identified as a barrier to the spread of the wildcat (Easterbee, Hepburn & Jefferies 1991). Re-introductions into other areas
of Britain would have to select cats from the least hybrid populations in Scotland, and only introduce these animals to areas which favour the survival of the wild form over the domestic.

Following the results of the genetic study, a study of the behavioural and ecological differences between wild and feral domestic cats should be undertaken, in order to gain an understanding of the barriers which have kept the two forms apart. Such as study would aid the protection of the wildcat in Scotland and benefit any introduction programmes.

4.4 Mustelids: Polecats (Mustela putorius) / Ferrets (Mustela fura)

4.4.1 Origin of the ferret, systematics, and the domestication process

The European (western, forest, dark or black) polecat, *Mustela putorius*, is distributed throughout western Europe. The Steppe (Siberian, Eastern Asiatic or light) polecat, *Mustela eversmanni*, is found in isolated populations in eastern Europe and through the former USSR to eastern China. Ferrets were domesticated from one or other or crosses between the two. Domestic ferrets were first recorded by Aristotle in the fourth Century BC but they could have existed before 1000 BC in Palestine (Zeuner 1963). In captivity ferrets hybridise to produce fertile offspring with both species of polecat. Because of the uncertainty over the origin of ferrets, they are given separate species status *Mustela furo* by some authors (Corbet and Hill 1991) but as their morphology and karyotype have suggested primarily European origins, other authors use a sub-specific differentiation, *Mustela putorius furo* (Ashton 1955; Rempe 1970; Gray 1971; Van der Horst et al 1991). Although ferrets have been domesticated for several thousand years, back-crossing to local wild populations of ferrets will decrease the expected difference, and ferrets from some geographical areas of their distribution may be more similar to *M. putorius* than *eversmanni*, this may not be the case for all ferrets. Ferrets are easily backcrossed to wild polecats in captivity, and the degree to which this occurs will depend on the practice of rabbit hunters (Pitt 1921).

Much of the controversy over ferret taxonomy, and the recognition of hybridisation, has been in the attempt to understand how much of the difference between polecats and ferrets is due to domestication, and how much is due to phylogeny. The other part of the argument has been over whether Steppe polecats and European polecats are separate species or sub-species, and thus even if ancestral ferrets were Steppe polecats, they may, like the domestic cat, only have initially had a sub-specific level of difference between the wild and domestic form found in Britain today.

Naturally occurring hybrids (individuals with intermediate characters) have been reported in the wild (Novikov 1962), but an extensive morphological investigation (Rempe 1970) found neither intermediate discriminating values, nor an increase in variation in sympatric populations, thus finding no support for introgression. Where the distributions of the two wild polecats overlap they are separated by differences in habitat preference (Blandford 1987). Despite their morphological similarity they are now generally considered to be separate species.

4.4.2 History and distribution in Britain

Polecats (*Mustela putorius*) probably colonised Britain during the early post-glacial, but the first definite records are from about 6,000 to 5,000 years b.p. (Blandford & Walton 1991). They were once widespread and common throughout England, Scotland and Wales but declined rapidly in distribution during the 19th Century. By 1915 there was little evidence of viable populations of polecats (as opposed to feral polecat ferrets) anywhere except Wales (Langley & Yalden 1977). Walton (1968) documented an expansion in Welsh polecat distribution but again found no evidence of polecats outside Wales. Polecats have continued to spread, and are now recolonising the English Midlands. Polecats of Welsh or mixed origin have been introduced into
Cumbria and parts of Scotland, although there is no evidence, as yet, that viable populations of these introduced animals survive (J. Birks, A. Kitchener, K. Walton pers. comm.).

Ferrets and polecat ferrets are widely kept and frequently escape. Feral populations have been established on the islands such as Mull, Lewis, Arran, Isle of Man, and on the mainland in Yorkshire (Blandford & Walton 1991). They may have been introduced to Britain by the Romans, but Owen (1984) suggests their arrival could have been as late as the 14th Century.

4.4.3 Recognition of hybridisation: a description of difference between European and domesticated polecats

Pelage. Domestic ferrets are either albinos, having pink eyes and cream coats, or have similar pelage to polecats but are generally lighter in colour with broader facial markings (the latter are more often called polecat ferrets). The darkest forms may be indistinguishable from wild polecats (Blandford & Walton 1991; J. Birks pers. comm.). Pitt (1921) found all hybrids of albino ferrets and European polecats in the F1 generation had the characteristic, dark, polecat markings, except (subjectively perhaps) in having more whitish underfur. Continued back crossing of hybrids to albino ferrets led to what appeared to be a 'washing out' of the overall pigmentation due to a general increase of cream coloured fur (Pitt 1921).

A red or erythristic variety of polecat occurs when the black pigment of the guard hairs is replaced by a reddish one, this is thought to be through natural mutation rather than introgression of domestic genes. Red forms occur in western Russia (Timofeef-Ressovsky 1940) and Wales, detailed in Blandford (1987). Variation or increase in the amount of white hairs is considered indicative of hybridisation (Walton 1968; Blandford 1987).

There is some support for the suggestion that populations of feral ferrets will revert in time to a darker coloration, as longer (25 years+) established island populations of ferrets are darker in colour than those on the more recently colonised islands in Britain (J. Birks, pers. comm).

Behaviour and ecology. Difference in behaviour between captive polecats and ferrets is described by Pitt (1921) and Poole (1972, 1973). Polecats are more nervous, more cautious in exploration, more aggressive and more readily emit musk, than the domestic ferrets. Recent increases in rabbit populations in many areas of Britain (Trout, Tapper & Harradine 1986) and the likelihood that the effects of myxomatosis on rabbit populations will diminish further (Ross & TintENSOR 1986) will probably affect both the spread of polecats and the ability of ferrets to survive in the wild (J. Birks pers. comm). Wild polecats appear to be less resistant to disease in captive situations (Pitt 1921).

Morphological. There is little morphological distinction between either species of polecat and ferrets. To try and discover which polecat the ferret was domesticated from, Ashton & Thomson (1955) investigated colour patterns and skull measurements of polecats and ferrets, incorporating the character differences which had already been noted by previous authors (Miller 1933; Pocock 1936; Ellerman & Morrison-Scott 1951). The ferret specimens were found to be more similar to the Asiatic polecat than the European polecat in only two characters: the proportion of individuals in which the frontal (white) band was continuous, and the degree of constriction of the post-orbital region of the skull (Ashton & Thomson 1955). Although their work was inconclusive, they suggested sub-specific status for the degree of difference found between any of the polecat forms, and that British ferrets appeared to be more like the European polecat than the Asiatic. In the ferret, brain, bulla, orbita and dentition are reduced (Pocock 1936; Rempe 1970), which is similar to findings in other domesticated animals (Kruska 1988). Rempe (1970) suggested that the post-orbital constriction, which had been used to imply similarity to the Steppe-polecat, was a result of the changes due to domestication and not phylogeny.
Since completing this review the authors have been made aware of a comparative study of the effect of domestication on skull morphology of polecats, ferrets and feral ferrets (Burrell 1981).

Genetic. The karyotype of the ferret is identical to that of the European polecat and different to that of the Steppe polecat, although a hybrid with an intermediate karyotype has been reported (Blandford 1987).

Sperm structure. The sperm structure of black-footed ferret, Steppe-polecat, domestic ferret, and a hybrid between the last two, was investigated (Van der Horse et al 1991). Scanning electron microscopy made it possible to describe distinct differences between the three species and the hybrid, although the hybrid sperm was of an intermediate nature. The authors suggest that although there is virtually no information on sperm structural differences of closely related mammals, sperm might be a fairly sensitive taxonomic indicator and useful in hybrid studies. Friend (1936) found it was possible to recognise any species of British murid rodent on sperm alone.

4.4.4 Current research

Expansion of the polecat out of Wales and into the English Midlands is being investigated by J Birks from English Nature, in a three year project funded by VWT. Although hybridisation is not a primary part of the investigation, the potential for hybridisation is acknowledged. Polecat and ferret specimens are collected by A Kitchener (Royal Museum of Scotland) for morphometric studies and funding is being sought for a genetic study of this material by Leeds University (H Griffiths, pers. comm).

4.4.5 European perspective

The continental range of the polecat has expanded in recent decades (Youngman 1982). The spread of the polecat into Finland since the 1860s has been documented, and it is now common in southern and south-eastern parts of the country (Skaren 1979). No information on hybridisation with ferrets as a conservation concern has been uncovered, as yet, in Europe.

There have been reports of polecat and mink crosses occurring in wild populations in Finland and France, with the suggestion that hybridisation with polecats has been a factor in causing the decline of European mink (Mustela lutreola) (Granqvist 1981). There has been much research into polecat and mink hybridisation in captivity for the purposes of fur farming in Russia (e.g. Grafodatstkiii, Ternovskaya & Ternovskii, 1985). Farmed hybrids, the inevitable escapees, could pose a considerable threat to the survival of the European mink. Romanov (1993) cautions against recent suggestions that either hybridisation with polecats, or matings between American mink males and European mink females in the wild (the embryos are reabsorbed but because of delayed implantation the female is not able to re-mate that year) are a root cause of the decline of the European mink. Hybridisation, or attempted mating, in the wild between American mink (Mustela vison) and polecats in Britain is unknown, as yet.

4.4.6 Protection

Polecats, Mustela putorius, are protected under Schedule 6 of the Wildlife and Countryside Act 1981. If ferrets are considered to be the domestic form of M. putorius putorius, and therefore Mustela putorius furo then, as the law stands, they would be granted the same protection.

Because of the extent to which ferrets are used for rabbiting in most areas of Britain, the genetic integrity of polecats may be under threat, particularly in those areas where polecats are expanding their range. It will be important to advise the public as to the distinction between the two, to gain recognition of the polecat outside Wales. The ability to distinguish hybrids will be important in this respect. The spread of native polecats, rather than hybrids, may be encouraged by actively discouraging the release of ferrets or polecat/ferrets into expanding polecat areas. Unless doubt is cast on the genetic integrity of the polecat population in Wales as a whole (as it has been for wildcat in Scotland), the captive breeding of polecats and polecat cross

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ferrets would gain known hybrid material for study.
5. Hybridisation in other British species

Hybridisation has been documented between various species of birds and fish in Britain and a brief outline of occurrence and current studies in these two groups of animals is given below.

5.1 Birds

There are many examples of hybridisation between bird species or sub-species in Britain. Most are reports of occasional sightings, but there have also been several studies examining hybrid survival and the mechanisms which usually prevent hybridisation. A summary of hybrid occurrence and previous studies are given below, and current studies are outlined.

5.1.1 Occurrence of hybrids

Reports of hybrids species of waterfowl are not uncommon (M. Marquiss, B. Hughes pers. comms.; Bengston 1968). Hybrids of ducks in the genus *Aythya* (pochard (*Aythya ferina*), tufted duck (*A. fyligula*), ferruginous duck (*A. nyroca*), and scaup (*A. marila*)) were described in detail by Gillham, Harrison & Harrison (1966) from specimens and field observations. Reports are, however, sporadic and do not appear to involve significant numbers of the overall populations of the birds. Greylag geese (*Anser anser*) (the only goose native to Britain) can hybridise with the introduced Canada goose (*Branta canadensis*), and there are sympatric populations in many areas, with occasional hybrids reported. Hybrids are often noted in association with escaped exotics, or vagrants, for example the only record of a great northern diver (*Gavia immer*) breeding in Scotland was with a black throated diver (*G. arctica*). Lack of a conspecific mate, and "making do with the next best thing" (Tinbergen 1953) is commonly suggested to account for sporadic interspecific pairing.

Hybrids also occur sporadically in other taxa, for example capercaillie (*Tetrao urogallus*)/black grouse (*Tetrao tetrix*) (Thom 1986), and herring gulls (*Larus argentatus*)/lesser black-backed gulls (*Larus fuscus*) (Harris 1970). There is a zone of hybridisation between carrion and hooded crows (*Corvus corone corone* and *C. c. cornix*) running north/south in Scotland, with hooded crows on the west coast and carrion on the east. The position of the zone is in flux, and thought to arise from secondary contact (Cook 1975). The hybrid zone for the crows also extends from Germany to the Alps (Coombs 1978). Studies in areas of overlap of the two in north-western Italy have suggested that both low fitness of hybrids and some differences in courtship maintained the distinction (Rolando 1993).

5.1.2 Studies of Aythya hybrids

Pairing between scaup and tufted duck was investigated by Bengston (1968). He found that most mixed pairs comprised a drake tufted duck and a female scaup. The tufted ducks formed pairs and bred earlier than the scaup, and so he hypothesised that unpaired male tufted ducks would more easily find unpaired female scaup, than male scaup would find female tufted ducks. To account for the mixed pairs in the first place he suggests a combination of factors: similar display of the two species, the unequal sex ratio (excess of males also occurs in other duck species), and the timing of pair formation. Wild hybrids of tufted ducks and pochard have been similarly explained (Bezzel 1960).

5.1.3 Cross-fostering and misplaced eggs

Herring gulls (*Larius argentatus*) and lesser black-backed gulls (*Larus fuscus*) are often quoted as an example of the 'Ring species', a chain of circumpolar populations, whose links show a clinical variation. In Western Europe the two gulls are sympatric, yet appear to hybridise infrequently even in mixed colonies, despite their wide ecological and behavioural overlap (Harris 1970). One striking difference between the two is that *argentatus* in Britain is large sedentary and *fuscus* is a migrant. However, cross-fostering experiments demonstrated that of the birds recovered, the cross-fostered *fuscus* had migrated as normal, but that the *argentatus* had migrated like their foster-parents (Harris 1970). After the cross-fostering, high numbers of mixed pairs occurred over a number of years, with a
strong suggestion that all birds in mixed pairs had been cross-fostered. Hybrids of these mixed pairs were later identified at breeding grounds, but their rarity, compared to the conspecific controls, suggested selective disadvantage of hybrids. (Harris, Morley & Green, 1978).

A three year study (Porter 1976) suggested that the species were usually reproductively isolated by differences in mantle colour and voice, acting chiefly through the mate choice of females, but males also to a certain extent preferred conspecifics. This supported the results of Harris (1970) that mate choice could be imprinted. Mate choice by imprinting has been documented for male ducks (Schutz 1965) and mixed pairs achieved by egg-switching in flycatchers (Lohrl 1955) and house sparrow (Cheke 1969). Thus, misplaced eggs in the wild can lead to mixed pairs and account for hybrids (Harris 1970).

5.1.4 Conservation questions and issues

5.1.4.1 Ruddy ducks and white-headed ducks

Feral breeding of North American ruddy ducks (Oxyura jamaicensis) was first recorded in Britain in 1960, amongst escapees from The Wildfowl and Wetlands Trust in Slimbridge. Numbers were estimated at 3,150 by 1990. A three year study suggested that these ducks were having no obvious detrimental effect on the British fauna (Hughes 1992). However ruddy ducks have since been reported in another 11 European countries, and in those where the endangered white-headed duck (Oxyura leucocephala) is present, they present a serious threat to that species. The white-headed duck has undergone a widespread reduction in world numbers. In Spain, following extensive conservation measures, there was an increase in numbers from 22 in 1977 to 786 in 1992 (Anon. 1992). Ruddy ducks, however, started to colonise Spain, and hybrids between the two species were noted in 1992. Ruddy ducks appear to be very successful colonisers, and complete introgression of the small populations of white-headed ducks has been postulated (Anon. 1993). Campaigns to eradicate ruddy ducks from Spain have started, and pressure put on British authorities to reduce their feral populations. Captive breeding at Slimbridge has confirmed that both first and second generation hybrids are fertile and there have been reports from Spain on first, second and possibly third generation crosses in the wild (B. Hughes pers. comm.).

5.1.4.2 Scottish Crossbills

At present there are four potential interpretations for the presence of a large-billed form of crossbill in Scotland

i That the Scottish crossbill is a distinct species, derived long since (Loxia scotica).

ii That it is a small-billed (island) subspecies of the parrot crossbill Loxia pytyopsittacus.

ii That it is the result of the ongoing selection for the larger-billed individuals of the common crossbill Loxia curvirostra i.e. that common crossbills with stronger bill morphology do well in pine forests and therefore stay behind as smaller-billed birds move out to a better food supply when the ripe seed is past (Marquiss & Rae, in press).

iv That it is the result of hybridisation between curvirostra and pytyopsittacus.

There is little in current published evidence to distinguish between these alternatives because there is no distinct bimodality for bill size. The depths of bills of birds caught on Deeside and Speyside vary from those typical of European caught curvirostra to those that are so large that they could be pytyopsittacus - with many birds lying between (Marquiss, Rae & Duncan 1992; Marquiss & Rae in press; Marquiss & Rae unpublished). Measurements of skins from the Royal Museum of Scotland also found a great diversity of bill sizes with indistinct bimodality, specimens with very small bills labelled as scotia and a specimen with a moderate sized bill labelled as pytyopsittacus (Marquiss, Jardine, Summers, McGhie & Proctor unpublished).
To investigate whether genetic difference is apparent between the two forms, (or between continental and British birds) and whether assortative mating or hybridisation occurs, a DNA study has recently been started (collaboration between Summers, Marquiss & K Sullivan, Dundee Institute of Technology).

5.1.4.3 Introduction of different races of Goshawks

The goshawks (Accipiter gentilis) now breeding in Britain are largely, if not wholly, derived from imported birds which escaped from hawk keepers or which were deliberately released (Marquiss 1981). Wild birds established in the 1960's stemmed mainly from the small forms from Central Europe, whereas those established in the early 1970's were larger and paler, mainly originating from Finland (Marquiss 1981). Mixing of the two stocks may now occur, as the populations of wild birds are approaching one another in places (Marquiss 1994). It is not known whether one phenotype will eventually dominate, or whether the two will remain discrete, or whether an intermediate form will develop.

5.2 Fish

Hybridisation between salmon (Salmo salar), trout (S. trutta) and farmed fish has recently received much attention through the potential problems associated with both unintentional and intentional releases of farmed fish into rivers and lochs.

Despite big difference in chromosome number between salmon and trout (Phillips & Hartley 1988), and lowered viability and fertility of crosses in captivity (Chevassus 1979) many studies have found hybrids (by typing using gel electrophoresis) in wild populations. Unlike mammals, fish can show large variation in protein alleles, both in frequencies and unique occurrences between species (Karakousis & Triantaphyllidis 1990). High natural levels of hybridisation between Atlantic salmon and trout were found in juvenile salmonid populations in south-west England, 1.2 and 2.0% respectively, (Hurrell & Price 1991). High levels of hybridisation have been connected with introduced populations and disturbance. In Newfoundland where brown trout were introduced, rates of hybridisation were higher in comparison to those in Europe where trout are native and relatively undisturbed (Verspoor 1988c). Rates of hybridisation in Spain, where extensive stocking of non-native salmon occurs, are 20 times higher than elsewhere in Europe. Interbreeding between anadromous and nonanadromous Atlantic salmon in Newfoundland was found not to occur where the two forms were in sympatry naturally, but did occur when anadromous salmon were introduced into an area formerly occupied only by nonanadromous salmon (Hutchings & Myers 1985). Hybridisation of salmon stocks has been connected with decreasing returns of ranched salmon (Bailey 1987). Verspoor (1989) suggests that it is too early to draw conclusions on the effects of releasing fish of a different stock or species in the water systems, but there is a need for concern. Hybridisation rates have been connected with disturbance of populations and extreme environmental conditions (Garcia de Leaniz & Verspoor 1989) and competition for limited spawing areas has also been postulated (Hurrell & Price 1991).

The effects of interbreeding between local populations of wild fish and farmed stock is difficult to assess. The level of genetic variability of Atlantic salmon in culture is often lower than that present in wild fish (Stahl 1983; Verspoor 1988; Youngson et al. 1989). Genetic differentiation between cultured and wild stocks would also seem to be common, and a positive association between genetic variability and fitness has been found in many species (Allendorf & Leary 1986). Genetic differentiation between farmed stocks and the wild stocks from which they are derived, is probably due to random changes caused by the use of small numbers of founding broodstock (Verspoor 1988).
Evaluating the Threat from Hybridisation

There are two perceived threats from hybridisation:

1. Reduction of viability through outbreeding

2. Incorporation of undesirable genes which change the character of the species, or lead to the complete introgression and loss of the species.

Inbreeding and out-breeding

When population numbers are low there are deleterious effects from inbreeding - by expression of homozygous recessives (Rails & Ballou 1983). Additionally, heterozygous advantage is often postulated. Although few studies have proved heterozygotes to be superior to homozygotes among populations in general (Nei & Chesser 1983) there have been studies showing support for heterozygote advantage, including survival rates, disease resistance, growth and development rates (Allendorf & Leary 1986). For these reasons, the mixing of subspecies which have reached very low numbers or which exhibit very low genetic diversity, such as the Florida panther or the African cheetah (Acinonyx jubatus) has been suggested as positively advantageous rather than disadvantageous (O'Brien, Wildt & Bush 1986; O'Brien & Mayr 1991).

Outbreeding depression between subspecies has also, however, been demonstrated, when animals are introduced to a managed population from a distant or captive stock (review in Templeton 1989). The degree to which populations have to diverge before outbreeding depression becomes important is not known and may vary between species (Amos & Hoelzel 1992). An often quoted extreme example is the Tatra Mountain ibex (Capra ibex) (Groombridge 1992). The local ibex in Czechoslovakia was hunted to extinction and re-established using Austrian stock. Subspecies from Turkey and Sinai were added to the herd two years later. The ibex now rutted in early fall and calved in February in contrast to the local ibex which had calved in the spring. The cold weather killed the kids and the entire population went extinct. Low survival of hybrids in hybrid zones may be due to naturally occurring outbreeding depression. Outbreeding between European mink and American mink, which although produces no progeny, results in the loss of a reproductive season for the European mink female, has been speculated to be involved in the decline of European mink, although there is no good data to support this theory (Romanov 1993).

Incorporation of undesirable genes

The introduction of exotic species has been found to lead to hybridisation, and the possibility of introgression of non-native genes into native species. In New Zealand the introduced mallard (Anas platyrhynchos) has hybridised with the native grey duck (Anas superciliosa), such that there is concern over the number of pure grey ducks remaining (Gillespie 1985). Mallard success was thought to be due to the newly created agricultural habitat (Gillespie 1985). In North America, introduced mallards hybridised to such an extent with Mexican ducks (Anas diazi) that all 'Mexican' like ducks are now considered hybrids and the species lost (Greig 1980). Hybridisation also occurs between domesticated strains of free-living animals and their wild counterparts. Wolf populations in Italy are very low and the greatest threat to their continued existence is thought to be interbreeding with domestic dogs (Boitani 1992; Blanco, Reig & de la Cuesta 1992). In Australia, dingoes have been persecuted for a long period of time, and low population numbers of dingoes coupled with the increase in feral domestic dogs in settled areas, may have led to hybrids becoming commonplace in south-eastern Australia (Newsome & Corbett 1985). Wildcat populations are low and scattered throughout much of Europe and hybridisation with domestic cats is thought to occur in many areas, and is perceived as a threat where populations were or are low. Population numbers of the wild form are therefore the key factor in the relation between the concern over the occurrence of hybridisation and the extent to which introgression of domestic types into the wild populations takes place.
Domesticated animals could be argued to be less fit in the wild and therefore introgression of domestic genes is unlikely to occur in large, well-established animal populations. If domestic genes have pleiotropic effects, such as resistance to disease (diseases possibly originating from large domestic or feral populations), or increased tolerance of individuals to one another, such that they can live in higher densities, where they are tolerated and aided by man, then suites of domesticated characters might establish in wild populations.

Hybridisation also occurs between distinct native populations and distinct species. In North America, wolves and coyotes have hybridised in areas in which persecution of wolves coupled with habitat changes favouring coyotes have led to low and fragmented wolf populations and an influx of coyotes (Lehman & Wayne 1991; Wayne et al. in press). Habitat change, either the result of climate change or anthropogenic factors has led to the spread of white-tailed deer into ranges previously occupied by mule deer, with hybridisation now occurring (Carr et al. 1986; Conin, Kyse & Cameron 1988). Natural hybrids have also been found between endangered species such as blue whales and fin whales (Arnason et al. 1991).

Does hybridisation and the threat of complete introgression suggest that the species is no longer valid as a distinct unit, worthy of conservation? The definition of a species taken from Barton & Hewitt (1989), as "a cluster of phenotypes that is stable to the invasion of foreign genes", could be modified to include the two important factors which render a species vulnerable to hybridisation, habitat and population numbers. Thus a species is a cluster of phenotypes that is stable to the invasion of foreign genes in its natural habitat and given an adequate population size. If there is no habitat anywhere in the world in which one form cannot be introgressed by another then, by this definition, they would be subspecies. However if the distinction between the two is a function of habitat and population numbers, then only the manipulation of these two factors will protect the two forms as separate species. In discussing the control of animal populations, Caughley (1977) concludes thus "The treatment of a population by changing to its detriment the key components of its habitat is the most powerful and elegant technique of population control". Thus he advocates management of the species through management of the habitat. The same would appear to be true to mitigate hybridisation, given an adequate population size of the endangered form.
Discussion and Conclusions

Examples of the genetic integrity of species being threatened by hybridisation are found worldwide. However, hybridisation not only results from the introduction of an exotic, closely related species, or the introduction of a domesticated form of the wild species; hybridisation also occurs between naturally occurring sympatric populations of animals which had been considered to be distinct native species, and may therefore play an important part in evolution. The link between the examples appears to be some man-induced perturbation of the previous balance, such that low population numbers or changed habitats alters the stability of the species involved.

We conclude that the two mammals native to the United Kingdom that are at greatest risk from hybridisation and subsequent loss of native type are the wildcat and the red deer. However, hybridisation itself is not, perhaps, the cause of the problem, but the symptom. Hybridisation underlines the message that species cannot be conserved in isolation from their ecosystems. Preservation of genetic diversity is misleading as it has us searching for ways and means of proving how genetically unique a species is, or proving its species rather than subspecies status, in an effort to gain for a population a greater 'right' to protection. The potential for hybridisation to occur emphasises the fact that species, subspecies and races are not static units which can be preserved in zoos, and that a changing environment will eventually change a native species as much as introgression with a closely related population does.

Mitigation is difficult. Clearly, preventing introductions or the movements of 'undesirable' genotypes throughout the country will help. (The deliberate release of sika deer is now prohibited under the Wildlife and Countryside Act.) However, in both the cat and deer cases, it is likely that spread can only be delayed rather than prevented for all time. Refuges, such as islands or peninsulas could be created and selective killing of 'undesirable' types could be attempted in the few areas where this may be possible.

The best way to ensure the survival of native red deer and wildcat, and all other native species, of course, would be to follow a land use strategy which protects and conserves the ecosystem which gave rise to the native flora and fauna of Britain, and made it unique in the first place. A species does not exist outside of a habitat; if the habitat is modified then the species will start to change and evolve to suit a new set of conditions. Thus the native, genuine, 'real' wildcat or red deer are only as native, genuine and 'real' as the habitat in which they are presently evolving. Manipulations of food supply as well as habitat will also allow an introduced form to compete favourably with the wild type. For example feral cat numbers may depend on a food supply associated with man and on high rabbit populations (rabbits being an introduced species and reaching high numbers according to farming practices).

Required action depends on philosophical argument that need to be resolved before proper management can be implemented. That is, does hybridisation or mixing of different gene pools, lead to increased genetic diversity, or hybridisation leads to the loss of distinct gene pools. Following from the latter it is necessary to determine whether wildcats and red deer are, in fact, discrete, accepting that hybridisation in cats has likely taken place already and there has been so many introductions of 'foreign' red deer into the native Scottish population. We recommend that studies are continued, using modern, molecular techniques, to investigate the variability within cat and red deer populations and to determine their distinctiveness, and that ecological and behavioural studies follow on from the results of the genetic studies.

It is for the statutory agencies to define the phenotype of animal protected, although governments should recognise that even using the best methods available to find a legal definition for a species, the definition will contain a degree of subjectivity.
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