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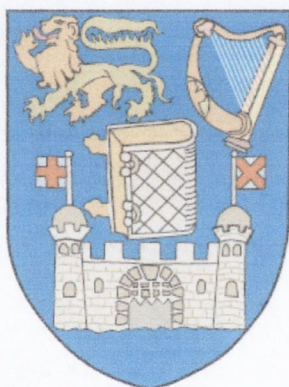
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AN INVESTIGATION INTO THE ECOLOGY OF THE
PINE MARTEN (*MARTES MARTES*) IN
KILLARNEY NATIONAL PARK.

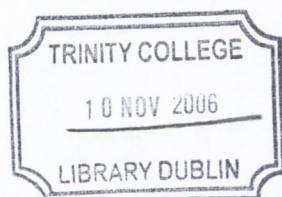
ÁINE B. LYNCH



TRINITY COLLEGE DUBLIN

A thesis submitted in the fulfilment for the Degree of Doctor of Philosophy to the
University of Dublin, Trinity College.

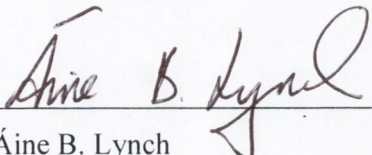
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DECLARATION

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Áine B. Lynch

SUMMARY

The majority of the research for this thesis was carried out on a reestablished population of pine marten in Killarney National Park (KNP). I explored the success of the translocation and used distributional, biometric and reproductive data as the criteria by which to measure that success. I also examined the diet of the pine marten in the park. Two novel methods for monitoring pine martens populations were also investigated (hair traps and the use of the SRY gene to identify sex from non-invasively collected samples) and the results of both these chapters have been published. Finally, I examined the current level of genetic diversity within the pine marten population in KNP compared to the source population from which the translocated animals were derived and to samples taken from animals located throughout the countryside. In addition I gave an evaluation of the usefulness of the genetic markers used in this study for describing different aspects of the molecular ecology of the pine marten.

The re-establishment of pine marten in KNP has been successful. Live trapping of animals discovered individuals belonging to all age cohorts, and furthermore many of the adult animals caught were found to be reproductively active. Adult males and females were sexually dimorphic with males significantly larger and heavier than females. Thus the criteria for a successful translocation were met. Though there was an observed female bias in the trapped population (which normally shows a male bias), this was ascribed to the trapping methodology and was not considered as an indication of an unhealthy population. The diet of the pine marten in KNP was similar to that recorded by other studies in Ireland and on other islands (Minorca), with all components being consumed more or less equally and with a wide niche breadth, but different to studies from continental Europe and Scandinavia where small mammals, particularly vole *spp.*, are the backbone of the diet throughout the year with the remaining components of the diet taken seasonally. The bank vole, a previously unrecorded item in the diet of pine marten in Ireland, was consumed as frequently as the woodmouse. Though a wide niche breadth may be considered a sign of ecological stress, in this study it was taken to reaffirm the generalist opportunistic nature of the pine marten's diet. The microsatellite markers used in this study proved problematic and were found to be unsatisfactory despite being used previously to study pine martens. Loci were found to be out of Hardy Weinberg Equilibrium and one locus showed linkage disequilibria. I would suggest that specific microsatellite markers be developed for the pine marten. I did, however, detect a significant decrease in the level of genetic diversity between KNP and the remaining

Irish samples. I could find no evidence to suggest pine martens were still present in KNP prior to the translocation which was also supported by the mitochondrial DNA. As similar mitochondrial DNA patterns were found for the Irish pine marten as the Scottish pine marten then this supports the hypothesis that the Irish pine marten was a post glacial coloniser.

An assessment of hair traps as an effective method for monitoring pine marten populations showed that animals were detected as quickly using hair traps as they would be by scat location or by live trapping and that hair traps were particularly useful in remote areas with no paths where the location of scats was difficult and live trapping was impossible. The use of the hair samples retrieved from the hair traps in DNA studies was possible even if samples were left in traps for up to 28 days. It was found that for the purpose of molecular studies DNA from at least three hair follicles was required for successful amplification. Springs routinely retained more than 10 individual hairs (approximately 55%) and those hairs retained were from the one individual, thus hair traps provide a viable approach to molecular assessment of pine marten populations. Sex primers developed for the otter were useful for identifying sex in pine martens and together with the non-invasive hair samples resulted in an additional five individuals being identified in KNP.

Overall the investigation into the population structure of the pine marten in KNP shows the population has re-established itself successfully. Furthermore my results have illustrated two new techniques for monitoring those populations. Though the population in KNP appears healthy, the reduction in heterozygosity may have serious consequences further along the line in terms of fitness and as such the monitoring of the population should be continued. A reassessment of the genetic diversity of this population and the entire island should be carried out should specific microsatellite markers be developed to observe whether the current pattern is true or an artifact of the methodology.

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And of course the pine martens, especially you Lucy!!!

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1. 1. Introduction

A quarter of the world's mammal species and a greater percentage of their populations are considered at risk from extinction owing to a combination of rarity, anthropogenic impacts and endemism (Ceballos *et al.*, 2005). Habitat destruction and fragmentation caused by human activity is one of the greatest threats to global biodiversity and for mammal species with restricted ranges or which are confined to a few habitat types, fragmentation poses an especially great threat (Bright, 1993). Only 8% of the world's mammals are restricted exclusively to hot spots, with 62% shared between hotspots and other areas. The remaining 30% of mammal species have ranges which occur outside these 'hotspot' areas, but many have small ranges (76% have a range < 1 million km²). Despite 95 % of all mammal species having ranges which overlap with at least one reserve listed in IUCN/UNEP (World Conservation Union/United Nations Environment Programme) world database of protected areas much of the ranges of mammal species occur outside protected or priority areas and as such overlap with areas used principally by humans (Ceballos *et al.*, 2005). It is imperative upon ecologists, in the face of limited resources, to gather information which will allow managers to make effective decisions regarding the conservation status of species or groups of species within landscapes used by humans (Zielinski & Kucera, 1995). It is not possible to redress declines in mammal populations unless the cause of the problem is known. Furthermore knowledge of species typical habitat requirements is essential as the habitat needs of rare or endangered species are often derived from areas of current distribution, which may not be the optimal habitat and thus lead to erroneous conclusions (Bright, 2000).

Thus many of the challenges associated with conserving mammal populations will not occur within protected areas but within the remainder of the landscape used by humans and principally those areas under agriculture. Ceballos *et al.* (2005) show that agriculture and human density are good estimators of biodiversity loss but that as many of their areas selected as priority for conservation have not been overly effected by agriculture (< 25% area has been altered from natural vegetation to agricultural vegetation in many cases) then they suggest there is room to reverse some of the damage which has been caused. Conversely those areas (South America, Africa and Asia) most affected by conversion of the natural vegetation to agricultural produce also tend to be areas with the highest number of mammal species and thus habitat loss poses

the greatest threat to species loss in these areas. It is also important to realise that areas designated as good for mammals, would not necessarily be so for another group of species and as such we cannot preserve biodiversity solely within protected areas.

Thus the conservation of mammal species globally will not be dependent exclusively on what occurs within protected areas but how we manage the landscape between protected areas (Bright, 2000). Hence schemes such as the Rural Environmental Protection Scheme (REPS) in Ireland, in addition to legal protection and protected areas, not only promote awareness to those most involved with altering the natural landscape but provides incentives to maintain land under their management for wildlife or to restore land thus redressing ecological damage (Bright, 2000). Similar schemes have been put in place globally, while other rich nations have also been able to conserve individually endemic mammal species with good results through intensive management, as has been the case in Australia. The challenge in conserving mammal diversity and indeed all biodiversity is daunting because of the great differences in the ability of nations to protect organisms within their borders, with mammals in poorer or politically unstable nations most at risk (Ceballos *et al.*, 2005).

Many individual species have benefited from conservation measures taken to readdress declines in their populations. For example the black footed ferret, reduced to 11 individuals, was taken into captivity and is now numbered at over 200 (Wisley *et al.*, 2002). Despite numbers having recovered, this population has not been successfully returned to the wild, though efforts are still being made to do so. Other species benefit from being protected from harvesting, for example the Newfoundland marten population which has recovered significantly once harvesting ceased (Kyle & Strobeck, 2003). As a group the challenge in protecting carnivorous mammals will always lie in gaining support from certain human interest groups opposed to their presence, such as farmers and field sportsmen while trying to overcome land constraints particularly with the more wide ranging species (Bright, 2000). Thus as a tool wildlife protection can be useful for aiding the recovery of species or groups of species once the threat or threats facing that species in the wild has been recognised and attempts are made to stop or reduce that threat.

The challenges associated with conserving mammal species belonging to the mustelid family, resource limitation aside, lie in overcoming many of the life history traits and

characteristics that make them difficult to study: wide ranging, low density, elusive, nocturnal, intelligent etc., (Messenger & Birks, 2000). Many of the species within the Mustelid family are impacted upon by man's activities, be it through water quality, habitat fragmentation, commercial use of forests and forestry practices, harvesting, road development, other land use practices (agriculture, mining, recreation), disturbance and man's perceptions of their threat to human commerce (O'Sullivan, 1983; Brainerd, 1990; Adamowicz & Condon, 1997; Prolux, Bryant & Woodward, 1997; Bright 2000). Not all of these practices are detrimental to populations, for example limited felling of forests produces increased edge effects thereby promoting small mammal densities, a principal prey item for martens (Hargris & Bissonette, 1997). The same threats face species in Ireland, with all mammal species including the mustelids facing similar challenges, though all are free from persecution (hunting or harvesting) except the badger (*Meles meles*), which is suspected in the transmission of tuberculosis to cattle, and the introduced American mink (*Mustela vison*) (Hayden & Harrington, 2000).

The genus *Martes*, to which the pine marten (*Martes martes*) belongs, has been extensively exploited over the centuries. The several species which belong to this genus (see section 1.2), are typically associated with undisturbed mature semi-natural forests (primarily coniferous or sub tropical) (Anderson, 1970; Brainerd, 1990; Brzeziński, 1994; Genovesi, Secchi & Boitani, 1996; Kyle & Strobeck, 2003; Vinkey, 2003) and are still harvested in some parts of their distribution for their pelts, which are considered valuable (Brzeziński, 1994; Adamowicz & Condon, 1997). In many countries, conservation awareness of these species has increased, with much research effort being placed on the requirements of these animals in commercially managed landscapes and on how to monitor populations effectively in order to assess trends (Zielinski & Kucera, 1995; Prolux *et al.*, 1997; Messenger & Birks, 2000).

Despite a good tradition of research and mammal monitoring in the UK, many species, the pine marten included, have no effective method for monitoring trends in their populations, either at regional or national levels (Harris & Yalden, 2004). Ireland is many years behind the UK in terms of mammal research, with no national biological records centre or tradition of voluntary mammal organisations. The fact that the pine marten is one of the few species to benefit from total protection under the Irish Wildlife Act (1976/2000) has not led to research being focused on this species. Since 1970 the main areas of research thus far have been predominantly on diet (Fairley & O'Gorman,

1974; Fairley, 1970; Fairley, 1975a; Warner & O'Sullivan, 1982; NíNeachtain, 1998), with one known study of distribution (O'Sullivan, 1983) and population structure (NíNeachtain, 1998). More recently genetic studies have been carried out primarily focusing on identifying the pine marten from other sympatric species (Statham, Turner & O'Reilly, 2005), or as part of larger scale projects investigating the European pine marten (Davison *et al.*, 2001; Kyle, Davison & Strobeck, 2003).

1.2. Taxonomic Status

The pine marten is a member of the family Mustelidae (Order Carnivora) which contains five sub-families, the Melinae (badgers), Mellivorinae (honey badger), Mephitinae (skunks), Lutrinae (Otters) and Mustelinae (weasels, martens, polecats) (Woodroffe, 2001). Within the Mustelinae the pine marten belongs to the genus *Martes*. Although specialisation occurs within the family Mustelidae to cope with particular modes of life e.g. aquatic otters (*Lutra lutra*), the same cannot be said of the genus *Martes*. Though adapted for a wide variety of environmental conditions throughout their range (boreal coniferous forests to tropical rain forests), the species within this genus have retained many of the primitive characters seen in the early mustelids (relatively small size, flesh eating dentition and generally unspecialised habits), and thus are thought to be very close to the basal stock (Anderson, 1970). Though the fossil record is incomplete, fossil remains have been found throughout Europe (Langley & Yalden, 1977; Charles, 2000; Sommer & Benecke, 2004; Richter, 2005), but not in Ireland.

The pine marten, the only member of the genus *Martes* present in Ireland, occurs throughout much of Europe (Fig. 1.1; see section 1.3.) Other members of this family are distributed across the northern hemisphere and are divided into three subgenera: *pekania*: fisher (*M. pennanti*) of North America and Canada; *charronia*: Nigilri marten (*M. gwatkinsi*) of south India and yellow throated marten (*M. flavigola*) of south east Asia; *martes*: Japanese marten (*M. melampus*) of Japan, American marten (*M. americana*) of North America and Canada, sable (*M. zibellina*) of Russia and Asia and pine marten and stone marten (*M. foina*) of Europe. More recently, based on genetic evidence, it has been suggested that the American marten is actually two species which restricted to the east and continental North America, and *M. caurina*, primarily occurring along the west coast of America. However where the two meet it is known that they hybridise (Stone, Flynn & Cook, 2002; Small, Stone & Cook, 2003).

It has been proposed that the four species: Japanese marten, American marten, sable and pine marten, exhibit a circumpolar cline of the genus *Martes* (Anderson, 1970). This is supported by the close similarity of their morphology and ecology. Indeed where the pine marten and sable populations meet the two species are known to mate and produce viable young known as kidases (Anderson, 1970). Of late it has been suggested that the pine marten may also hybridise with the American marten, evidence of introgression between the two species being recorded in the U.K. The likely source of the American marten animals is from escapees or deliberate releases from fur farms (Davison *et al.*, 2001). The fifth member of the subgenera *Martes*, the stone marten (also referred to as the beech marten) though sharing similar morphology and ecological traits, is clearly a different species as evidenced by a different dental pattern (Anderson, 1970; Reig, 1992). Furthermore no evidence of hybridisation between the pine marten and stone marten, despite the ranges of these two species overlapping extensively across much of Europe (Marchesi 1989; Genovesi *et al.*, 1996), has been found (Davison *et al.*, 2001).

1.3. Distribution

The group '*martes-zibellina-melampus-americana*' ranges from the west of Europe, through Asia and to the east coast of North America and Canada. The pine marten is replaced by the sable in Russia, which is then replaced by the Japanese marten. The American marten is the only species of this group to occur across North America and Canada (Anderson, 1970). The earliest known occurrence of the pine marten is from the Eemian age beds (130,000 years ago) in Central Europe. This species is believed to have spread throughout the forested regions of West Central Europe and Britain and by post-glacial times to have reached Scandinavia (Anderson, 1970). Based on genetic evidence, Davison *et al.* (2001) suggest that due to the absence of ancient lineages in the pine marten, this species colonised Europe from a single refugium after a recent glacial event. However, Sommer & Benecke (2004) contradict this finding, reporting that fossil evidence suggests at least three glacial refugia (1. Iberian Peninsula, 2. Italian Peninsula, and 3. Moldova) and possibly more.

Today pine martens occur throughout most of Western Europe, with Ireland as its most peripheral group to the west and including Fennoscandia, but excluding some of the southern countries (Fig. 1.1; Kyle *et al.*, 2003). Though populations of pine marten are distributed widely across Europe they are not necessarily abundant. A reduction in numbers across most of their range has been caused by centuries of hunting this species

for its fur and for sport, poisoning as vermin, and habitat loss and fragmentation (Langley & Yalden, 1977; O’Sullivan, 1983; Rudge, 1987; Strachan, Jefferies & Chanin, 1996; NíNeachtain, 1998). Predation by foxes (*Vulpes vulpes*), particularly in Scandinavia, is not thought to be a threat to pine marten populations, but where predation occurs it can severely limit the number of animals in an area (Lindström *et al.*, 1995). The golden eagle (*Aquila chrysaetos*), eagle owl (*Bubo bubo*) and lynx (*Lynx lynx*) are also known to prey on pine martens, but once again are not thought to be significant threats to populations (Brainerd, 1990).

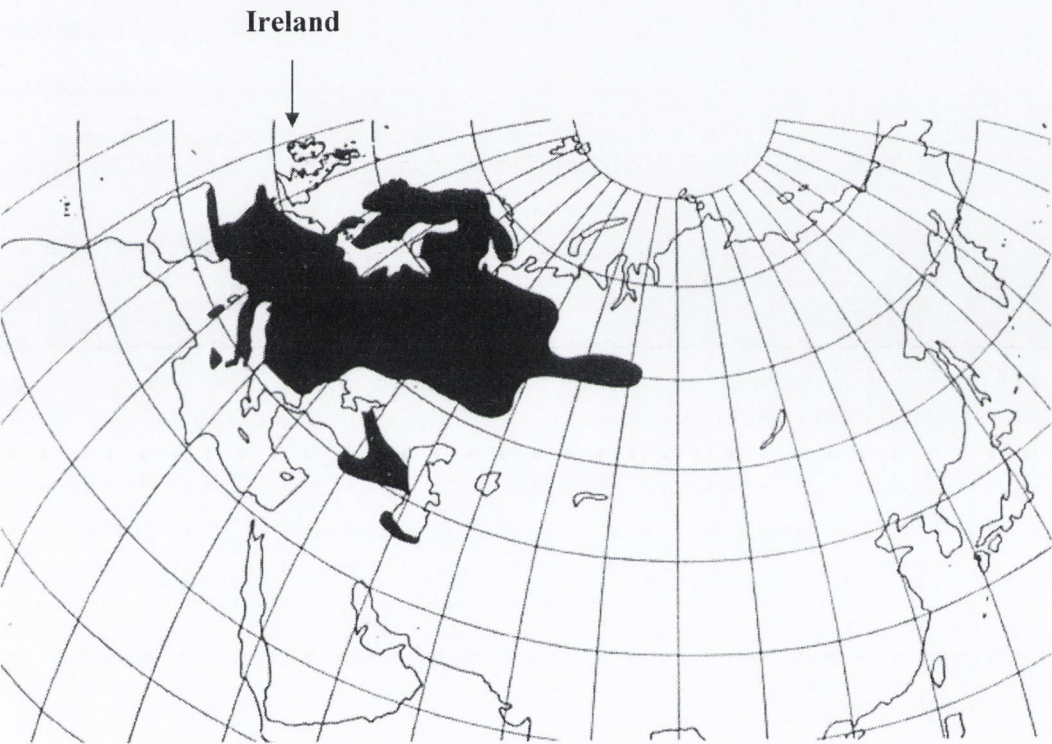


Fig. 1.1. Map of the global distribution of the pine marten, *Martes martes* (from Velander, 1991).

1.3.1. Irish Distribution and Status

Much debate and speculation still surrounds the colonisation of Ireland by its mammal fauna. Several routes have been suggested which include 1) remaining here through the last glaciation in a refugium in the south of Ireland 2) colonisation by a land bridge, 3) accidental introduction and 4) deliberate introduction. Ireland has an unusual mammal

composition, having many more predators (*circa* 10, some now extinct: bear, wolf and wild cat) than would be typically supported by a small prey base (*circa* 5) (Woodman, 1983). Today good evidence exists for a refugium which almost certainly sustained stoat (*Mustela erminea*) and pygmy shrew (*Sorex minutus*) populations and may have supported hares (*Lepus timidus*) (R. McDonald *pers comm*). The argument for a land bridge is supported by the presence of large carnivores such as bears and wolves which are unlikely to have survived through the last glacial period primarily due to climatic constraints and were too troublesome to have been introduced either accidentally or deliberately by man (Woodman, 1983). Though likely to have crossed by a land bridge, the otter could also have swum across while bats would have flown possibly using the same bridge as a corridor. Similar speculation surrounds species like red squirrels, foxes and badgers which could equally have colonised via a land bridge by themselves or been introduced. Others such as rats and mice are likely to have been accidental introductions, arriving with man, while the bank vole is a known recent accidental introduction (Smal & Fairley, 1984).

It is unknown when the pine marten arrived in Ireland, though it is unlikely to have survived through the last glacial period due to climatic restrictions (*circa* 10,000 years ago). Consequently it either arrived on this island soon after the melting of the ice via one of the supposed land bridges (*circa* 10,000 years ago) or, owing to the lack of archeological evidence to support this claim, was brought here by one of the many waves of settlers that arrived on these shores since. Consequently it has been suggested that this species was introduced for its fur (Fairley, 1975b). Man is first thought to have arrived on these shores sometime around 9,000 B.P., with continual contact being kept with Europe through trade (Woodman, 1983). Woodman (1983) notes that a barbary ape (*Maca sylvaus*) was found at a Late Bronze Age dig (Emain Macha, Co. Armagh) and Sleeman (1983) proposes that the 22 species man managed to introduce to New Zealand, all in a short time span, support the idea that introduction via boat to Ireland was possible.

Genetic evidence may support the idea of an introduction or introductions. Low levels of genetic diversity found for the Irish pine martens indicate a small founder population, consistent with introductions, though anthropogenic pressures and island effects are also likely to account for some of this genetic homogeneity (Kyle *et al.*, 2003, Chapter 6). By contrast only one control region haplotype has been found for the Irish marten (N =

34 individual samples sequenced) (Davison *et al.*, 2001; Chapter 6), which may suggest natural colonisation. If man had introduced this species then it is likely several haplotypes may exist within the population, as sources would have surely come from several different populations. Furthermore Irish pine martens have a haplotype which is linked to the Iberian peninsula (Davison *et al.*, 2001) which was supposedly linked to Ireland via a land bridge. A similar pattern to that observed for the Irish pine marten is reported for the Scottish marten (Davison *et al.*, 2001) and archaeological evidence points towards the Scottish pine marten being one of the earliest post-glacial colonisers (Strachan *et al.*, 1996). Despite the ambiguities surrounding the arrival of the pine marten it is considered indigenous to Ireland (Woodman, 1983; Hayden & Harrington, 2000).

Once considered widespread and abundant throughout Ireland, the fate of the Irish pine marten would appear to mirror that of its European counterparts, with a steady decrease over centuries (14-20th) due to persecution and habitat loss (see chapter 3, section 3.1.1.). There is no up-to-date map for the distribution of pine marten in Ireland (Republic), the only census so far being carried out during 1978-1980 (O'Sullivan, 1983; Fig. 1.2.). The national survey indicated that the pine marten's main range was restricted to the western seaboard counties extending north from Limerick to Sligo, with isolated populations occurring at several locations around the country (1. Boyne Valley, Louth; 2. Portlaw, Co. Waterford; 3. Slieve Bloom Mountains, Co. Laois; 4. Gougane Barra, Co. Cork; 5. Killarney National Park, Co. Kerry). In Northern Ireland the last census (1996) listed the pine marten as only occurring in Derry (also on the border with Tyrone), Antrim-Down border, and Fermanagh (Fig. 1.2.; CEDaR Map, Ulster Museum).

Though the same cannot be said for Northern Ireland, in the Republic of Ireland the pine marten would appear to be extending its range and making a come back since the mid 1970's (O'Sullivan, 1983; Smiddy & Berridge, 2002). Several reasons have been suggested to account for this apparent recolonisation of the former range which is believed to coincide with a cessation in persecution and secondarily with an increase in wooded habitat. Firstly, the pine marten has been fully protected since 1976 under the Irish Wildlife Act, making it illegal to trap or kill this species, even in the face of damage to livestock, without a licence. In the latter case a licence must be obtained first and not in retrospect. The protection afforded the pine marten was further extended in

2000 under an Amendment to the Wildlife Act (1976, 2000), which now makes it illegal to disturb its den (this includes photography or video) without a licence. Secondly, the pine marten also occurs on Annex V of the Habitats Directive (a species of special interest to the European Union), which is the driving force behind wildlife legislation in the Republic of Ireland and also is on Annex III of the Berne Convention (requiring that it be monitored). Thirdly, the use of strychnine poison was banned in 1991. This poison was used for predator control by landowners protecting livestock, and pine martens who will readily consume carrion were thought to have died after feeding at baited carcasses. Foxes and dogs (*Canis familiaris*) were the main targets of this campaign (O'Sullivan, 1983). Lastly the increase in land covered by commercial forestry plantations is thought to be facilitating the spread of the pine marten, thereby providing an increasing amount of favourable habitat (O'Sullivan, 1983).

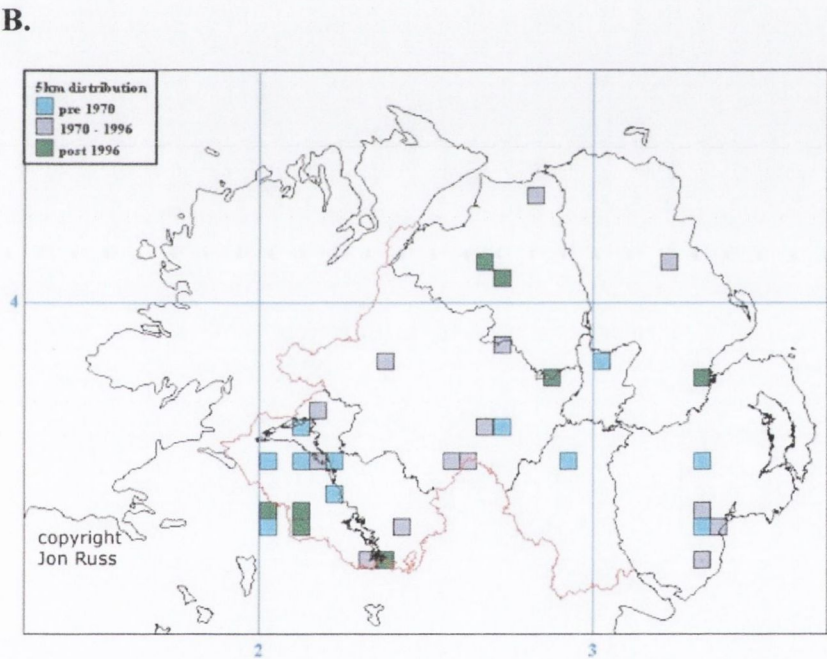
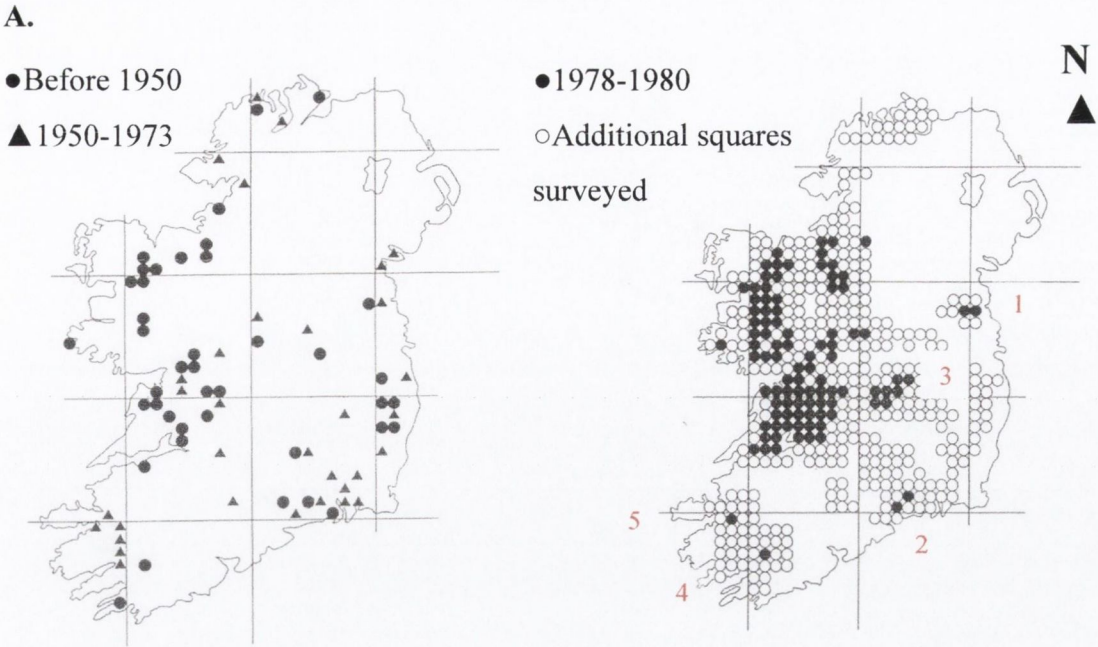


Fig. 1.2. A. Map of the distribution of pine marten in Ireland prior to 1973 and as found during the census 1978-1980 (from O’Sullivan, 1983). Isolated populations: 1.Boyne Valley, Louth; 2.Portlaw, Co. Waterford; 3.Slieve Bloom Mountains, Co. Laois; 4.Goungane Barra, Co. Cork; 5.Killarney National Park, Co. Kerry. Each square surveyed was 10km x 10km. **B.** Map of the distribution of pine marten in Northern Ireland (taken from CEDaR website, Ulster Museum).

1.4. Biology

1.4.1. Biometrics

Studies on geographic variation of a species provide an opportunity to examine morphologic responses of species to different regimes of habitat, food and coexistence with potential competitors (Reig, 1992). Species with overlap or potential overlap in resource requirements are assumed to have coevolved in some way as to minimise competition between them, thus competition is viewed as having a decisive ecological, biogeographical and evolutionary role (Dayan & Simberloff, 1998). The use of analogous food resources by similar species is argued to be factor that might cause morphological changes, allowing the exploitation of different prey sizes by species or sexes, due to interspecific competition and is referred to as character displacement. Community wide character displacement is studied within the framework of ecological guilds and examines groups of species that use the same class of resources in a similar way and are assumed to be potential competitors. Researchers have also suggested that morphological variation should be related to the number of competing species, with niche width increased in the absence of competitors and increased variation reflecting the increased niche width. This is known as the 'niche variation' hypothesis. Another morphological phenomenon related to competition is sexual size dimorphism. Niche partitioning between sexes is frequently adduced as a possible cause of such dimorphism and has been verified for numerous taxa such as weasels (Dayan & Simberloff, 1994).

Anderson (1970) reported that across the distribution of the pine marten there is an east-west cline of increasing size, based on measurements of bones from museum species. Reig (1992) reports that this is not the case, but that there is a gradient of increasing skull size but this extends from north to south (see Table 1.1 for biometric measurements of pine marten across Europe). He cites the absence of material from eastern and southern European countries in Anderson's (1970) data of causing her to reach the above conclusion. In comparison with the sympatric stone marten, Reig (1992) also found no evidence to suggest competitive release where each species occurred solely compared with areas where both occurred together and cites the more generalist habits of the stone marten compared with pine marten as a reason for this. Reig (1992), also not finding a conclusive relationship between morphological characteristics and climatic variation, suggests that the observed increase in size from

north to south is due to prey densities of small mammals which decrease from north to south. The gradual increase in skull size observed may be attributed to an adaptation to exploit other food resources, particularly larger mammals e.g. lagomorphs. Clevenger (1993a) explains the incidence of gigantism observed within a population of pine martens on the island of Minorca as possibly the result of resource limitation or different prey assemblages compared to those observed on the mainland. In Minorca the pine martens consumed all food groups equally throughout the year, with rats (*Rattus norevgeticus*) and rabbits (*Oryctolagus cuniculus*) the main components of the mammal intake in the diet, while small mammals (primarily woodmouse: *Apodemus sylvaticus*) were the mainstay of the diet in the comparable mainland population in Spain.

The differences in the morphologies of mammal species on islands compared with mainland populations has led to a general rule being deduced such that small mammals get larger and large mammals get smaller (Dayan & Simberloff, 1998). There are several proposed explanations for this which take into account the size of individual colonisers, dispersal ability and opportunity, predation, absence of competitors, food availability and generalist or specialist habits of the individual species (Dayan & Simberloff, 1998). Work has been carried out examining pine martens in Britain and Ireland (Reig, 1992) and also the mustelid guild on these two islands (Dayan & Simberloff, 1994). Reig (1992) found that within these two islands Scottish pine martens were the largest, while though Irish pine martens were the smallest they were closer in size to the intermediate English specimens. He suggests that the apparent contrast in size between populations from England and Scotland might be a consequence of respective isolation from each other, considering the scattered distribution of the pine marten within the United Kingdom. He states that red foxes from Scotland are also larger than specimens from Southern England. The work of Reig (1992) was primarily focused the differing biometrics of the pine marten across its range and whether these differed in relation to the presence and absence of the stone marten. He did not draw any conclusions as to why the pattern of biometrics of this species differed between the three the populations.

By contrast Dayan & Simberloff (1994) specifically focused on the mustelid guild within Britain and Ireland of which the pine marten is a member to account for different observed sizes amongst its members. Ireland has three native species of mustelid: pine marten, stoat and badger, while the United Kingdom has five: pine marten, polecat,

stoat, weasel and badger. The otter, another member of the mustelid family, found on both islands was excluded from the study as it does not compete with the other members as it is solely aquatic and feeds primarily on aquatic organisms. The American mink, now present on both islands having been deliberately or accidentally introduced was also included. Dayan & Simberloff (1994) found by simultaneously examining the relationship between species and between the sexes of individual species that there was good evidence for character displacement amongst the Irish guild of mustelids. They suggest that in the absence of the polecat the female pine marten has decreased in size (based on canine size) to fill this niche and likewise the female stoat (similarly on canine size) now exploits the niche of the absent weasel. Thus both these species show increased sexual dimorphism compared to their British counterparts and this sexual difference was also more variable. McDonald (2002) has however shown that there is no difference in diet between male and female pine martens in Ireland and thus exploitation of differential prey sources cannot explain the greater degree of sexual dimorphism in pine marten between the two islands. Dayan & Simberloff (1994) ask if the generally greater within sex canine variation in the Irish mustelids reflects a population-wide broader food niche. The dietary niche breadth of Irish pine marten compared with the British pine marten may suggest so (Warner & O'Sullivan, 1982; Balharry, 1993b; Appendix 5).

Table 1.1. The biometric measurements and weights recorded (means and/or ranges) for European pine marten. Table is adapted from that presented in NíNeachtain 1998. L/D indicates if measurements were taken from live (L) or dead (D) animals.

Source	Country	Weight (Kg)		Head & Body		Length (cm)		Hind Foot		L/ D
		Male	Female	Male	Female	Male	Female	Male	Female	
Marchesi (1989)	Switzerland	1.47 (1.24-1.85)	1.06 (0.86-1.26)	45.73 (43.5-48)	42.73 (40.5-45)	23.58 (20.5-26)	21.89 (20-20.37)	8.96 (8.5-9.8)	8.21 (7.85-8.6)	L
	Russia	(0.67-1.05)	(0.48-0.85)	(40.5-47.3)	(36.5-42)	(18.5-22.8)	(17-21)			L
	Germany	(1.2-1.6)	(0.8-1.3)	(48-53)	(40-45)	(25-28)	(23-26)			L
	Poland	(0.8-1.5)	(0.98-1.07)	(42-59)	(38-42)	(18-25)	(16-22)			L
	Italy		1.5	(42-57)	49	(19-23)	19			L
	Germany	(1.05-1.9)	(0.9-1.4)	(42-51)	(41-45)	(21-28)	(18-22)			L
Velandar (1991)	Scotland	(1.63-1.68)	(1.28-1.48)	(51-54)	(46-54)	(26-27)	(18-24)			L
	Russia	(0.67-1.05)	(0.48-0.85)	(40-47)	(36-42)	(18-22)	(17-21)			L
	Ireland	(1.5-1.9)	(1.1-1.45)	(46-51)	(41-45)	(20-22)	(20-21)			L
Mal'dzhyunaite (1957)	Lithuania		0.94	42.4						L
Ryabov (1962)	Russia	1.36	0.95	46.6	43	24	22.7	9.9	8.7	
NíNeachtain (1998)	Ireland	1.72	1.28	46.8	42.6	20.4	18	8.7	7.8	L
		(1.38-2.00)	(1.2-1.4)	(45-50)	(41.5-45)	(18-25)	(15-20)	(8.2-9.3)	(6.6-8.3)	

1.4.2. Social Organisation

The pine marten is considered to be a solitary animal that lives by an intrasexual territorial system (Powell, 1979). In such a system it has been found that while male and female home ranges overlap with each other, they tend not to, or at least to any great extent be overlapped by members of the same sex (Marchesi, 1989; Balharry, 1993a; Clevenger, 1993b; Schröpfer, Wiegand & Hogrefe, 1997). The same has also been found for the American marten (Hawley & Newby, 1957). In contrast to the above, Pulliainen (1981) found that pine martens in the sub-arctic regions of Finnish Lapland show a range of resource land-use patterns, including intrasexually exclusive and overlapping home ranges. This study was however restricted to winter time, which may have affected the patterns of activity observed and thus the conclusions reached.

Throughout the distribution of the pine marten, home range size varies (Table 1.2.), with male home ranges always larger than that observed for females (Pulliainen, 1981; Marchesi, 1989; Balharry, 1993a). The variation observed in studies on home ranges can generally be attributed to a function of the method used to determine the size of the area utilized by the animal (live trapping or radio telemetry), the time of year the study was carried out and the habitat within which the observations were made. Though the number of studies carried out investigating the annual patterns of home range utilization by pine marten is limited it appears of the six, the time of year at which the determination of home range occurs would appear significant. Balharry (1993a), carrying out a detailed investigation into home range utilization in pine martens found that animals used more of their home range in summer than in winter. However it is agreed that male home ranges generally overlap at least one female home range, though two is not uncommon (Pulliainen, 1981; Balharry, 1993a).

Common to all reports in the literature is the use of scent marking by pine martens throughout their home range (Marchesi, 1989; Balharry, 1993a; Clevenger, 1993b; DeMonte & Roeder, 1993; Hutchings & White, 2000). It has been suggested that scent marking has evolved for several reasons in mammals: 1) as an alarm signal, 2) epideictic display, 3) sex attractant, 4) individual recognition, 5) orientation and familiarity, 6) warning conspecifics away and 7) to indicate dominance (in Pulliainen, 1981). Pulliainen (1984) suggests that scent marking in the pine marten is not in response to the active

defence of a territory but rather it is primarily to orientate the owner to its surroundings within its home range. He further suggests that as the animal marks throughout the range and not just at the borders then it is not actively defending the boundary; this system he termed ‘martelism’. Brainerd (1990) suggests that as foxes also do the same but are considered territorial then this is not quite correct. Pine martens are however regarded as highly tolerant of conspecifics, with aggressive interactions rarely being observed or reported (Pulliainen, 1981). The same has been found for the American marten, which the authors noted as highly tolerant of transients and dispersing juveniles (Hawley & Newby, 1957).

Table 1.2. Table of reported pine marten densities (means and or ranges) (taken from NíNeachtain 1998).

Source	Country	Woodland Habitat	Density (individuals/km ²)
Marchesi (1989)	Switzerland	Coniferous	0.1-0.8
Zalewski <i>et al.</i> , (1995)	Poland	Coniferous	0.83-1.74
Jedrzejewski <i>et al.</i> , (1993)	Poland	Deciduous	0.49-0.8
Pelikan & Vackar (1978)	Poland	Mixed	0.93-1.39
Halliwel (1997)	Scotland	Mixed	0.58-0.82
O’Sullivan (<i>pers. comm.</i>)	Ireland	Deciduous	0.78

1.4.3. Reproduction

The pine marten follows a typical mustelid pattern of breeding, with mating occurring between mid-July and late August (Hayden & Harrington, 2000). During this time the

female comes into oestrus; the male on the other hand will show signs of breeding (testes visible and enlarged) between May and October. It is also during this time that the normally solitary pine marten may be seen engaged in playful behaviour (chasing, mock fighting) with members of its own species (Birks, 2002; Appendix 1: 16/07/2002). Though it has been suggested that mating is promiscuous for both sexes (Velandar, 1991), others have reported that this is only for the male and that for the female the relationship is monogamous (Schröpfer *et al.*, 1997). In the latter case the evidence was based on paternity testing using DNA markers.

Schröpfer *et al.* (1997) also reported that contrary to the belief that females (N = 6) raise the kittens solely by themselves, males (N = 6, based on a 6 year study) are indirectly involved through maximising their summer territory in early spring to encompass those females they have bred with the previous summer, thus exhibiting mate guarding behaviour. The same authors further advocate that the increase in scat deposition, or territorial marking behaviour that occurs at this time is indicative of a male-mother-family system and is not solely to warn off other males from entering their territory and mating with their potential mates. They argue that as this increased territory size begins in early spring and continues until late November, by which time the kittens are independent sub-adults, then this gives strong evidence of an indirect role of the male pine marten in the rearing of their off spring by protection from other males through their exclusion from the area. Though a plausible explanation for why males expand their territories from April to November, the small sample size together with few annual investigations into home range size merits further investigation before this annual pattern of behaviour can be explained adequately.

The implantation of the embryo, which ceases to grow a short period of time after conception (August), does not occur until early spring, usually February or March. This period of implantation is thought to bring about a second period of 'heat' during which increased social interactions between individuals occur. Helldin & Lindström (1995) suggest that this observed second period of social interaction is actually the female initiating the dispersal of sub-adults from her territory prior to the new litter being born. The increase in hormones associated with the implantation of the embryo is also likely to make her more attractive to resident males. Schröpfer *et al.* (1997) make no mention of

whether the increase in female hormones at this time is a potential trigger to the male, who also begins to show increased hormone levels (Balharry, 1993a; Schröpfer *et al.*, 1997), to increase its territory size and scent marking.

Once implanted the embryos grow quickly with the gestation period being approximately 30 days. The young, usually a litter of 1-3 kittens, are born both blind and naked (Fig. 1.3.). The eyes of the kittens open after about 40 days and the milk teeth descend by seven weeks. These are replaced with the adult teeth at about four months (Birks, 2002). The young kittens are weaned by 12 weeks, generally by mid/late June (*pers. obs.*) at which time they will leave the den for the first time. Once weaned the young often accompany their mother on hunting trips within her home range (it is at this time that young kittens may be caught in live traps, Appendix 3) but do not become independent till they are about six months of age (MacDonald & Barrett, 1993). The young of the year are thought to begin to disperse in October (Birks, 2002), but may remain within their mother's home range over the winter and then leave (Helldin & Lindström, 1995).

As slow breeders (males may breed in their first year but it is not usual and females are typically 2 or 3 years old before they do) with small litter sizes (1-3 kittens per litter annually) pine marten populations are extremely susceptible to exploitation and disturbance (Balharry, 1993a; Birks, 2002). Thus successful breeding in newly colonised areas may take some time. By comparison stoats exhibit a different life history which is characterised by prolific breeding in a short period of time. Female stoats are impregnated prior to leaving the nest (but not by their father or brothers) and typically have 10-12 young per litter thus when they disperse and a suitable territory is found they can establish quickly (Hayden & Harrington, 2000). Other mammal species such as otter are not constrained to a breeding season but may have young whenever resources are suitable and can therefore breed throughout the year (Woodroffe, 2001).

1.4.4. Habitat Use

The pine marten, as its name would suggest, is typically associated with woodland across much of its range (Velandar, 1983; Marchesi, 1989; Brainerd, 1990; Ozoliņš & Pilāts, 1995), though the type of woodland differs between countries and regions (Brainerd, 1990). In Britain and Ireland it has been hypothesised that this species has adapted to a gradual

reduction in woodland cover over the centuries by making use of alternative habitat such as rocky areas and scrub woodland (NíNeachtain, 1998; Birks, 2002). Observations on pine marten movements suggest that this species does not cross open spaces and those areas can also define territory boundaries (Pulliainen, 1981; Brainerd, 1990; Balharry, 1993a). The same has also been recorded for the American marten (Hawley & Newby, 1957; Brainerd, 1990). Predator avoidance, mostly of foxes and eagles, has been suggested as the reason for the apparent avoidance of open areas (Pulliainen, 1981; Marchesi, 1989; Clevenger, 1993b; Birks, 2002).



Fig. 1.3. Two pine marten kittens in the attic of a house, Co. Clare.

The selection of den sites as opposed to resting sites, are also determined by the risk of predation. Brainerd *et al.* (1995) found that females significantly preferred cavities in trees and rotten snags as dens (quality dens) for the early stages of offspring development. It would appear that animals are less choosy about the location of resting sites but predation and protection from the elements are still significant factors driving the selection (Zalewski, 1997). Similar patterns have been observed for the American marten, with up to 85% of

natal dens being located in trees (Gilbert *et al.*, 1997). A hypothesis put forward by Birks, Messenger & Halliwell (2005) suggests that current woodland conditions in Britain do not favour the creation of quality arboreal dens, an essential resource for pine martens. They have also suggested that the observed use by pine martens of attic spaces in occupied and unoccupied homes within the main range of the pine marten in the UK (Scotland) supports this claim.

A similar pattern has been observed in Ireland, where animals have taken up residence in holiday homes, schools and fulltime occupied residences (NPWS staff *pers. comm.*; Fig 1.3). In some cases little damage has been caused, while on other occasions substantial damage resulting in monetary loss and subsequent distress to the owner have resulted (NPWS staff *pers. comm.*). While it is typical of pine martens to forage at compost bins, bird tables and/or disturb and kill domestic fowl (Marchesi, 1989; Halliwell, 1998; Appendix 1: 26/05/2003), conflicts arising from the use of residential or commercial property by this species is increasing in Ireland and as in the UK leads to much distress for the property owner. It is reasonable to suggest that though the increase in commercial forestry in Ireland maybe providing good hunting grounds and cover for the pine marten, that the structure of these monoculture stands cannot provide quality arboreal dens for females, thus forcing them to seek suitable neo-natal dens elsewhere. Nothing is known of the denning requirements of this species in Ireland and without proper research such conflicts generated from the use of buildings as dens cannot be resolved satisfactorily.

1.4.5. Diet

The diet of the pine marten will be extensively covered in Chapter 5 of this thesis and a review of some of the literature appears in Appendix 5. In brief, throughout much of its range the pine marten is considered to be an opportunistic generalist feeder, with small mammals being the back bone of the diet but with it also consuming fruit, birds, invertebrates, frogs, lizards and earthworms (Goszczyński, 1976 & 1986; Pulliainen, 1981; Lindström, 1989; Marchesi & Mermod, 1989; Jedrzejewski, Zalewski & Jedrzejewska, 1993). The description of an opportunistic feeder is maintained when the diet of this species was investigated on islands but small mammals no longer make up the back bone of the diet, rather all food groups are exploited equally (Warner & O'Sullivan, 1982; Clevenger, 1993c; NíNeachtain, 1998). The different dietary strategy observed is thought to be a result

of the reduced mammal prey species commonly associated with islands. Thus on islands the pine marten exhibits a wider dietary niche breadth. Where studies of the diet of the American marten have been carried out on islands a similar picture of resource exploitation is observed (Nagorsen, Morrision & Forsberg, 1989; Nagorsen, Campbell & Giannico, 1991).

As mentioned in section 1.4.1 differences in pine marten size observed on islands, particularly the skull, have been attributed to differences in the diet. Clevenger (1993c) found that pine martens on Minorca exhibited a distinct cranial morphology compared with a population on the mainland, the island animals being larger than mainland populations. Nagorsen *et al.* (1991) hypothesised that the larger cranial morphology of the American martens of the Queen Charlotte Islands is because they feed extensively on hard-shelled intertidal animals such as crabs. The components of the diet taken from stomach contents did not support this hypothesis but as the authors correctly point out the study only covered the winter period. Neither did Nagorsen *et al.* (1991) nor Nagorsen *et al.* (1989) find any difference between the diets of male or female pine martens on Queen Charlotte Islands or Vancouver Island respectively.

1.5. Molecular Ecology

The study of molecular ecology, specifically the use of DNA and the associated tools within this discipline, is now an integral part of the conservation of wildlife species and provides an important avenue through which information can be gathered about species and their populations. Investigations have been carried out to examine the origins of populations (Davison *et al.*, 2001, Stone *et al.*, 2002). Given the historic and present day persecution of many mammal species, high levels of habitat loss and habitat fragmentation worldwide, molecular tools have also been used to investigate the problems associated with small populations, isolation of populations, relationships between fragmented populations and reintroductions (Paetkau, Shields & Strobeck, 1998a; Kyle, Davis & Strobeck, 2000; Kyle, Robitaille & Strobeck, 2001; Waldick, *et al.*, 2002; Vinkey, 2003). In addition molecular techniques have been used in more practical ways to gain information about the specific dynamics of individual populations such as dispersal (Broquet *et al.*, 2005), sex ratios (Dallas *et al.*, 2003; Chapter 7), population estimates (Mowat & Paetkau, 2002),

parentage (Schröpfer *et al.*, 1997) and to identify species by the traces (e.g. hair, scats) they leave behind (Davison *et al.*, 2002; Domingo-Roura, 2002; Palomares *et al.*, 2002).

The molecular ecology of the pine marten is adequately covered in Chapter 6. In brief, extensive studies of this species using molecular tools have not been carried out. One European wide study examined the postglacial colonisation of Europe by the pine marten (Davison *et al.*, 2001) and reported an absence of ancient lineages suggesting that the present day distribution of martens is made up of animals that have colonised from a single refugium. As sample sizes were limited from many of the populations examined, the authors suggest that the full picture may not have been presented and that ancient lineages may well exist. Another study using microsatellite analysis revealed a high degree of structuring within the European pine marten primarily due to anthropogenic impacts and isolation by distance (Kyle *et al.*, 2003). The same authors report similar levels of genetic variation for continental populations but reduced levels for island populations sampled, Scotland and especially Ireland. Once again uneven sample sizes between populations and low sample sizes for some populations have made the data hard to interpret accurately. By contrast American martens show little structuring and high genetic variation, except for island populations which exhibit lower levels similar to that reported for European pine marten (Kyle & Strobeck, 2003; Kyle *et al.*, 2003). Others have focused on developing techniques for the identification to species level from non-invasive samples in order to allow positive determination of the pine marten from other species which may leave similar traces behind (Davison *et al.*, 2002; Domingo-Roura, 2002; Statham *et al.*, 2005).

1.6. Aims and Objectives of this study

The main aims of this project were:

1. To investigate the success of the translocation of pine marten to Killarney National Park (KNP) and comment on the current status of the population.
2. To explore the use of hair traps as tools for assessing pine marten presence and abundance in broadleaf woodland.
3. To examine the annual and seasonal diet of pine marten in KNP.
4. To investigate the level of genetic diversity within the Irish pine marten, to include the population at KNP, and whether there is mitochondrial evidence for sub-population structure within the Irish pine marten.

5. To assess the feasibility of sex primers developed for otters for identifying sex in pine marten and to apply this to invasive (tissue) and non-invasive samples (hair).

Main hypotheses.

Hypothesis 1

The pine marten population has established itself in Killarney National Park following the recent translocation of animals to the park.

Hypothesis 2

The pine marten population in Killarney National Park shows no reduction in genetic variation compared with animals from the source of the translocation or the rest of Ireland.

Hypothesis 3

It was hypothesised that given that pine martens were over hunted and reduced to isolated populations for the previous hundred years or more that some degree of sub-structuring would have occurred owing to this fragmentation of the populations. It was further hypothesised that if such sub-structuring had occurred then it would be possible to determine if pine martens were extirpated from KNP or was a remnant population present during the re-establishment process. The alternative hypothesis is that fragmentation was either too brief or some level of connection remained between populations or no sub-structuring has occurred.

With the exception of chapter 2 (General Material and Methods) and Chapter 8 (General Discussion) the following chapters (3-7) are presented as discrete units. Chapter 3 explores the history of the pine marten in the Killarney Valley and gives an appraisal of the effort to translocate and re-establish the pine marten population in KNP. The current status of the pine marten in KNP is investigated through an analysis of sightings, scat location, hair trapping and live capture data, which includes the distribution within the park boundary. As high capture rates may not reflect a healthy population (Hargris & Bissonette, 1997), I used reproductive status, weight, body condition and recapture in subsequent years to assess the overall status of the pine marten population. The results of my findings are discussed with recommendations for continued monitoring and woodland management to promote the long term viability of this species in KNP.

Chapter 4 investigates the use of hair traps for assessing pine marten presence and abundance. This chapter has been accepted as a paper to the *Journal of Zoology, London* and is currently in press. For the thesis minor modifications have been made to the material and methods section, which now give a brief account of the work carried out. A full appraisal of all the methods used can be found in Chapter 2. The rest of this chapter remains as it will be printed in the journal and discusses an alternative method for monitoring pine marten.

In chapter 5 the annual and seasonal diet of the pine marten in KNP is explored. Of special reference in this chapter is the occurrence of the bank vole in the diet, which has until now been absent from other study areas where pine marten diet has been investigated in Ireland. The difference in diet of the pine marten in Ireland, occurring on an island, compared with mainland European populations is also discussed.

Chapter 6 investigates the genetic structure of, and levels of genetic diversity within the Irish pine marten, with specific reference to the recently re-established population in KNP. Current levels of genetic diversity within this population were compared with the rest of Ireland to examine whether translocation has had an impact on genetic diversity. The possibility of discovering whether pine martens were present in KNP prior to the translocation is also investigated by comparing mitochondrial DNA haplotypes from KNP and county Clare (origin of the translocated animals) with published sequences.

In chapter 7 the feasibility of using sex primers developed for otters for sex identification in pine martens was investigated. This chapter has been accepted as a note to the journal *Molecular Ecology Notes* and is currently in press. The chapter has been modified to include two extra tables and one figure, which owing to word constraints, were omitted from the paper. The possibility of using this technique to sex non-invasively collected hair samples and the missassignment errors in correctly identifying males are discussed.

Chapter 8 is a general discussion of the topics presented in this thesis and recommendations for further work.

2.1. Study Area

Killarney National Park (KNP).

The Killarney area, apart from possessing outstanding natural beauty, is one of major scientific importance. There is a great diversity of ecosystems, ranging from mountains and moorlands to the fertile lowlands, an extensive river and lake system and the largest expanse of native woodland in Ireland (Smal, 1978). Killarney National Park (KNP) is located in the south west of Ireland in County Kerry (Irish national grid reference (NGRef): V9487). KNP is approximately 10,289 ha of which 14% is semi natural woodland [native oak woodland (1200 ha), yew woodland (25 ha) and wet broadleaf woodland (155 ha)] the remainder is made up of lakes (24%) and upland blanket bog, heathland, rivers and streams (62%). Today the care and management of KNP, a national park, a special area of conservation (SAC) and UNESCO Biosphere Reserve, lies with the National Parks and Wildlife Service (NPWS).

The climate of the Killarney area can be considered oceanic and is characterised by mild winters and cool summers with rainfall spread out throughout the year. High rainfall is distinctive of the region with more than a twofold increase in rainfall between the town centre and the summit of Mangerton (839m; NGRef: V807981; highest summit within the park), a distance no less than three miles (Kelly, 1975). Little variation in air temperature occurs between summer and winter with mean values ranging from 5-15°C (NPWS, 2004).

Study Sites Description

All study sites are described in order as they are listed in each chapter (1-12) except for chapter 4, which for publication purposes were listed as 1-5. The habitat codes used in this study are following the description set out in Fossit (2000) and where any of those habitats occur on Annex I of the Habitats Directive this code has been included (Table 2.1). The grid reference quoted is the national grid reference for Ireland, and the map number in all cases is 78 (Discovery Series, Ordinance Survey of Ireland).

One transect line was established in each of the twelve sites selected and was approximately 2.5km in length ($\mu = 2.175\text{km}$, $SD = \pm 0.433$, $N = 12$). 2.5km was chosen as an appropriate length as the only study carried out in Ireland (lowland broadleaf

woodland) estimated home range size to be 0.42km² and 0.20km² for male and female pine martens respectively (Birks, 2002; Chapter 1, Table 1.2). While it was expected that home range size would increase with increasing altitude owing to a decrease in habitat quality, I assumed that 2.5km would still cover at least one adult male/one-two female pine marten home ranges in lowland and 1male/1female in upland sites. Sites were selected so as to cover all the major woodland areas in the park that could be accessed more or less without any assistance. Transects were positioned through the areas selected so they were easily accessible and to avoid any hazardous obstacles (e.g. cliffs, very wet boggy areas etc.)

1. *Tomies Wood*

Tomies woodland is situated at the northwest of the park (Fig. 2.1). This woodland was clear felled in 1800 but was replanted also immediately with oak (*Quercus spp.*) and thus the trees appear in straight lines in the woodland (Caruthers, 1998). There are blocks of conifers in this wood, a legacy of when parts of the park were under the control of the forestry service. The dominant tree species are oak (*Quercus petraea* or *Q. robur*) with an understorey of holly (*Ilex aquifolium*) and birch (*Betula pubescens*) (Table 2.1). A forestry road runs through the entirety of this wood. The area is very much over grazed (red deer, sika deer and sheep) and is infested with rhododendron (*Rhododendron ponticum*).

2. *Muckross Forest*

Muckross forest is dominated by coniferous trees of which the dominant tree species are sitka spruce (*Picea sitchensis*), lodgepole pine (*Pinus contorta*), Norway spruce (*Picea abies*) and larches (*Larix spp.*) (Fig. 2.1; Table 2.1). In many areas the stands have been thinned and good light penetrates to the woodland floor where there is an abundance of great wood rush (*Luzula sylvatica*). Elsewhere there are still dense blocks of conifer trees typical of commercial plantations. The whole area is accessible with forestry roads. The area is very much over grazed (deer and sheep) and is infested with rhododendron.

3. *Muckross Peninsula*

The Muckross peninsula has two dominant types of woodland occurring on it (Fig. 2.1) owing to the geological boundary which bisects it; limestone occurring to the east and old red sandstone occurring to the west. On the calcareous limestone pavement one of

the remaining yew woods, Reenadinna wood, in western Europe occurs (Table 2.1). This area is dominated by yew (*Taxus baccata*) with a good understorey of ash (*Fraxinus excelsior*), hazel (*Corlyus avellana*) and holly (*Ilex aquifolium*). Many of the yew trees have been damaged by stags rubbing their antlers off the trunks. Adjacent to Reenadinna yew wood on the acidic soils overlying the old red sandstone is Camillan wood which is an oak-holly-birch dominated woodland (Table 2.1). The strawberry tree (*Arbutus unedo*) is in abundance in this area of the park. There is a network of nature trails running through the whole of the peninsula in addition to a private road (Dinis road, authorised personnel only). This area of the park receives many visitors, being both accessible and close to Muckross House. The whole of the Muckross peninsula has been overgrazed primarily by sika which have recently been culled extensively (194 individuals in 2004/05 season, P. O'Toole *pers. comm.*). The Muckross peninsula is largely free from rhododendron.

4. Ullauns Wood

Ullauns wood occurs in the south west of the park and is typical of upland broadleaf woodland, dominated by oak-holly-birch assemblages (Fig. 2.1; Table 2.1). Once again the area is overgrazed by deer and sheep but also goats. The hiking trail, the old Kenmare road, runs through part of this woodland. Ullauns wood is still infested with rhododendron but has been sprayed (Roundup weed killer) recently to control this pest.

5. Derrycunnihy

The part of this woodland which was used in this study is the area covered by lower Derrycunnihy (Fig. 2.1), which runs parallel to the Upper Lake. This is also an oak-holly-birch dominated woodland (Table 2.1). There is a hiking trail, the Kerry Way, running through the length of this woodland, which also doubles as a private road (access for KNP staff). This woodland is very much infested with rhododendron, which for the most part has not yet formed dense stands and thus the remnants of good ground flora still persists but is heavily overgrazed.

6. Tower Wood

The Tower wood is an oak-birch-holly woodland (Fig. 2.1; Table 2.1). There is no ground flora to speak of as this area has been or still is heavily infested with mature rhododendron (up to 7m in length when cut at the base have been recorded *pers. obs.*). At the time this work was being carried out large areas had been cleared by Peter

O'Toole and the KNP volunteers and exclosures created to exclude grazing animals to allow regeneration to occur. The area covered by the transect extended through both recently cleared and still heavily infested areas. There are no established paths or roads through this woodland.

7. Looscaunagh Wood

Looscaunagh wood is located in the south west of the park and is an oak-holly-birch woodland (Fig. 2.1; Table 2.1). There are some old stands of scots pine (*Pinus sylvestris*) to be found within this woodland. The area is devoid of pathways and nature trails and remains virtually unvisited by humans. Looscaunagh wood is moderately overgrazed but a good diverse ground cover still exists and rhododendron is largely absent. Adjacent to this woodland is a Coillte conifer plantation.

8. Torc Face

The north face of Torc mountain was once part of the park that came under the control of the forestry service (Fig. 2.1; Table 2.1). The main stands are conifers and the whole area is very much infested with rhododendron, forming impenetrable stands in many areas. An old scenic nature trail which contoured around the face of the mountain was used as the transect for this area. Once very overgrown, this nature trail was reopened to the public in 2004.

9. Blue Pool

This small area of the park is dominated by a wet willow-alder-ash (willow: *Salix spp.*; alder: *Alnus glutinosa*; and ash) woodland and areas of conifer plantation (Fig. 2.1; Table 2.1). The field layer here is dominated by greater tussock sedge. The water level in this wet woodland is maintained constant throughout the year due to the presence of active springs. There is a good network of nature trails throughout this site which made up the walking transect in this area. The Blue Pool area is badly infested with rhododendron, though effort has been made to remove some of it, and is overgrazed.

10. Gardens

This is a mixed woodland containing both native and non-native species close to and surrounding Muckross House (Fig. 2.1; Table 2.1). The area also covered a small area of yew woodland which the Old Boathouse trail extends over. There are a good network of paths and roads running through this area.

11. *Glaisín na Marbh*

Glaisín na Marbh (little stream of the dead) is an upland oak-holly-birch woodland (Fig. 2.1; Table 2.1). This entire woodland has been infested by rhododendron, but has been cleared by the voluntary organisation Groundwork. The area is heavily overgrazed by both deer and sheep and ground cover is primarily a covering of bryophytes over the rocks and or wood sedge. There is no field layer to speak of. Glaisín na Marbh has no established pathways and is rarely visited by humans except those dealing with the rhododendron or overgrazing problem.

12. *Canichaun*

This is a narrow piece of oak-holly-birch woodland which runs parallel to the northern shore of the long range (Fig. 2.1; Table 2.1). There is no field layer to speak of and ground flora is restricted to a covering of bryophytes. Canichaun is entirely overgrazed, primarily by sheep and sika deer, but is for the most part free of rhododendron (area covered by transect was). There is no established pathway in this woodland and it is most easily accessed by boat.

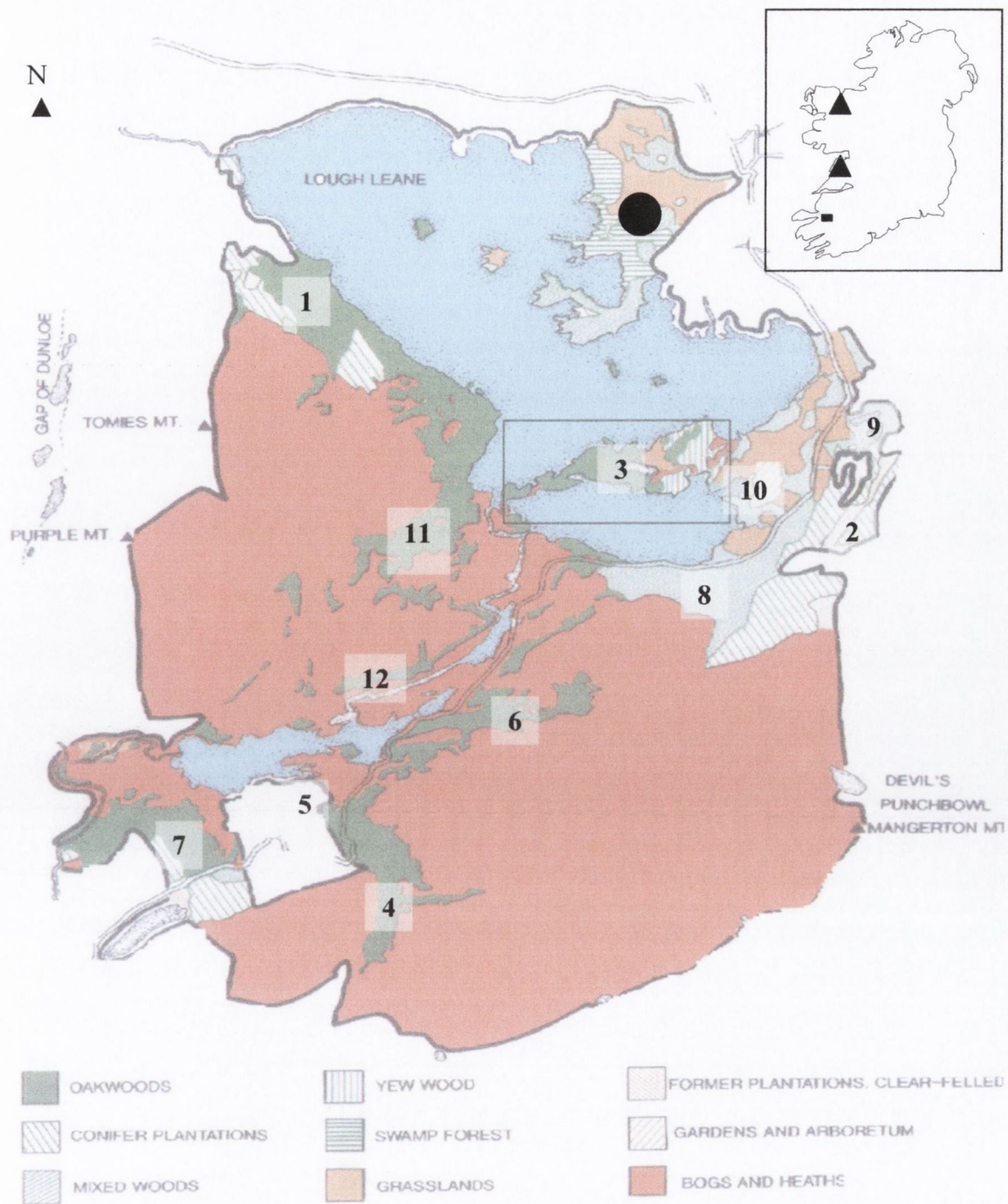


Fig. 2.1. Map of Killarney National Park showing the location of transects (1-12) and dominant vegetation type. Location of the Game and Reen woods is indicated by circle (black). Inset a map of Ireland showing the approximate location of KNP, with triangles showing the location of County Mayo and County Clare.

Table 2.1. The description of the twelve study sites where field work was carried out during this study in KNP. N indicates transects 1-12 and the site name is the local name given to the woodland. Grid reference is the national grid reference and approximately gives the centroid position of the wood. The Irish code refers to the codes set out in Fossit (2000). The European code refers to its link on Annex I of the Habitats Directive. S is scat location, H is hair trapping and L is live trapping.

N	Site Name	Grid Reference	Irish Code	European Code	Work carried out
1	Tomies Wood	V9188	WN1 & WD4	91A0	S, H, L.
2	Muckross Forest	V9784	WD3	-	S, H, L.
3	Muckross Peninsula	V9586	WN3 & WN1	91JA	S, H, L.
4	Ullauns Wood	V9279	WN1	91A0	S, H, L.
5	Derrycunnihy	V9181	WN1	91A0	S, H, L.
6	Tower Wood	V9382	WN1	91A0	S, L.
7	Looscaunagh Wood	V8981	WN1	91A0	S, L.
8	Torc Face	V9584	WD3	-	S.
9	Blue Pool	V9887	WN6 & WD4	-	S.
10	Gardens	V9686	WD1 & WN3	91JA	S.
11	Glaisin na Marbh	V9285	WN1	91A0	S, H.
12	Canichaun Wood	V9283	WN1	91A0	S, H.

2.2. Fieldwork

2.2.1. Initial Set-Up Phase

Prior to the commencement of fieldwork permission was sought from and given by the then divisional manager Mr. Paddy O'Sullivan (NPWS). Fieldwork (systematic effort) was carried out between April and September (inclusive) in 2003 and 2004. An initial two month period (February and March 2003) was used to organise the transport of equipment to KNP and to find suitable study areas within the park to carry out the project. This also allowed me to become familiar with those areas. It was during this time that scats, if encountered, were collected for future dietary analysis. They made up the opportunistic collection of data as opposed to the systematic collection of data. The collection of scats at this time was carried out in the exact same manner to scats collected during the systematic survey.

Of prime concern was access to areas. As equipment was heavy and would have to be moved into and out of sites regularly (hair traps, live traps, processing equipment and bait) it was vital that those areas, particularly where live trapping was going on, could be easily reached. Furthermore it was imperative that where animals were to be live trapped that the area could be accessed at all times, even in times of heavy rainfall and any subsequent flooding. Thus it was decided that live trapping could only be carried out on transects 1-7 (Table 2.1). Transects 8-10 were too public and it was felt that traps may be interfered with. Transects 11-12 were too remote and can be cut off by flooding. Furthermore transect 12 is most easily reached by boat and therefore given resources available could not be visited daily as required for live trapping. Hair trapping was carried out on transects 1-5 and 11-12 (Table 2.1). Scats were collected when encountered on all transects (Table 2.1).

An additional area was surveyed for the presence of pine marten, Reen and Game woods (Fig. 2.1). These woodlands are primarily wet woodland (flooded in winter but dry out in summer) and are situated close to Killarney town centre. It was felt that given that this area is heavily used by the public and that no evidence of pine marten was found when initially investigated then it would not form part of the systematic surveying of the park.

The initial set-up phase was also used to build up stocks of bait for hair and live traps. The bait used in this study was deer offal (heart, liver and lungs). The bait used in this

study was deer offal (heart, lungs and liver) as this was a readily accessible source of bait which could be frozen until use. Furthermore pine martens have been observed eating at carcasses left in the field (P O'Toole *pers. comm.*).

2.2.2 Systematic Surveying

Scat Location

Recent work in the UK reported that, even in the case of experienced field workers, confusion with other species, primarily fox, may arise when identifying pine marten scats in the field (Davison *et al.*, 2002). In Ireland pine marten scats may be confused with fox, stoat (*Mustela erminea*), badger and mink (*Mustela vison*) all of which occur in KNP. Pine marten scats were distinguished from the above species primarily by smell, which NiNeachtain (1998) described as aromatic but not pungent or unpleasant, and secondarily by size and shape. Davison *et al.* (2002) suggest that scats from wild captive pine martens may be used to train field workers in their correct identification in the field. Animals were live trapped during the same period as scats were collected, and scats often found in the cages were compared to those collected for dietary analysis to ensure correct identification.

Scats were collected if they had the characteristic pine marten sweet odour and were of the correct size (5-12cm) and shape, though morphology (Fig. 2.2) does vary greatly (Birks, 2002). O'Sullivan (1983) further recommends that scats should be collected within seven days of deposition to ensure correct identification but may need to be collected sooner depending on weather and content of the scat. I observed after heavy showers of rain that scats could be partially or completely disintegrated. Scats considered likely to be pine marten (lacking odour or shape) were also collected but were only used for dietary analysis and not as proof of positive presence (this only occurred on transects 11 & 12; N = 12).

(a)



(b)



Fig. 2.2. Two scats located during field work in Killarney National Park showing how content may effect the morphology. Scat (a) contained mainly animal and bird material while scat (b) was mostly ivy (*Hedera ilex*).

To ensure correct identification of scats, transects were cleared of all faecal material on day one and only scats on subsequent visits were included for consideration. Subsequent visits were every second day for a further three times. All sites were visited three times each year once in May/April, once in June/July and once in August/September and a constant route was walked on each occasion. The exceptions to this were transects 11 and 12 which were visited fortnightly and monthly respectively owing to their remote locations. On these transects scats were collected as encountered. Any scat identified as pine marten was done so based firstly on smell and secondarily on size and shape. For each scat I recorded presence or absence of smell, length (cm) and a picture was taken for reference.

Hair Trapping

The hair traps used in this study were designed by John Messenger of VWT (Vincent Wildlife Trust) and are described in detail in Messenger & Birks (2000). In this study each trap had the internal dimensions of 15cm height and 10cm breadth and was 70cm in length. An additional length of wood was left at the front of the trap (20cm) to act as a platform. The wood used was marine ply and all wood was treated with a wood preserve prior to assembly. The wood preserve being brown also acted as a good camouflage (Fig. 2.3). A wire mesh was fixed to the back of the trap so the trap was not entirely enclosed. The spring used was a common furniture spring (supplied by C & C Springs, Ireland). Each spring was permanently attached to one side of the trap and

hooked to the other with a small hook, so that when set the individual coils of the spring were separated. Hooks were made from old wire clothes hangers and bent into shape as required. The traps function by removing a sample of hair from the dorsal surface of the animal as it is retreating from the trap (Fig. 2.4).

Given the impoverished mammal fauna in Ireland distinguishing between pine martens and other potential hair donors (mink, cat and possibly stoat) was quite easy. I estimated two non-target species could potentially have triggered the spring: mink and feral cat (*Felis domesticus*). Cat hair has bands and is easily distinguished from pine marten. Mink guard hairs are shorter than pine marten (Guard hair 1: 18-28 mm and up to 90mm respectively). Furthermore the pattern of the medulla differs from pine marten (Teerink, 1991; Tóth-Apathy, 2002). Only 1 sample of each of these species was recorded. The feral cat had been live trapped at the same location prior to the finding of the hair sample. No mink were ever live trapped. Badgers and foxes were considered too large to enter the trap. In any case the hair of these two species is readily distinguished from pine marten by eye.

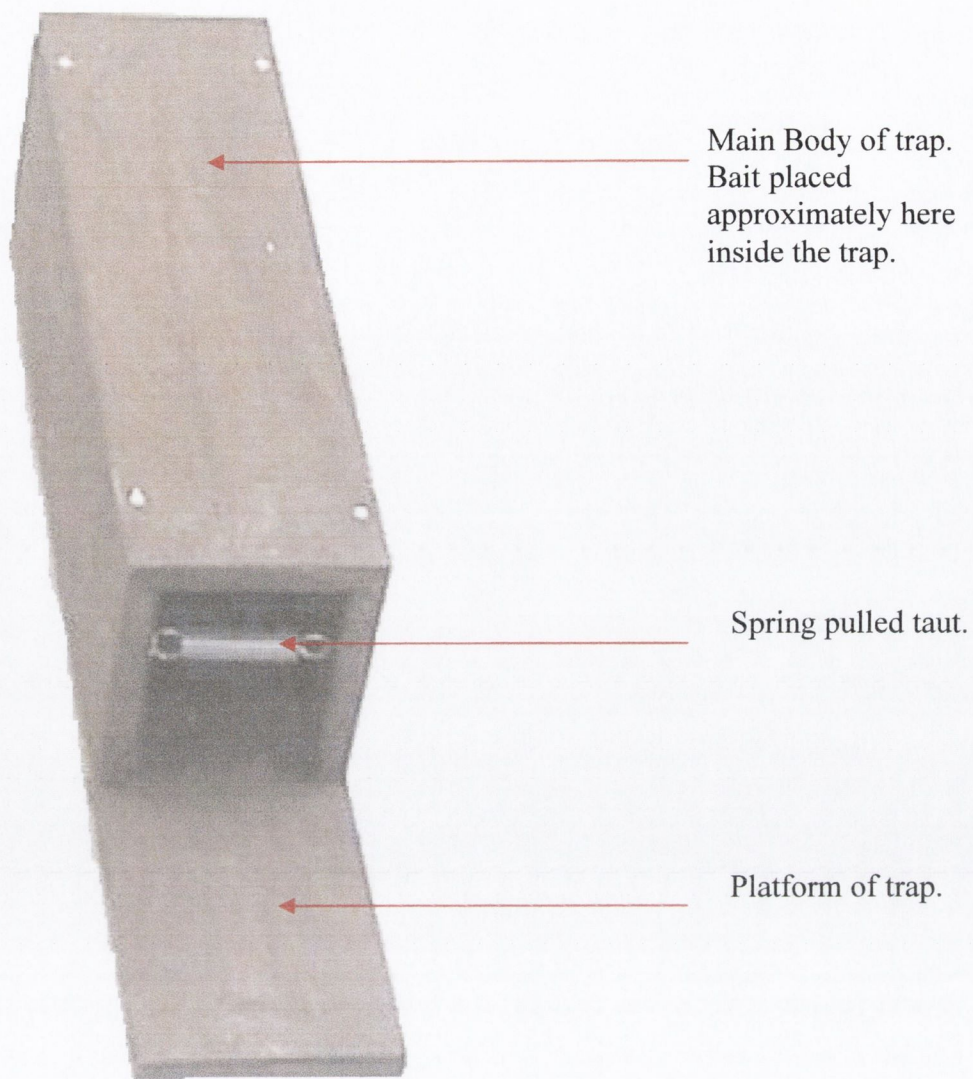


Fig. 2.3. Hair trap used during field work in this study (design by John Messenger of the VWT). The animal pushes underneath the spring as it enters the trap, which is pulled taut, to retrieve the bait. Thus hair can enter into the spaces between individual rings of the spring. As the animal is backing out of the trap the spring becomes dislodged on one side and closes tight. A sample of hair is removed from the dorsal surface of the animal.



Fig. 2.4. Hair trap with pine marten hairs caught in the spring.

Removal of bait by non-target species was most likely from stoats, small mammals (wood mice, bank voles (*Clethrionomys glareolus*) and rats) and slugs. Stoats were considered too small to set off the spring and no samples were found. However the guard hairs of stoat are much shorter (Guard hair 1: 9-13mm) than pine marten (Guard hair 1: up to 90mm in length) so this distinguishes them from pine marten. Slugs were often observed feeding on the bait, which was always replaced when this happened.

In the laboratory a single hair was removed from each sample to confirm species identification made in the field using the key in Teerink (1991) and my own reference collection. A sample of hairs was collected during live trapping to coincide with the different phases of moulting between April and September. Pine martens typically begin to moult their winter coat (light brown) in April and have their summer coats (dark brown almost black looking) by the end of May. The winter coat begins to appear in September and is completed by the end of October (Birks, 2002).

Systematic surveying resulted in hair traps ($N = 10$ per transect) being placed along the transect line at intervals of between 200 and 400 m. While I aimed to place traps at 300

m intervals, distribution of appropriate sites led to variation. Prior to placing traps in the field all sites were assessed for potential pine marten presence and/or features classically used by martens: walls, crossing points, fallen tree trunks, animal trails, nature trails or forestry roads and within mature woodland. Traps were then placed alongside or at these locations. Care was always taken to place traps where the general public could not see them or to camouflage them using moss when this was not possible. Hair traps were set on day one, baited with deer offal with no effort being made to disguise the bait, and were checked three times (every second day for six days). Hair trapped in the spring was collected using a forceps, a preliminary identification made and the number of individual hairs in the sample counted. Traps were reset and/or rebaited depending on the outcome at checking. All sites were visited three times each year, once in April/May, once in June/July and once in September/October except transects 11 and 12 which were visited fortnightly and monthly respectively.

On transects 1-5 the number of trap nights remained constant over the two years (30 trap events per session, 90 per year). The frequency of visitation to the remaining two transects were once fortnightly for transect 11 and once monthly for transect 12. This resulted in 13 visits in 2003 (130 trap events) and 10 visits in 2004 (100 trap events) for transect 11 and was owing to the remote location of this transect which involved an hour's hike across bog to reach. Furthermore the area covered by transect 11 can get cut off in times of heavy rainfall. Transect 12 was visited 7 times in 2003 (70 trap events) and once in 2004 (10 trap events) after positive presence had been established.

Live Trapping

Prior to the commencement of live trapping a licence was sought from NPWS each year for the purpose of live trapping a protected wild animal (Appendix 10). Live capture traps used in this research were specially constructed for the purpose by John Walsh, from a design provided by P. O'Sullivan (NPWS; NíNeachtain 1998). Traps were made of a galvanised wire mesh, 2.5cm mesh and 3mm gauge and each had the dimensions of 20 x 20 x 60 cm and weighed approximately 4 kg (Fig. 2.5). Traps were non-collapsible. A treadle-spring mechanism activated the door. The door was reinforced by having it double back upon itself, with the spring threaded through the partition. Chewing and scratching a heavy duty wire mesh door may damage the teeth and claws of the animal, so a piece of wood was attached to the inner side of the door to protect the animal from damaging itself. This trap design was used in the NíNeachtain (1998)

study and the exact same traps were used for this study. No fatalities or injuries were recorded during that study. A further 25 traps were made up to the same specifications by John Walsh in March 2003.

Regrettably two animals died as a direct result of trapping efforts during session 1 of 2003. I note above that in previous work using these traps no fatalities had occurred hence my decision to use this trap design for the study. On the first occasion a female animal (transect line 5) broke her back trying to escape from a trap and was found dead when traps were checked at first light. This animal had been trapped two days previously and was in good health. At this point trapping was suspended and advice sought both from the university and park authorities (NPWS). It was decided trapping could continue but not to use that individual trap again as the spring may have become weakened (this trap had been used in the NiNeachtain 1998 study). A second animal (male transect line 4) was found dead with a broken back two weeks later. At this point trapping was suspended once again until a solution could be found.

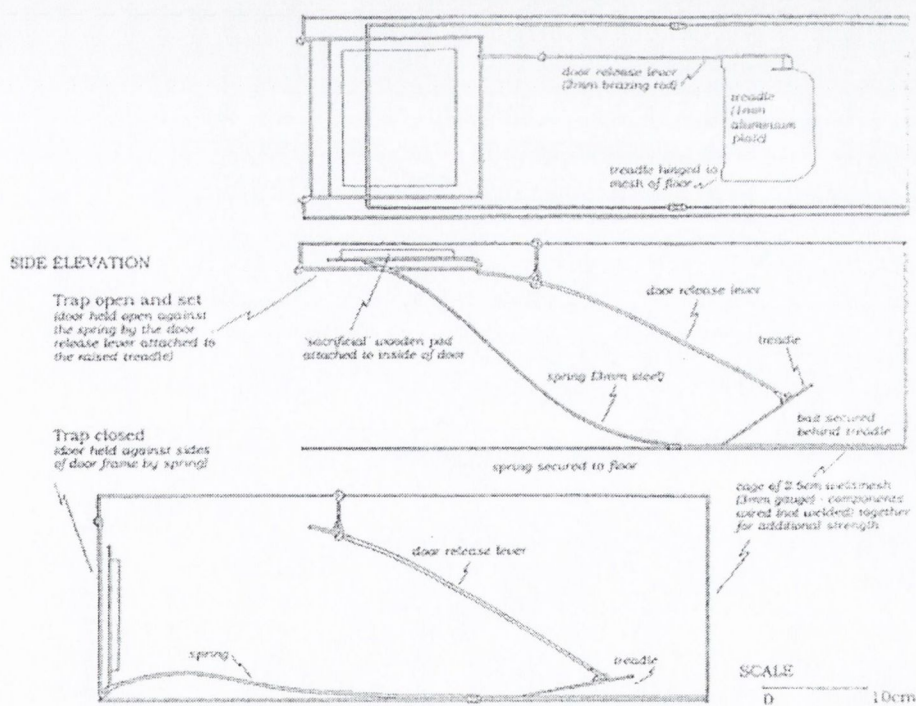


Fig. 2.5. Schematic drawing showing the design of live trap used in this study taken from NiNeachtain 1998.

It was determined that animals were able to get their nose under the door and then push their bodies underneath but were getting caught at the hips. At this juncture all traps were taken into the workshop and two hooks (bull wire was used to form the hook part of which was securely fitted to the door) were fitted to the front to of the door and held up with garden wire. The amendment to the traps was designed by Peter O'Toole (NPWS) and myself. The force of the door shutting caused the hooks to fall out of the garden wire and latch under the trap, thus preventing the animal from pulling the door back even a little bit. After these were fitted no further escapees or injuries or fatalities occurred. No hooks were ever dislodged from the trap door (See Fig. 2.6.).

Prior to the fitting of the hooks a number of escapees had occurred, but no sign of injury was observed (blood on trap). Furthermore all trap doors were checked and reinforced while fitting hooks. For the duration of the study a total of 30 animals were trapped. The traps used in this study had been used in a previous study where no trap deaths were recorded (NiNeachtain, 1998; N = 9 animals live trapped). While I deeply regret the loss of both animals the KNP pine marten population appears to be relatively abundant and so the deaths are unlikely to damage the dynamics of the population (Chapter 3). The modification of the traps rectified the situation and prevented any further damage to the animals.

Live trapping was carried out on transects 1-7 between April and September in 2003 and 2004. Traps (N = 10 per transect; approximately 60 trap nights (T_n) per session; Table 2.2) were placed in exactly the same position as the hair traps (moved to one side for the duration of the trapping session), on transects where hair trapping (transects 1-5) had been carried out. All traps were generously covered with moss inside and outside to keep the animal both dry and warm (Fig. 2.6). This also acted as a natural concealment for the trap. Indeed on most occasions when the locked trap was checked the animals appeared to be curled up and asleep inside. The bait was placed behind the treadle and covered with moss. Traps were left locked open and pre-baited for two days prior to each trapping session starting. Each session lasted six days and each site was visited three times each year once in April/May, once in June/July and once in August/September. No live trapping was carried out on transects 6 and 7 in 2003 due to time constraints, which arose following the trap deaths and subsequent time spent in the workshop fixing traps (Table 2.2). Traps were set in the evening and were checked every morning starting at first light. The same transect line was used for both years

except for transect 6 and 7. In these sites the transect line was moved to a lower elevation within the woodland to facilitate access. Live traps were left *in situ* on both these sites for the duration of the study.

When an animal was caught it was processed in the following manner. Equipment was set out beside the trap. The bar-tool, a specially constructed tool with vertical bars, narrow enough to fit through the wire mesh and long enough to reach the wire mesh on the floor of the trap, is used to keep the animal at the back of the trap while work was being carried out at the front (Fig 2.7.). The transfer bag with handling cone attached was fixed to the front of the trap (Fig. 2.7). The bar-tool was then removed and the animal allowed to pass into the bag and then the handling cone. Once secured in the handling cone the animal could be processed (Fig. 2.8). It was found that animals if recaptured were reluctant to go back into the handling cone. If this was the case the animal was scanned while in the transfer bag. If a marked individual was recorded, then this animal was allowed to pass back into the trap from where it was promptly released. Unfortunately under Irish Law it was not possible to sedate animals, all veterinary sedatives are VSO (Veterinary Surgeon Only), thus animals were restrained. It is likely that animals associated the handling cone with being processed before, which involved an ear biopsy, and were thus reluctant to re-enter a second time.

Table 2.2. Live trapping sessions for 2003 (1. April/May, 2. June/July and 3. August/September) and 2004 (4. April/May, 5. June/July and 6. August/September). Tn is the number of trap nights per session (60 was the maximum). SD is the standard deviation.

Transect	1 (T _n)	2 (T _n)	3 (T _n)	4 (T _n)	5 (T _n)	6 (T _n)	Total (T _n)
1	60	60	60	60	60	60	360
2	30	60	60	60	60	60	330
3	60	60	60	60	60	60	360
4	30	60	60	60	60	60	330
5	50	60	60	60	60	60	350
6	0	60	60	60	60	60	300
7	0	60	60	60	60	60	300
Total (T _n)	230	420	420	420	420	420	2330
Average (T _n)	32.85	60	60	60	60	60	332.86
SD	25.63	0	0	0	0	0	25.63

(a)



Garden Wire

Hook

(b)



Fig. 2.6. (a) Live trap with trap door shut concealed with moss. The covering of traps with moss was carried out for two reasons. Firstly to keep the animals dry and warm and secondly to conceal the traps from members of the public. Also shown are amendments to trap design (b) animal inside live trap prior to being handled.

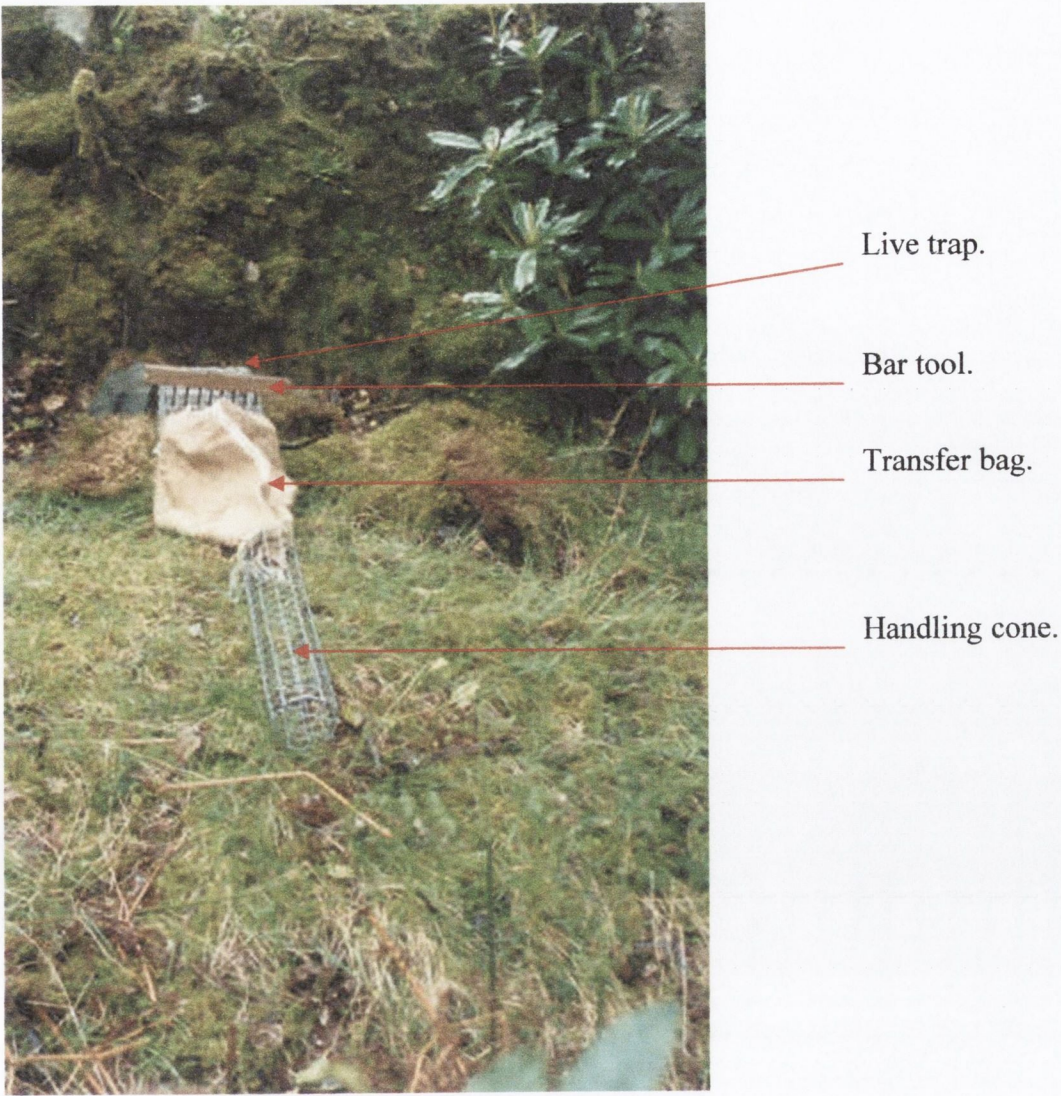


Fig. 2.7. The trapping equipment used in this study and how it was set up.



Fig. 2.8. A live captured animal being restrained in the handling cone, with bib clearly visible.

All animals captured were marked with a PIT (Progressive Integrated Transponders, Trovan, supplied by The Pet Chip Company, 4-5 Coleridge Gardens, London NW6 3QH, U.K.). Each tag was approximately 1cm in length, came fitted in a sterilised needle and was individually wrapped. The needle is fitted to an injection gun and the tag inserted subcutaneously in the scruff of the neck. Needles were only used once. It was felt that this was the best system available as ear tags may become caught or snagged and cause damage to the animal. For the duration of the study, no tag migrated or lost signal. These tags had been used previously by a colleague studying red squirrels (*Sciurus vulgaris*) and were found to be of excellent quality. They are currently being used on otters and stoats by other colleagues.

Each animal had an ear biopsy (2mm diameter disc) taken and a minimum of 20 hairs removed for DNA work (Chapter 6 & 7). Biometric measurements were taken of all live captured animals. Measurements taken included, the total length of the animal (from tip of nose to end of tail, cm), the body length (from tip of nose to beginning of tail, cm), the tail length (the distance from the base of the underside of the tail to the end of the tail bone, cm) and hind foot length (the distance from the end of the carpal bone to the end of the middle toe excluding the nail, nearest mm). The weight of an individual was measured using a 5Kg Pesola balance (Appendix 3).

The sex of the animal was determined by looking at the distance of the urinary tract from the anus, this distance being greater in males. Sex could also be confirmed by looking at the overall size of the animal and by their behaviour in the trap. Adult male animals are larger than females and the distance between the eyes is visibly greater. Males are also instantly aggressive when the trap is approached, vocalisations are louder and they will bluff charge in the trap. Females tend to be quieter and only begin to get aggressive when the trap is moved. Similar patterns of behaviour were also observed with juveniles. Breeding status was assigned to each animal based on presence of testes in males and lactation in females. Unfortunately breeding females could only be identified while nursing young owing to the presence of nipples. Lactation appeared to end in KNP by mid to late June. Certainly no females live trapped from July onwards were seen to be lactating. Animals were then released (Fig. 2.9).

Only on two occasions was it not possible to process animals. These two animals were both males. On the first occasion (Transect 7, Session 2, 2003), the animal refused to enter the handling cone. It was let go after about five minutes to avoid unnecessary stress but not before a hair sample was taken and a drawing of the bib made. On the second occasion the male (Transect 4, Session 3, 2003) was too big for the handling cone. It was decided to make a second handling cone specifically for males to avoid this situation arising again. All animals live trapped in 2004 were processed without any problems.



Fig. 2.9. Pine marten being released from handling cone after being processed. The animal was allowed back out without interference.

Carcass Samples

Letters were issued to each conservation ranger in the country, taxidermists were contacted and appeals by word of mouth were made for pine marten carcasses or samples to be sent to the Department of Zoology, Trinity College Dublin, Dublin 2. This resulted in 26 samples being collected from around the country (Appendix 4). The other two animals were the trap accidents during the survey period.

Sightings in KNP

All sightings from KNP of pine marten were taken from the wildlife records book at the ranger base (Appendix 1). Only sightings recorded by individuals with sufficient wildlife knowledge were used.

2.3. Laboratory Work

2.3.1. Dietary Analysis

Each scat was defrosted in the lab and dried to a constant weight in a 50°C oven and the weight noted (Appendix 5). The scat was then soaked overnight in detergent (steradent) to help break it up prior to being washed through two sieves (Manufactured by Retsch in Germany), 500µm and 125µm simultaneously. The run off from the two sieves was not collected for further analysis.

The microscopic component was considered to be that fraction retained in the 125µm sieve. This was collected and allowed to settle and was later investigated for the presence of earthworm chaetae. The macroscopic fraction was collected in the top sieve and was examined under a dissecting microscope. This fraction was divided into its separate components and identified using my own reference collection and following published keys (Day, 1966; Yalden & Morris, 1990; Chinery, 1993; Teerink, 1991). An attempt was made to identify each component to order (invertebrates, birds and mammals) or species (mammals, fruit, amphibians), though this was not always possible. No attempt was made to subdivide the beetles beyond Order Coleoptera.

It was found that the separation of components was most easily achieved using a fine paint brush and dissecting needle or forceps. Each of these components was then dried (oven @ 50°C, overnight was usually sufficient) and weighed separately (Goszczyński 1976 & 1986). All components were weighed while remaining in the small tube into which they were put after being dried. The balance used was a Mettler Toledo Balance AG245 and was correct to 4 decimal places. In order to account for the variation in weight that occurred with the sample tubes, ten tubes were weighed and the average weight calculated ($\mu = 4.1966$, $SD = 0.1645$). It was this weight that was then used to subtract from the total weight to get the weight of the component in grams. It was decided to use this method for two reasons. Firstly because once placed in the tube the static electricity inside meant that it was very hard to remove the entire sample, particularly fruit remains and secondly there were many samples (883). It is recognised that this is not wholly satisfactory but the errors that were generated would have applied to all the samples.

2.3.2 Molecular Work

Molecular work was carried out at the new molecular facility at the Department of Zoology, Trinity College Dublin. No work on pine martens had been carried out in the laboratory previous to this project. Pipette tips used in this study were unfiltered and were sterilised in the lab using autoclaving facilities available.

DNA Extraction

All tissue samples were extracted using a DNeasy Tissue Kit (Qiagen, Appendix 11). Samples were stored at -80°C and were allowed to come to room temperature prior to extraction [Samples 1-28 ear biopsy samples from KNP and Samples 29-56 RTA (Road Traffic Accidents) from around Ireland]. Irrespective of tissue types all samples were processed following the protocol: Isolation of Total DNA from Animal Tissues (kit handbook). A number of minor alterations were made to the protocol.

1. Ear biopsy (all) and leg muscle (25mg) were placed in a 1.5ml micro-centrifuge tube and 20 µl of Proteinase K added. The tissue was macerated using a pestle and then allowed to incubate at room temperature for twenty minutes.
2. Buffer ATL (180 µl) was then added to each tube, the tube vortexed and centrifuged at 7000 rpm for 5 secs (spun down) before being placed in a thermal block set at 55°C. All ear biopsy samples were left to incubate for two days (vortexed intermittently) and leg muscle was left to incubate for 3-4 hours or overnight as required (sample had completely broken down).
3. The sample was then vortexed (15 s) and Buffer AL (170 µl) added and the mixture incubated in a preheated thermal block (70°C) for 10 minutes.
4. Ethanol (95%; 200µl) was added to each sample, vortexed (15 s) and spun down.
5. The entire contents were then transferred into the DNeasy Spin Column which was sitting in a 2 ml micro-centrifuge tube. The sample was then centrifuged for 1 min at $\geq 6000g$. The flow through (liquid which passes into the 2 ml tube) and tube were discarded. The spin column was placed in a new 2 ml tube.
6. Buffer AW1 (500µl) was then added to the column and step 5 repeated.
7. Buffer AW2 (500µl) was added to the column and the above repeated, except that on this occasion the sample was centrifuged for 3 min at $\geq 20,000g$.
8. Buffer AE (200µl) was added, incubated for 1 minute at room temperature and centrifuged for 1 minute at $\geq 6000g$. This step was repeated resulting in 400 µl of eluate which contained the extracted DNA.

Only 6 samples were processed (steps 3- 8) on each occasion to prevent cross contamination or mix-up between samples. All extractions that were carried out were done so with a blank to control for contamination and for use as a -ve sample in future PCR work. 50µl of each extracted sample was aliquoted into a 1.5 ml micro-centrifuge tube and was used as the working sample. This was done as DNA may degrade when repeatedly defrosted and to control for contamination. All samples, both working and stock solutions were stored frozen at -20°C. To check for DNA quality and quantity each sample was run out on an agarose gel (1.5% Ethidium Bromide (EtBr) stained), if no band was observed or DNA was badly degraded then the sample was extracted a second time (Fig.2.10). This was only possible for samples 29-56 as I had an abundance of tissue. Samples 1-28 were extracted once.

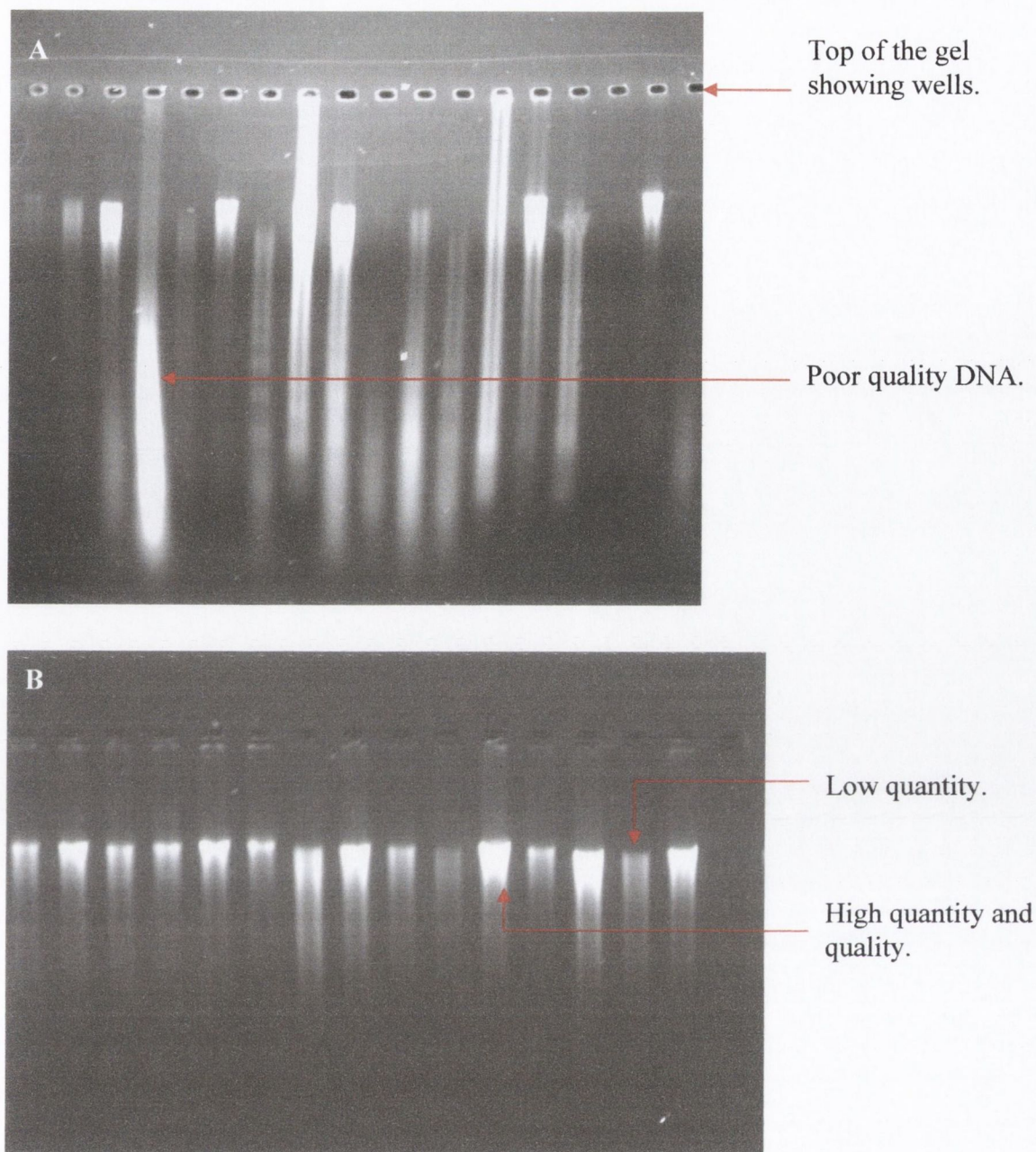


Fig. 2.10. Agarose gel showing DNA extraction from tissue samples. Lanes with DNA streaked the entire length is highly degraded, while lanes with DNA concentrated mostly at the top is of good quality. The intensity of the band is an indication of quantity. Figure a) shows DNA of varying quality and was taken from samples 29-56. Figure b) shows good quality DNA which is highly concentrated and was taken from ear biopsies (Samples K14-K28).

Two methods were used to extract DNA from hair samples (non-invasively collected hair samples). An attempt was made to use Chelex (a resin when suspended in water binds to the cells and causes the release of DNA from the nucleus by agitation) and proteinase K to extract DNA from hair. Many authors have used this method with good

success and it appears to be the standard method (with some variation on method between studies) for the extraction of DNA from hair samples (Walsh, Metzgar & Higuchi, 1991; Gagneux, Boesch & Woodruff, 1997; Taberlet *et al.*, 1997; Goossens, Waits & Taberlet, 1998; Frantz *et al.*, 2004). The following protocol was used, as it had successfully been used on red squirrel hair in the lab previously and is typical of the protocols using Chelex in the literature.

1. The roots of at least 5 hairs were added to each micro-centrifuge tube (1.5ml).
2. Proteinase K (5 μ l) was added.
3. Chelex (5%; 200 μ l) was added to each tube. Prior to adding the Chelex it was heated up on a hotplate and resuspended with the aid of a stirrer.
4. All samples were left to incubate over night at 56°C on a rocking platform.
5. Each tube was then incubated at 100°C for 10 minutes before being finally spun down at $\geq 20,000g$ for 2 minutes.
6. The supernatant was transferred to a clean 1.5ml tube.

All extractions were carried out with blanks to control for contamination. Samples were run out on an agarose gel (1.5%, stained with EtBr). As no bands were observed for this method another extraction method was used and gave good results.

The second method used was a DNeasy Tissue Kit (Qiagen) which had been used previously by Valderrama *et al.* (1999) with good results. The same protocol was used as for tissue samples with a couple of exceptions. Samples were always incubated overnight (8-14 hours) and for the initial incubation at 55°C this was carried out on a rocking platform and not in heated thermal block. The rest of the protocol remained the same. All extractions were carried out with blanks to control for contamination. Samples were run out on an agarose gel (1.5%, stained with EtBr) (Fig. 2.11). The use of the Qiagen kit though expensive, €200 for 50 preps (approximately), is a standardised method for the extraction of DNA and produces good clean DNA which is important for future PCR.

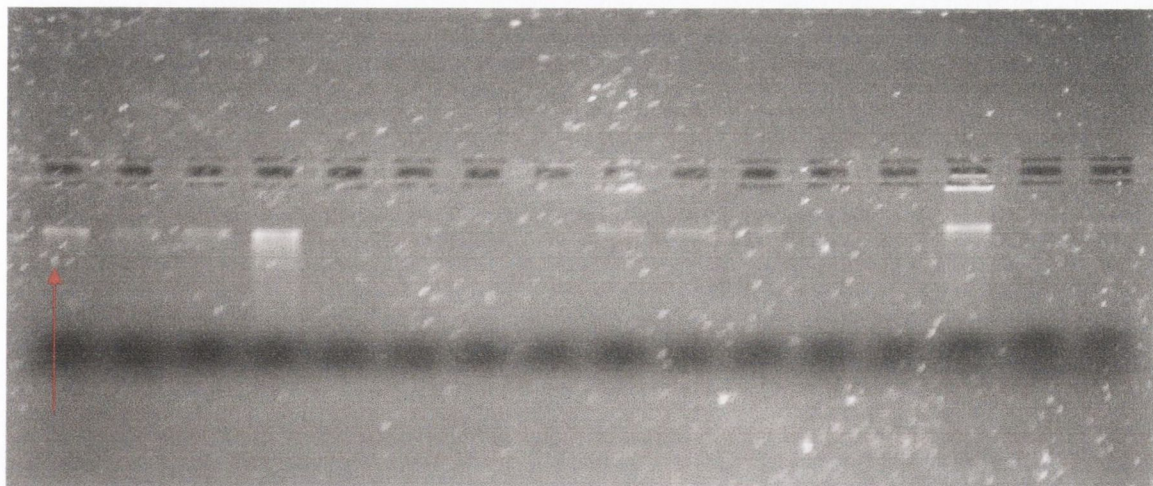


Fig. 2.11. Agarose gel showing DNA extracted from hair samples using Qiagen DNeasy tissue kit. Bands, indicated by the arrow, are low in quantity of DNA but of high quality.

PCR (Polymerase Chain Reaction)

PCR (Polymerase chain reaction) is a tool used in laboratories whereby defined segments of DNA can be amplified to microgram quantities from as little as a single template molecule. Prior to PCR occurring short oligonucleotide sequences, usually 15-35 bp (base pairs) known as primers, are designed, homologous to flanking regions either side of the sequences to be amplified. For the PCR reaction mix (master mix) excess amount of primer is added compared with DNA template in addition to the other reaction components (PCR-buffer, $MgCl_2$, dNTPs and Taq polymerase) (Hoelzel & Green, 1998). Primers can be used to target specific sections of any genes or nucleotide sequences and in this study were used to amplify microsatellites, areas of the mitochondrial genome (control region and cytochrome *b*) and a section of the SRY gene. The list of the primers used in this study is presented in Table 2.3. It is desirable to use several microsatellite markers, particularly those which are polymorphic (more than 1 allele at a locus). Such definition can allow for individual identification and good estimates of genetic variation. Two sets of primers were chose for the mtDNA work to determine if there was any variation within the mitochondrial genome not previously detected.

Table 2.3. Primers used in this study. All primers were degenerate primers having been developed for a species other than pine marten.

Primer Name	Primer Sequence (5'→ 3')	Size Range	Target Gene	Species developed for	Reference
MA-1-F	ATTTTATGTGCCTGGGTCTA	206-219	Microsatellite	American marten	Davis & Strobeck (1998)
MA-1-R	TTATGCGTCTCTGTTTGTCA				
MA-2-F	ACCCATGAATAATGTCTTAT	168-180			
MA-2-R	ATCTTGCATCAACTAAAAAT				
MA18-F	TGGGTGGGTGTATTTGTGTAT	151-165			
MA18-R	TACTCAGTGGGGAATCTGCT				
MA19-F	AAGGCTTATGGATACCACAT	201-211			
MA19-R	GATCATTTGGTATTTGTCTTTC				
GG7-F	GTTTTCAATTTTAGCCGTTCTG	154-172		Wolverine (<i>Gulo gulo</i>)	
GG7-R	GTTTATCTCCCTCTTCCTAACCC				
GG14-F	ACTGTGAGAGCAGTGGGAG	132-142			
GG14-R	GATCTCTCTCTGTCCAATAAAAT				
Ggu454-F	CTTCTTACATAGTCAATGTTTTG	137			Walker <i>et al.</i> (2001)
Ggu454-R	TGCCATTTTCTCCAGAA				
Lut604-F	TATGATCCTGGTAGATTAACCTTGTG	197-211		Otter	Dallas & Piernney (1998)
Lut604-R	TTTCAACAATTCATGCTGGAAC				
H15149	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA	364	Cytochrome <i>b</i>	Mammals	Irwin <i>et al.</i> (1991)
L14771	CAACATTCGTAAAACCCACC				Shields & Kocher (1991)
H16498	CCTGAACTAGGAACCAGATG	320	Control Region		Davison <i>et al.</i> (2001)
LRCB1	TGGTCTTGTAACCAAAAATGG				
LRCB3	AGACTCAAGGAAGAAGCAAC				
Lut-SRY-F	GAATCCCCAAATGCAAACTC	70	SRY	Otter	Dallas <i>et al.</i> (2000)
Lut-SRY-R	GGCTTCTGTAAGCATTTTCCAC	70			

The first temperature for the PCR program is necessarily high (95°C approximately) to cause the DNA template to denature, that is force the double helix to separate. The following temperature is lower (40-65°C) and is known as the annealing temperature of the primer; the optimum temperature at which the primer will bind to the DNA template. The temperature is then adjusted to the optimal temperature for the DNA polymerase (enzyme that catalyses the reaction; usually at 72°C) and this is known as the extension phase. This cycle of three temperatures are then repeated somewhere between 25-40 times. In the initial cycle, copies are made of the target sequence and, thereafter, more and more copies are made from other copies. The expansion is roughly exponential.

All work regarding the PCR reaction was carried out in the PCR hood: primer resuspension, making up of working solutions and preparation of master mixes for PCR. As the PCR hood was a communal facility strict rules regarding its use were in place to avoid cross-contamination between projects and within projects. The following procedure was always adhered to when using the PCR hood.

1. Remove all contents from PCR hood. Wipe hood down with ETOH (ethanol 95%). Wipe all containers to be placed in PCR hood with ETOH (95%): racks, pipettes (use 70% ETOH to prevent corrosion of pipettes), tips, water bottle etc.
2. Replace contents. UV for 1000 secs.
3. If making up working solutions or master mixes for PCR, aliquot DH₂O (sterilised deionised water) into the required tubes and UV hood again for 300 secs.
4. When finished using hood wipe down hood and contents with ETOH again and UV 1000 secs.

Resuspension of Primers

All primers used in this study were supplied by Invitrogen through their custom primers service. Primers were supplied desiccated and had to be resuspended in DH₂O. The volume of DH₂O to be added was calculated given the concentration of the desiccated primer and a requirement to make a stock solution of 50µM solution (Table 2.4). Working solutions (10 µM) were made by mixing the given primer (200 µl) and DH₂O (800 µl) in a separate micro-centrifuge tube (2 ml).

Table 2.4. The volume of DH₂O added to each desiccated primer to resuspend them. The numbers in parentheses refers to where a second set of primers were purchased and resuspended.

Primer	Concentration (nmoles)	Volume of DH ₂ O added (µl)
MA-1-F	42.6	852
MA-1-R	41.4	828
MA-2-F	99.4 (21.3)	1988 (426)
MA-2-R	62.2 (24.2)	1244 (484)
MA-18-F	50	1000
MA-18-R	42.3	846
MA-19-F	37.3	746
MA-19-R	58.2	1164
GG7-F	56.3 (36.8)	1126 (736)
GG7-R	51 (26.5)	1020 (530)
GG14-F	57.7	1154
GG14-R	39.1	782
Gg454-F	66 (34)	1320 (680)
Gg454-R	47.2 (23.6)	944 (472)
Lut604-F	46.9	938
Lut604-R	41.3	826
H15149	56.7	1134
L14771	47.9	958
H16498	50.6	1012
LRCB1	46.7	934
LRCB3	43	860
Lut-SRY-F	23.2	464
Lut-SRY-R	27.7	554

PCR Reactions

A list of the reagents, their initial concentrations and storage instructions can be found in Appendix 11. As a rule, working solutions were made for most reagents to prevent contamination and the degradation of stock solutions from repeated defrosting and refreezing. All the reagents and DNA template necessary for PCR were removed from the freezer and allowed to defrost on ice. When fully defrosted they were vortexed and spun down. All PCR's included a positive control (same sample throughout the study) and a negative control (blank used in the extraction of DNA). For each PCR master mix the following protocol was used.

1. After the initial UV phase, DH_2O was added to the master mix tube (2 ml micro-centrifuge tube), all PCR tubes (0.2 ml) were labeled and the UV light put on again (300 s).
2. Each of the components of the master mix was added to the micro-centrifuge tube with the *Taq* polymerase being added last. This mix was then thoroughly vortexed and spun down and placed on ice.
3. The required volume of master mix was added to each PCR tube. DNA template was then added, lids sealed and the contents vortexed and spun down before being placed in the PCR machine. Mineral oil was not needed as all PCR machines used in this study had heated lids.

The PCR machine had been switched on and was ready prior to the master mix being made. It was vital that everything was kept as cold as possible prior to being placed in the PCR machine to avoid the *Taq* polymerase becoming activated. The concentrations for each component for all PCR reactions are listed below.

PCR Programs

All PCR work was carried out on the same machine (Whatman Biometra Thermocycler) except where difficulties arose with individual primers and samples (see below). If problems arose with PCR then reactions were carried out on a PCR Express Thermal Cycler (Thermo Hybaid) firstly and then on a PTC 200 Peltier Thermal Cycler (MJ Research) secondly. The former of these had a temperature gradient so optimum annealing temperatures and MgCl_2 concentrations could be checked simultaneously. The latter machine was used, after all the reagents and DNA template had been checked for viability and were deemed to be working appropriately, as it was felt that the machine may be responsible for the consistent PCR failure. Appendix 9 presents the number of PCR's carried out for each sample for each set of primers. Chapter 7 deals

specifically with the SRY gene as the number of times a sample was typed was part of the experiment carried out and therefore is not included in Appendix 10.

Problems Encountered with PCR

A number of problems were encountered with PCR work and was primarily a concern for microsatellite work. For samples 1-28 problems arose with primer sets MA2, GG7 and Ggu454. After five rounds of PCR no product had been visualised for any of these primer sets. Initially the *Taq* polymerase was checked for viability but this was not the problem. A $MgCl_2$ concentration curve (1→6mM) together with an annealing temperature gradient was carried out (PCR express Thermocycler) but once again this was not the problem. After this it was decided to purchase new primers as primer sets can degrade quickly. It was found that the new primer sets worked well at all concentrations except for primer set Ggu454 which worked best at 1.5mM for positive control samples. The PCR was rerun for all samples but no band was observed except for the positive control. The DNA template was checked for DNA degradation and the PCR rerun varying the amount of template (1:25 dilution →5µl of undiluted template). This again had no effect. Finally it was decided to rerun the PCR using a hotstart reaction and a different PCR machine (PTC 200). A Qiagen HotStart PCR kit was used (Appendix 11). Positive PCR occurred for primer sets MA2 and GG7 but not for Ggu454. The PCR was rerun again and on this occasion the remaining samples for MA2 and for primer set Ggu454 amplified (Appendix 10).

For samples 29-56 only samples Ce2, Ce5, G6 and L1 failed to amplify for one or more of the microsatellite primers. It was decided that this was probably due to the presence of null alleles (see Chapter 6, section 6.1.2) at these loci, as they had amplified at other loci. Only the Ireland samples were used for mitochondrial work. It was decided that as sequencing of samples was expensive and resources were limited then samples which did not amplify after two rounds of PCR would not be used. This resulted in 25 samples being sequenced for the control region and 19 for the cytochrome *b* region. Davison *et al.* (2002) used a combination of the primers sets H16498 and LRCB3 or H16498 and LRCB1 to amplify the control region, citing that the primer set which worked best was sample dependent. After a trial run with both sets of primers, for samples Kel, Ky1, Oy1, Ce6, Ce7 and Ce8, the best results were found with the primer set H16498 and LRCB3. Given the expense involved with sequencing it was decided to use this primer set on all subsequent samples.

Sequencing

All samples were sent to Lark Technologies Ltd., UK to carry out sequencing. Samples were sent as PCR products and were sequenced using the Silver Service. Sequencing was carried out on an automated ABI system. Results were returned by email.

Gels

Two types of gels were used in this study to visualise DNA and products of PCR.

Agarose Gels

Agarose gels were used to visualise DNA following extraction as stated above and for the visualisation of PCR products. Only for the SRY work were agarose gels used for the sizing of PCR products. Gels differed in the % agarose depending on the product being analysed. Typically 1.4 % gels were used for visualising total genomic DNA, 1.7% gels for visualising MtDNA PCR products and microsatellite products and 2 % gels for visualising PCR products from the SRY reaction. Agarose gels were used to check for positive PCR success for microsatellites as the spreadex gels used for microsatellite analysis are both costly and take a long time to run. All agarose gels were made up in the following manner.

1. The correct mass of agarose was placed in a conical flask (250 ml) with TBE (Tris Borate EDTA; x 1; 50ml). DH₂O (20 ml) was added as this was determined to be the approximate volume of water lost due to evaporation when boiling. The contents were swirled to mix and then placed in a microwave and boiled at full power for 2.5 min.
2. The flask was then removed and the contents allowed cool till it was possible to keep the base of the conical flask against the back of the hand without a burning sensation.
3. The comb (creates the wells into which the DNA is placed, size of comb is dependent on number of wells required) was placed in the mini gel tank (VWR International) and when the agarose had sufficiently cooled down it was poured into the gel tank and allowed set.
4. Once set, the combs were removed and TBE (x 1; 50 ml) was poured over the gel (running buffer).
5. Each sample (5 µl) to be loaded was mixed with loading dye (2 µl) and 5µl of this mixture was added to each well. A sizing ladder may or may not have been included depending on the samples being run.

6. The lid was then replaced on the gel tank, ensuring the electrodes matched up and the gel was run at 90 V. The length of time gels were run for varied, dependent on the samples and if two rows of samples were loaded in each gel, but was generally between 15 -30 min.
7. All gels were visualised using the Alpha Imager 3300, which is a gel documentation system.

Spreadex Gels

Spreadex gels were used to size microsatellite products as they allow for the separation of bands to the 2 bp (base pair) level, which can then be visualised using the Alpha Imager 3300. These gels (S-2 x 25 or S-2 x 13, Appendix 11) come ready prepared and are specifically designed to be used with SEA 2000 (Advanced Submerged Gel Electrophoresis Apparatus, Elchrom Scientific). The following protocol was used for running gels on this gel apparatus.

1. The gel tank was preheated to 55°C. The running buffer (TAE (Tris-Acetate-EDTA) 30mM) was replaced after every 5-6 gels that are run.
2. Gels were placed in the tank with plastic backing still attached (either S-2 x 13 or S-2 x 25 depending on number of samples to be run).
3. Samples (4 µl) were mixed with loading dye (6 µl) and added to the appropriate well. Two sizing markers were also used. The M3 marker (0.2 µl) was added to loading dye (6 µl) and always added to the first well on the gel. The M3 marker allows correct sizing of the DNA ladder should the end of the ladder run off the gel. The DNA ladder used for microsatellite analysis was a 10bp ladder and was always placed in the second well and last well. When used with S-2x25 ladders were frequently run in the middle well (13). The lid and temperature probe were replaced.
4. Gels were allowed to run for 2→2.5hrs depending on product size at 120 V.
5. Once finished running, gels were taken from the tank and the plastic backing removed using the easy peel system. Gels were then placed in a staining tray with TAE (50 mls) and sybr gold stain (5 µl). The tray was completely covered with a cardboard box and placed on a rocking platform (room temperature) and allowed to stain for 40 minutes, after which they were washed with DH₂O three times and then placed back on the rocking platform for 1 hour submerged in DH₂O (50mls).
6. Gels were visualised using the Alpha Imager 3300 system.

Some problems arose with gels regarding clarity and when this occurred the samples were run a second time. Samples were either homozygous (1 single band) or heterozygous (2 bands). Shadow bands also occurred but these could readily be told apart (Fig. 2.12). Within the population a number of alleles could be observed at the same locus. On occasion it was not possible to size bands if they were too faint or too bright (Fig. 2.12). In this case the gel was rerun using more or less of the PCR product as required. On occasion slippage was observed. Slippage refers to an error that occurs during replication when the new DNA strand pairs incorrectly with the template strand. It can be caused by incorrect annealing temperature. Thus PCR was repeated and if slippage occurred again, a range of annealing temperatures were examined to find one at which slippage no longer occurred. This was only a problem for primer set GG7.

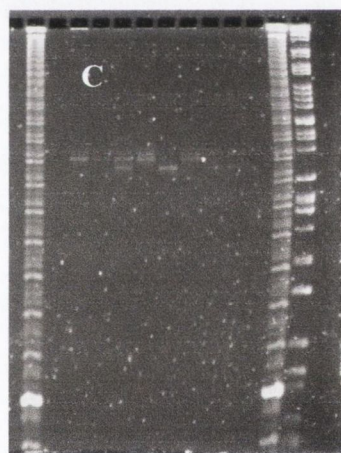
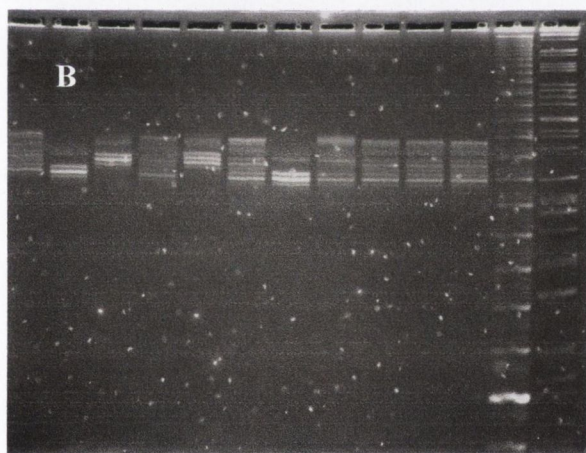
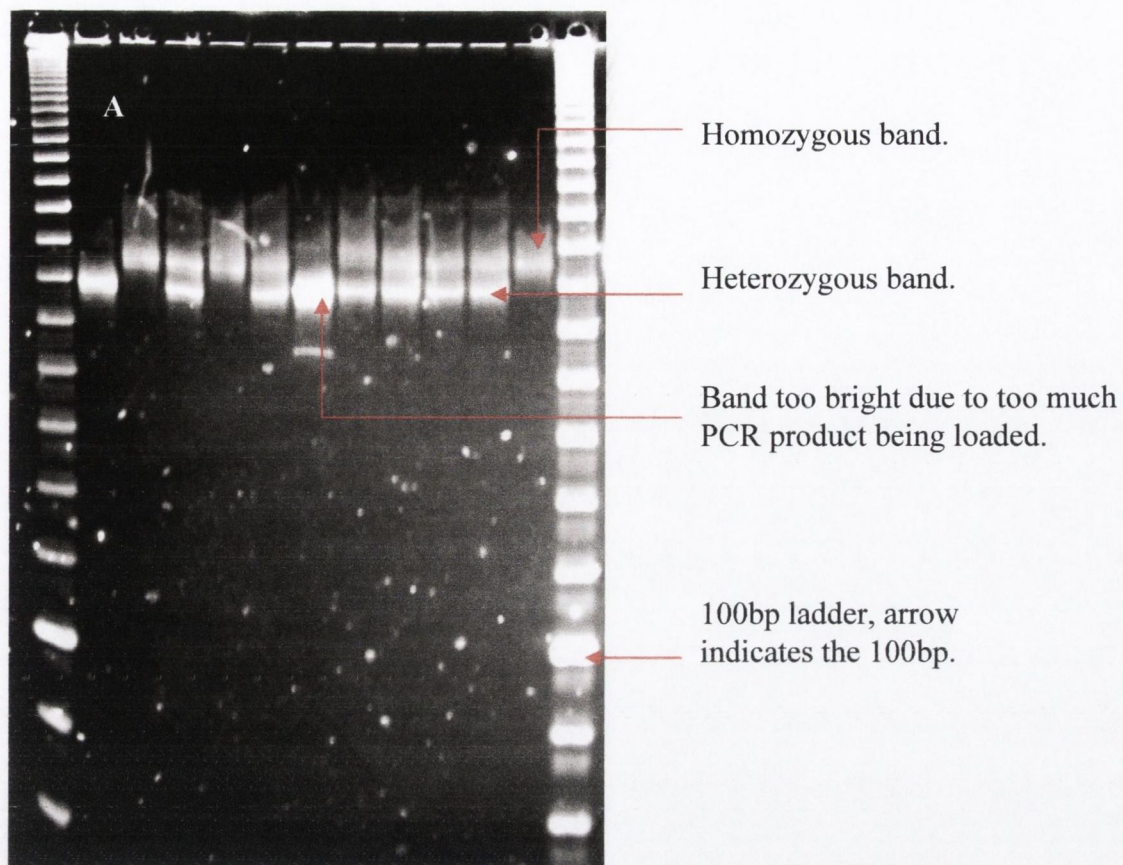


Fig. 2.12. Spreadex gels showing A) homozygous, heterozygous, bright bands and 100bp marker. B) spreadex gel showing slippage (multiple bands). C) Spreadex gel showing faint bands.

CHAPTER 3. CURRENT DISTRIBUTION AND STATUS OF THE PINE MARTEN IN KILLARNEY NATIONAL PARK: AN ACCOUNT OF EXPLOITATION, EXTINCTION AND RE-ESTABLISHMENT.

Abstract

A multi-evidence approach (sightings, scat location, hair trapping and live trapping) was used to determine the current status of pine marten within KNP between April and September in 2003 and 2004. Sightings of pine marten are now a regular occurrence. Scat location revealed positive proof of presence on transects 1-10 but not on transects 11 and 12, and they were most easily found in April/May compared with June/July and August/September. Hair traps were useful in detecting pine marten presence in the most isolated sites, transects 11 and 12. A total of 30 animals were live trapped over the two year period with a female bias in the live trapping (1.18:1) and was attributed to the methodology used. Both adult males (N = 9) and females (N = 13) were found to be fecund though it was not always easy to tell whether females had bred in a given year as this was dependent on time of first capture. Six sub-adults and two kittens were also identified during live trapping. Males were found to be significantly larger and heavier than females. Pine martens are now widespread throughout KNP. It is apparent that the population is now reproducing and holding its own within the park boundary. Though the pine marten has shown itself to be adaptable in the face of a changing environment there is no reason to think that it will be able to adapt to meet all the changes and thus healthy source populations like the pine marten in KNP will be necessary to maintain the longevity of this species on our shores.

3.1. Introduction

Many species of mammals worldwide face serious threats to their existence due to anthropogenic factors: hunting, illegal trade, habitat loss and fragmentation and pollution. Recent estimates suggest that current rates of species extinctions are at least 4 to 5 times background rates recorded from the fossil record (Mace *et al.*, 1996). In the developed world, where there are abundant resources available for mammal conservation, emphasis is placed on the protection of rare and endangered species, though it can be argued that enough is not being done. In the developing world the problems associated with mammal conservation are more complex, complicated by issues such as high human population growth rates, poverty and disease.

Mustelids have been and still are exploited worldwide, primarily for their pelts, but also as species to be hunted for sport and occasionally for food or controlled as pests or vermin (Harting, 1894; Fairley, 1975b; Brainerd, 1990; Strachan *et al.*, 1996; Adamowicz & Condon, 1997; Charles, 2000; Ryan, 2001; Vinkey, 2003). There is a small amount of evidence to suggest that pine martens were used as food in prehistoric times (Mesolithic era) in Europe. The remains of two animals were found at Starr Carr in Britain, a well known Mesolithic site, displaying marks consistent with butchery and meat removal, but this species was primarily sought for its fur (Charles, 2000). Some proof exists to imply that they may have been consumed as a delicacy during historic times also e.g., pine marten occurred on a bill of fare for a wedding in Yorkshire during the reign of Henry VIII ('Martens to Pottage'), but once again the pine marten was chiefly sought for its luxurious pelt (Strachan *et al.*, 1996). Such was the demand for its fur that the same authors report that by the fourteenth century pelts were being imported from Wales, Scotland, Northern Europe and Scandinavia to meet consumer desires in England; the pine marten being apparently scarce there now (Strachan *et al.*, 1996). The net result of this level of exploitation coupled with habitat loss (deforestation and increasing and expanding human populations) is that the pine marten has been reduced in number across its European range, so much so, that it is now considered rare, vulnerable or endangered in many European countries (Rudge, 1987; Kyle *et al.*, 2003).

3.1.1. The Pine Marten in Ireland

The gaelic name for pine marten is 'cat crann' which translates into english as tree cat. Indeed many references to cats in old Irish poetry and place names with cat in them are thought to be associated with this species: Carrickacat (County Mayo) & Carrigacat (County Cork) both meaning rock of the cat; Craignagat (County Antrim) meaning crag of the cats; Glennagat (County Tipperary) meaning glen of the cats; Knockaunacat (Counties Mayo, Galway and Waterford) meaning hill of the cat (Hayden & Harrington, 2000). Originally called marterns, Sleeman (1989) suggests that this was changed to martin and then subsequently changed to marten to avoid confusion with the bird (martin); marten is apparently a recent innovation.

Historic accounts primarily dating between 1700 and recent times, point towards the pine marten being abundant in Ireland till the beginning of the 20th century despite this species being extensively persecuted for its fur. An early account narrated in Fairley (1975b) by Giraldus Cambrensis in 1183 tells of a hunting scene in Ireland involving

marten, which are described as plentiful in the woods, but Fairley (1975b) describes the author's work as an 'amalgam of fact, fibs, and fantasy, much of it is patently absurd'. While the hunting scene was possibly exaggerated, in all likelihood martens were plentiful in the 10th century. Two later accounts in Fairley (1975b) indicate that marten's pelts were exported to the UK as early as 1430:

'I caste to speke of Irelande but a lytelle.
Commoditees yit I woll entitelle,
Hydes, and fish, samon, hake, herynge,
Irish wollen, lynyn cloth, faldyng,
And marternus gode, bene here merchandyse,
Hertyns hydes, and other of venerye,
Skynnes of otere, squerel, and Irysh hare,
Of shepe, lambe, and fox, is here chaffre.'

Harting (1894) reports that at one time martens were considered to have been distributed throughout Ireland, and gives an account from a Dr. John Ruddy in 1772 which states that 'the Martern or Marteron, *vulgo* Marten, is found at Lutterel's town [County Dublin], that it destroys rabbits and poultry, and is almost as mischievous as a fox'. County Dublin would have been one of the most densely populated areas in Ireland at that time. Furthermore, Ryan (2001) reports on two gentlemen (landed gentry) wearing coats lined with the hides of Scottish wild cats (*Felis sylvestris*); the collars and cuffs being made from pine marten and polecat (*Mustela putorius*) as being characteristic of their class and period (*circa* 1800). Heavy exploitation for the fur of the pine marten (13-20th centuries) and habitat loss (17-19th centuries) had by 1894 gradually restricted their range to the north and west of Ireland (Harting, 1894).

There is little evidence in the Irish literature to suggest that martens were actually hunted for sport ('hunting the mart') like other species such as foxes, otters and deer or eaten as they were in U.K. (Strachan *et al.*, 1996). Stringer (1977) in his book 'The Experienced Huntsman' (first published in 1714) gives detailed instructions on how to find, chase down and kill martens with hounds, sticks and guns but warns that it is the sport of young men as it is all on foot. However no further account of 'hunting the mart', despite Strachan *et al.* (1996) uncovering hunting diaries and sporting journals in Britain that gave extensive and detailed accounts of the hunt [marten] and its excitement, was found for Ireland. It is however entirely possible that pine martens were

frequently the quarry of gamekeepers. In all of Ireland in 1851 there were 591 individuals registered as gamekeepers (Ryan, 2001). The pelts of the pine marten were probably sold for profit, Lord Kenmare's gamekeeper (Killarney area, County Kerry; Fig. 3.1) in 1877 reported them as no uncommon thing often fetching 7s 6 d (Harting, 1894), but whether this revenue was returned to the estate or kept by the gamekeeper is unknown.

Interestingly, in Ryan's (2001) in-depth analysis of game stalking and shooting on 4 estates in Ireland, he uncovered only one gamekeeper's vermin list (1905/06, Grehan Estate in County Cork) which, amongst other species included 31 cats, but these were considered to be domestic cats (*Felis domesticus*) and not pine martens. It is entirely possible by the 1900's that the pine marten had become so rare in much of Ireland that they no longer featured as part of the gamekeeper's gibbet or were so just on this particular estate. Both Harting (1894) and Barrett-Hamilton (1894) had listed them as present in County Cork ten years previously.

A similar gamekeepers list from the Lissadell estate in County Sligo describes six cats being shot between 1861 and 1864, two of these at 'Cat Corner' (Gamebook 1862-1864, Public Records Office, Belfast). Between 1938 and 1945 a further 65 cats were recorded (Gamebook No 7, Public Records Office, Belfast) as 'removed' from the estate, but these were domestic cats while the earlier accounts may well have been about pine martens as these animals were killed by 'the party while out shooting'. This also supports the idea that pine martens weren't actually the main target of a hunting party, but once encountered became the quarry.

By the 1940's the pine marten was regarded as 'scarcely holding its own' in Ireland, occurring in only nine counties (Kerry, Waterford, Clare, Tipperary, Kilkenny, Wexford, Wicklow, Galway and Mayo; Fig. 3.1), this scarcity was attributed to the high price set on its fur (Moffat, 1938). Moffat advocated that, should the pine marten be given protection from the 'mercenary war being waged upon it for its fur, then it would recover its numbers. Pine martens were not to receive full protection from hunting, trapping or illegal killing till the late 1970's.

Trapping and hunting, the main contributory factors to the decline of the pine marten in Ireland, were only part of the problem. Large scale deforestation throughout much of

Ireland between the 1600's and 1800's (Aalen, Whelan & Stout, 1997) removed much of the habitat that this species makes its home in (Birks, 2002). Gerard Boate writing on the natural history of Ireland in 1726 indicates that there was great destruction of Irish woodlands primarily for economic reasons of which there were many: charcoal for iron smelting, timber for houses, clearance for agriculture and fire wood. Boate (1726) describes how in some parts of Ireland you could travel 'whole days long without seeing any trees except a few about gentleman's houses'. He also notes that 'for the great woods which the maps do present unto us upon the mountains between Dundalk and the Newry, are quite vanished, there being nothing left of them there many years, but one only tree, standing close by the highway, at the very top of one of the mountains, so as it may be seen a great way off, and therefore serveth travelers for a mark'. This would suggest that forestry clearance was so rapid that the maps of the day were out of date. Today woodland cover in Ireland, both private woodland and state forests, account for about 7% of land-cover; substantially more than would have been present between the 16th -19th centuries (Aalen *et al.*, 1997).

However gloomy a picture Boate (1726) paints of the destruction of the woodlands in Ireland, he also states that were still great forests to be found, even in Leinster despite it being the most populated area in Ireland at that time and also at present. In Ulster there appeared to have still been large areas of forests in Donegal, Tyrone, Antrim and Down, while he describes Connaught as still mostly forested, except for Sligo and Mayo. In Munster and particular Cork and Kerry, despite immense efforts by the landlords there to 'make great havoc' of the woods, forests were still remaining.

Notwithstanding the large clear-felling of woods in Ireland, as early as the mid 18th Century landowners were reforesting their lands (Carruthers, 1998; Ryan, 2001). The survival of the pine marten in areas like the Burren, where mature woodland was never a feature of the landscape (NíNeachtain, 1998), is evidence that the pine marten does not need old growth woodland to persist so long as it has an alternative for a den (rocky crags as opposed to tree hollows), though such features may not have been common in some areas which were deforested. Furthermore the testimony of many above indicated pine martens were abundant throughout this period of widescale deforestation and only became scarce towards the end of the 19th century. Perhaps deforestation played a secondary role in the demise of this species while game keeping activities and persecution for fur were the main threat in Ireland?

O'Sullivan (1983) while carrying out the only national census of pine marten in Ireland thus far specifies that ordinary people had for generations trapped the pine marten for the money they would receive for its fur but had given up by the 1950's when the animal became so scarce it was no longer worth trapping. A law dating from 1787, still in force in 1894, stated that any person who took, killed or destroyed a marten was to be rewarded with 5 s (shillings) (Barrett-Hamilton, 1894). Such trapping allegedly continued in County Clare (Fig. 3.1) and actually increased in this county until the pine marten was afforded full protection under Irish law in 1976 (Wildlife Act 1976, Amendment 2000). County Clare is still to be considered stronghold of the Irish pine marten. O'Sullivan (1983) states that habitat loss and sheep farming and the associated poisoning for predator control (strychnine poison was placed on baited carcasses mainly targeting red foxes and dogs throughout the 1950's and 1960's) were important factors in the disappearance of the pine marten from much of its former range due to the fact that pine marten readily includes carrion in its diet.

It is reasonable to assume that given poison was a feature of gamekeeping on estates in Ireland (Ryan, 2001) and that ordinary people had trapped this species for monetary gain (Moffat, 1938; O'Sullivan, 1983), the pine marten had disappeared from much of its range long before the advent of intensified sheep farming in the 1950's. Thus the pine martens may have survived, albeit at low density, in areas where intensified sheep farming and the associated predator control using poisoned bait (this was banned in 1981) was not widespread or practiced, Co. Clare for example (O'Sullivan, 1983). Langley & Yalden (1977) report a similar pattern of decline in Scotland, suggesting that the evidence of decline coincides with the advent of the game estate and keeping activities and was not due to deforestation. They further suggest that observed expansion by pine martens during World War One, during which time keeping activities lessened, supports their hypothesis.

3.1.2. The Pine Marten in Killarney

Pine marten were once very abundant in Killarney and its surrounding areas. Cusack, (1871) lists them as common near the Lakes of Killarney in her history of the Kingdom of Kerry. Barrett-Hamilton (1894) narrates a number of accounts from Killarney and surrounding areas that indicate pine marten were plentiful. Charles Akroyd shooting

(fowl) in 1879 on lands currently encompassed within KNP, was told by the beaters that marten cats were plentiful and was brought one the next day (Ryan, 2001).

On both the Kenmare and Muckcross estates which now make up KNP, the control of vermin was part of the duties carried out by the gamekeepers, the style of which was heavily influenced by gamekeeping principles in England and Scotland. Gamekeeping started in the mid to late 1700's for pheasants on the Kenmare estate and continued on for the next 270 years on both estates, through the famine (1845-1850), World War I and World War II and on the Muckcross estate when it was given to the state as a national park (Bourn Vincent Memorial Park: BVMP) (Ryan, 2001).

Activities of game keepers included game preservation, even in the remotest parts of the estates, and extermination of vermin through trapping and poisoning. Ryan (2001) reports on an incidence in which a gamekeeper's wife used his arsenic (for controlling vermin) instead of bread soda when making bread, resulting in six people needing medical attention. The fact that the pine marten readily takes carrion would have made it susceptible to such a campaign of poisoning. Given the Killarney valley has to some extent always remained wooded and pine marten were considered plentiful there till the turn of the 20th Century (main period of deforestation was 1600-1800), it is likely that gamekeeping activities and trapping as mentioned previously were primarily responsible for the near or total extinction of the pine marten from the Killarney Valley.

3.1.3. Translocations of Pine Marten to KNP

The intentional release of organisms into the wild is often considered by wildlife professionals as a tool for increasing population numbers and has been called by various names of which translocation is one (Griffith *et al.*, 1989; Wolf *et al.*, 1996; Vinkey 2003). Thus translocation broadly encompasses the deliberate release of captive or propagated and or wild caught animals into the wild with the intention of restoring or repatriating an extinct population, augmenting an existing endangered population, to facilitate range expansion of populations and / or to avoid conflict with humans (in Wolf *et al.*, 1996). Translocations as a conservation tool have been prevalent in the last thirty years. Of the 116 cases examined by Fisher & Lindenmayer (2000) the vast majority occurred within North America, Australia and New Zealand. Surprisingly at least one third of all the translocation programs carried out on these two continents were either done for unknown reasons or not for conservation. By comparison such programs

made up a small percentage of the translocation programs in Europe, Central & South America, Asia and Africa (Fisher & Lindenmayer, 2000). Overall the vast majority of mammal translocations (approximately 91%) were for conservation.

There are disadvantages to carrying out translocation programs with the express intention of wildlife conservation. Translocations are generally unsuccessful with programs using captive bred animals even less successful than those sourced from wild populations (Wolf *et al.*, 1996; Fisher & Lindenmayer, 2000). They are also expensive, with costs for mammal translocations and subsequent reintroductions in North America being well over a million dollars especially for high profile programs such as the gray wolf (*Canis lupus*) project (US \$ 6, 700, 000 over a period of 8 years) (in Fisher & Lindenmayer, 2000).

Several considerations need to be borne in mind prior to a translocation program commencing. Generally a large number of animals are required for release, which may not be possible if the population is extremely rare or endangered without first embarking on a captive breeding program. It is also essential to have public support for the project particularly when releasing a species that is likely to pose a threat to human safety (e.g. large mammalian carnivores) or compete with humans for resources they use themselves (e.g. game species) or are likely to attack livestock (Wolf *et al.*, 1996). In Australia 69% (11/16) of translocation programs failed when animals were released into areas with predators, thus the risk of predation to the new population must also be considered (Fisher & Lindenmayer, 2000). Furthermore the program is also not likely to succeed unless the cause of decline has been specifically identified and removed. Fisher & Lindenmayer (2000) found that in the cases examined none of the programs which identified the cause of decline but failed to remove it were successful. By contrast when the cause of decline was stipulated nine out of the 41 programs were successful while only 3 were considered to have failed. The outcome of the remainder was still unknown at the time of publication. In addition to predation the role of disease must also be taken into account, both to the translocated animals and recipient ecosystem or population that will be receiving them (Wolf *et al.*, 1996). The potential effects of reduced population size and or small founding populations and subsequent inbreeding include reduced fecundity and survival (in Bodkin *et al.*, 1999).

To aid the success of translocations the IUCN/SSC (Species Survival Committee) drafted guidelines for reintroduction or translocation programs with the express intention to act as a guide for procedures useful to such programs. The guidelines outline that the manager should have clear knowledge of the pre-project aims, which include a feasibility study and background research, knowledge of previous reintroductions of the same or similar species, choice of release site and type (which should occur within the historic range of the species), evaluation of translocation site to check for suitability and removal of any the factors which caused the decline, availability of suitable release stock. The guidelines require a budget which takes into account the long-term nature of the project and which also has the full support of all interested parties (e.g. local communities, landowners, governmental agencies etc.). The guidelines also call for the soft-release of animals which tends to be another factor in successful translocation programs (Wolf *et al.*, 1996). They finally require that follow up monitoring be carried out to include ecological and behavioral studies of released individuals, causes of mortality to be investigated, continued public relations to increase awareness, evaluation of the cost-effectiveness of the project and publication in scientific journals.

Thus a key issue associated with translocations is defining how to measure success of a translocation program. The establishment of a self-sustaining population will be a key measure of success, while conversely a more pragmatic approach may be taken and success assigned if initial signs of ecological success are observed (e.g. use of a man made den or burrow) (Fisher & Lindenmayer, 2000). Ultimately success can only be determined if initial criteria for success are laid down and at a later stage followed up to see if the population meets all the criteria. As mentioned above the success of a translocation program can occur anywhere along a continuum from use of man-made structures to continued presence some time after the last release, to establishment of a viable population. Thus as no criteria were given to evaluate the success of the program I have laid down suitable criteria for success of the program. The program was deemed successful if the presence of pine marten was located throughout the woodlands of the park, if animals were found to be breeding, young were found in the population, and biometrics were typical of animals studied elsewhere.

Over a period of nine years (1987-1995) pine martens were translocated to KNP for the purpose of re-establishing the population. Approximately 30 animals (Table 3.1; exact

numbers unknown, data taken from wildlife ranger's diaries and wildlife book, ranger base, KNP) were moved from Counties Clare and Mayo (Fig. 3.1) and released using IUCN soft release guidelines. These animals were considered 'problem' animals, having been caught in people's houses or attacking poultry (Paddy O'Sullivan *pers. comm.*). All animals upon arriving in the park were placed in an enclosed pen for up to one month (unless they escaped) prior to release (Fig. 3.3, Transect 3, location of release site). Once released, food was placed at the pen till the animals stopped returning (evidenced by food not being taken). Only one of the released animals is known to have died having consumed rat poison in a nearby shed.

3.1.4. Objectives

The objective of this study is to evaluate the success of the translocation of pine marten to KNP using a multi-evidence approach to detect their current distribution (sightings, scat location, hair trapping and live trapping). I also assessed the health of the population through the biometrics and reproductive status of live trapped individuals. Together this information will provide an evaluation of the translocation program and will give the current status of the pine marten population within KNP. I hypothesised should the population of pine marten meet the above criteria then the population could be considered to be established. If they did not meet all the above criteria then they would not have.

Table 3.1. List of animals translocated to KNP between 1987 and 1995. Where the area has been left blank, this indicates there was no information available. Apart from the two male animals that arrived in July 1993 (County Mayo) the rest of the animals had come from County Clare. 27 animals are listed below but it is believed that approximately 30 animals were translocated. All animals were released from the same point, which was located in the area covered by transect 3.

N	Arrival Date	Release Date	No. Animals	Comments
1	Oct 1987	Nov 1987	10	1, a female, died (12/12/1987) as a result of rat poison.
2	Sep 1989		2	
3	Dec 1989		1	Male
4	Oct 1991		1	
5	Dec 1991	Feb 1992	1	
6	July 1993	Aug 1993	2	Both male & from Mayo
7	June 1994	Aug 1994	2	
8	June 1995	Aug 1995	5	1 adult female & 4 kittens.
9	Sep 1995	Oct 1995	1	
Total			27	

3.2. Methods

Chapter 2 has detailed descriptions of each of the four methods (sightings, scat location, hair trapping and live trapping) used to determine pine marten distribution and status of the population.

3.3. Statistical Analysis

A one way ANOVA was used to test for differences between the numbers of scats collected per transect each year. The minimum number of martens alive (M.N.A.) in the different sites was used because the number of captures and recaptures was very small, which prohibited the use of population estimates such as the Jolly-Seber or Fisher-Ford methods. Population estimates are given as the number of animals per km² of transect. The biometric data were normally distributed, thus pooled t-tests were carried out on the data to determine if there were any differences due to sex or age. The significance level was $p = 0.05$.

3.4. Results

3.4.1. Sightings results

Between 20/06/1985 and 01/08/2004 a total of 45 sightings of pine martens occurred within KNP (Fig. 3.1, Appendix 1), with 87% of sightings occurring after the translocation of pine marten ended (1995). Of the six sightings that occurred during the translocation time period (1987-1995) two were on transect 3 (where the release site was), two were on transect 1, one was on transect 6 and one was on transect 8. Sightings of animals ranged from fleeting glimpses as they dashed across the road to watching two individuals apparently fighting, though this may have been early courtship behavior as it occurred in mid July (Appendix 1). Three of the sightings were animals that had been killed on the road (1999 and 2000). A further two animals have been killed on the road since, though these were not recorded in the wildlife book and are not part of Fig. 3.1. Many of the sightings were during the day and there was one account of a pine marten chasing two squirrels across a field. Over the nine years of recording, the greatest number of sightings was in area 3 of the park while no sightings were recorded in areas 7, 11 & 12.

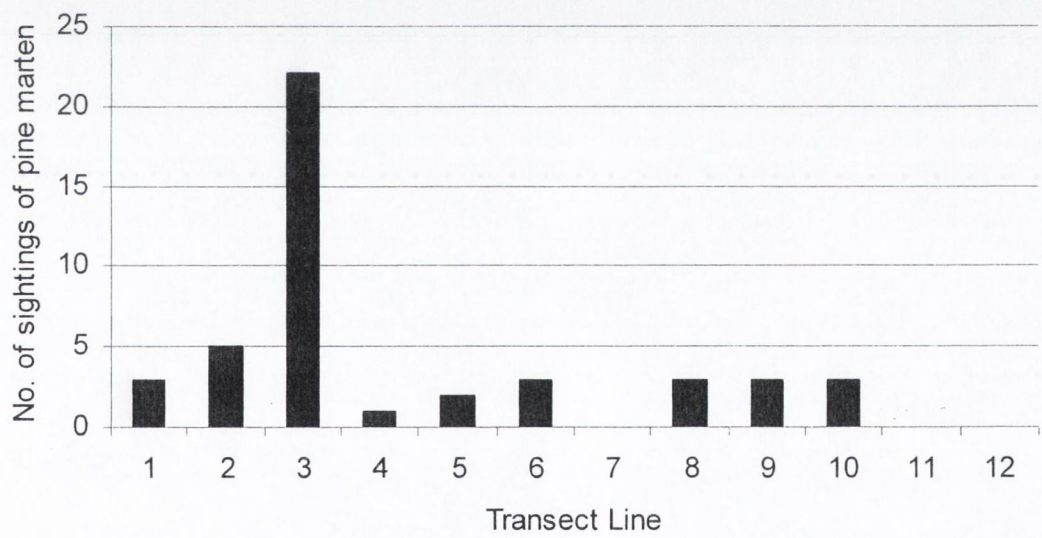


Fig. 3.1. The number of sightings of pine martens recorded between June 1985 and August 2004 for each of the twelve transect areas within the park. No sightings were recorded in areas 7, 11 and 12.

3.4.2. Scat Location

A total of 212 scats were collected during the systematic surveying of the 12 transects, which resulted in 193.3 km of transect being walked over the two years. Of the 212 scats collected 150 were located in 2003 and 62 in 2004 (Fig. 3.2). Overall there was no significant difference found between the number scats collected in 2003 and 2004 for the 10 transects (One way ANOVA: $F = 1.44$, $DF = 8$, $P = 0.18$; transects 11 and 12 were excluded from analysis as scats were collected at a different frequency, they accounted for 13 samples). Post hoc tests (LSD) showed that there was a significant difference in the number scats collected between transect 1 and transect 2 ($P = 0.009$). No significant difference was found between sessions and transects (One way ANOVA: $F = 7.6$, $DF = 5$, $P = 0.27$), though there was a trend for most scats to be collected in the first session (April/May) of each year.

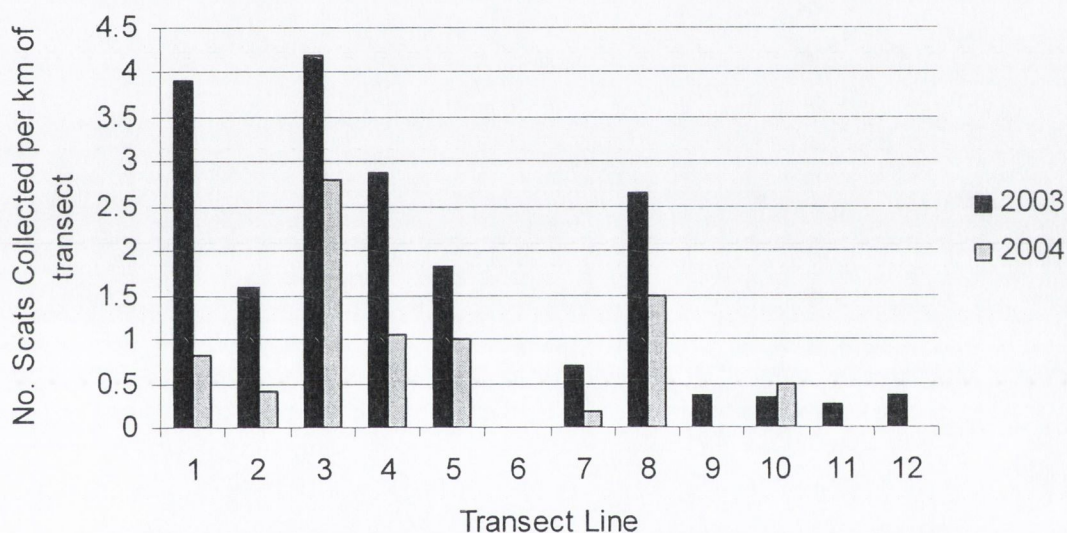


Fig. 3.2. The density of scats collected on each transect expressed as the number of scats collected per kilometer (km) of transect walked for each year. No scats were located on transect 6 either year.

3.4.3. Hair Trapping

The overall sampling effort resulted in 1,210 trap nights for hair traps, which broke down as 650 trap nights in 2003 and 560 trap nights in 2004. The difference in the sampling effort for the two years is accounted for by transect 12 being visited once in 2004, owing to its remote location (access was by boat only). Transect 11 had been visited 13 times in 2003 but only 10 times in 2004. Of the 7 transects where hair trapping was carried out (transects 1-5, 11-12) a total of 100 hair samples were retrieved

during the systematic surveying. 52 samples were collected in 2003 and 48 samples in 2004 (Fig. 3.3). Chapter four contains the data (trapping success and latency to detection) for the hair traps. The data from transect 2 and 12 were not included in that data set (Chapter 4) and accounted for a further 12 samples being recorded.

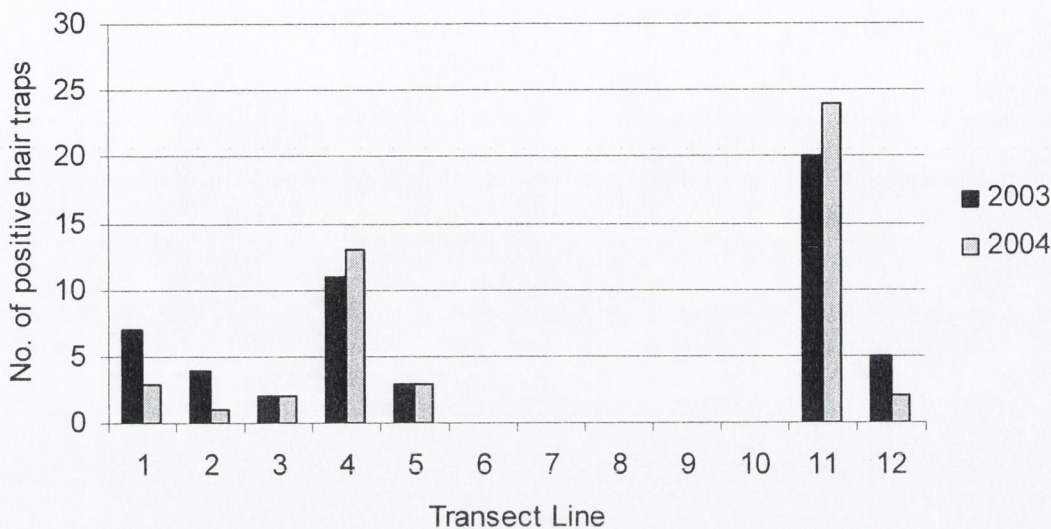


Fig. 3.3. Number of hair samples collected for each transect between April and September in 2003 and 2004. No hair-trapping was carried out on transects 6-10.

3.4.4. Live Trapping

The total effort for live trapping resulted in 2,330 trap nights, which broke down as 1,060 trap nights in 2003 and 1,260 trap nights in 2004 (average trap night per session: $\mu = 55.24$, $SD = 14.69$). The average number of trap nights, across all transects for both years, taken to identify a new individual was approximately 127 ($\mu = 127.11$, $SD = 144.73$), while the average number of trap nights taken to catch a pine marten was approximately 36 ($\mu = 36.5$, $SD = 12.97$). Most animals were identified first in the period April/May for both years ($N = 17$), while the rest were done so during the June/July session ($N = 10$) and August/September trapping session ($N = 3$).

A total of 30 individual pine martens were live trapped throughout the two years while field work was being carried out (Appendix 3). 11 animals, five males and six females, were caught in 2003 and 22 animals, eight males and 14 females (three of these females had been identified in 2003), in 2004 with all transects being represented both years except transect 6 in 2003 (Table 3.2). The sex ratio for the trapped population was 1.2 and 1.75 female to male in 2003 and 2004 respectively and overall it was 1.5. A further

5 individuals (minimum, four males & one female) were identified through hair sampling and typing of the SRY gene (Chapter 7), bringing together a total of 35 animals identified within the boundary of KNP. When these animals are included the sex ratio for the trapped population is 1.18 females to 1 male. By comparison the sex ratio of the road killed population was 1.3:1 to males (Appendix 4). There was no significant difference in the sex ratio of the trapped population and that generated through the collection of road kill (Fisher's Exact Test: $\chi^2 = 1.7$, DF = 1, P = 0.15).

Population estimates varied between years and for transects (0.42 - 2 animals per km², Table 3.2.). The available woodland cover in KNP is approximately 12km². Though it is entirely possible animals are not solely restricted to woodland, this was the area taken to calculate the overall densities of pine martens in the park. Density estimates were based on known adults (2003: adults = 10; 2004: adults = 18). The density of adult pine martens in 2003 was 0.83/Km² and in 2004 was 1/Km².

Table 3.2. Indicates the number of animals live trapped per survey unit in both years. T is the transect. Survey periods correspond to the following times: 1- April/May, 2- June/July, 3- August/September and figures in the columns below refer to the number of animals caught. The figure in parentheses refers to retrapped animals in the following year. * refers to animals that were retrapped in the following year.

T	Year	No. Trapped	Survey Period			Sex	Total Caught
			1	2	3		
1	2003	1	-	1	-	♂	1
	2004	4	1	3	-	♂♀♀♂	4
2	2003	3	1	2	-	♀♀*♂	4
	2004	0(1)	1	-	-	♀	1
3	2003	1	-	1	-	♀	1
	2004	6	3	2	1	♀♀♀♀♂♂	14
4	2003	3	1	-	1	♀*♂	14
	2004	3(1)	3	-	1	♀♂♀	15
5	2003	2	2	-	-	♀♀*	3
	2004	2(1)	2	-	1	♀♀♀	4
6	2003	0	n/t	-	-		0
	2004	3	2	1	-	♂♀♂	4
7	2003	1	n/t	-	1	♂	1
	2004	1	1	-	-	♂	4
Total		30	18	10	5		70

3.4.5. Biometrics

The biometric measurements recorded in the field for the pine marten population in KNP are presented in Table 3.3. As pine martens are known to be sexually dimorphic the sexes were separated for analysis. To minimise stress caused to animals as they were restrained and not sedated, measurements were only taken once each year. As such all the data have been pooled together and differences arising from seasonality or locality have been ignored. Data are absent from two of the males caught in 2003 as these were too big to fit into the handling cone and therefore had to be released without measurements being taken.

For the entire trapped population males were significantly heavier than females (Pooled T-Test: $t_{\text{weight}} = 3.2$, $DF = 26$, $P = 0.002$). Overall males were significantly larger than females ($t_{\text{total length}} = 1.9$, $DF = 26$, $P = 0.03$) but when lengths were divided into body and tail this relationship ceased to be significant ($t_{\text{body}} = 1.7$, $DF = 26$, $P = 0.05$ and $t_{\text{tail}} = 1.4$, $DF = 26$, $P = 0.09$). The hind feet of males were significantly longer than females' ($t_{\text{hind foot}} = 4.2$, $DF = 26$, $P \leq 0.001$).

I removed the animals from the population that were considered to be sub adults ($N = 6$, 2 males & 4 females) and kittens ($N = 2$, 1 male & 1 female) and redid the analysis for the adult populations. Adult male pine martens were still significantly heavier than female pine martens ($t_{\text{weight}} = 7.9$, $DF = 18$, $P \leq 0.001$), were significantly larger overall ($t_{\text{total length}} = 4.1$, $DF = 18$, $P \leq 0.001$) and had longer hind feet ($t_{\text{hind foot}} = 5.4$, $DF = 18$, $P \leq 0.001$). When body measurements were broken down into body and tail, it was found that male pine martens were significantly longer than females on both counts ($t_{\text{body}} = 3.35$, $DF = 18$, $P = 0.002$; $t_{\text{tail}} = 2.01$, $DF = 18$; $P = 0.002$).

Table 3.3. Biometric data for all pine marten caught within KNP between April and September 2003 and 2004. All length measurements are given in cm (centimetres) and weights in kg (kilograms). SD is the standard deviation. Two of the males live trapped were not measured. Individual weights and measurements for trapped animals can be found in Appendix 5.

Measurement	Sex	Mean	SD	Range	N
Weight	Male	1.55	0.38	0.85-2.05	10
(Kg)	Female	1.22	0.16	0.8-1.35	18
Total Length	Male	68.1	10.27	51-79	10
(cm)	Female	62.2	6.23	48-73	18
Body	Male	45.1	7.01	33-54	10
(cm)	Female	41.3	5.12	33-50	18
Tail	Male	23.0	4.7	16-30	10
(cm)	Female	21.1	2.77	15-26	18
Hind Foot	Male	9.58	0.62	8.5-10.5	10
(cm)	Female	8.71	0.46	8-9.8	18

3.4.6. Reproductive Status

All adult males (N = 9) trapped during this study showed signs of being reproductive, that is, their testes were visible. Testes were not visible in younger males (sub-adult and kittens). The determination of reproductive status of females was more problematic as this is only possible while they are lactating, which in KNP appeared to stop by mid June. Of the adult females (N = 13) trapped during the two year period six of these were found to show signs of lactating while seven showed no signs. Of these seven all were trapped between July and September and it was therefore impossible to determine if they had reproduced that year. Three of the females caught in 2003 were retrapped in 2004 (transects 2, 3 & 4). The female trapped on transect 2 (Fig. 3.3) was first identified in August of 2003 and when retrapped in May 2004 was not found to be lactating. The remaining two females (transects 4 & 5) were both identified first in May 2003 and then again in May 2004, in each year they showed signs of feeding young. In 2004 two kittens were trapped in July on transect 3 (Fig. 3.3) and were assumed to be out hunting with their mothers during the night when they got caught. In each of these areas of

transect 3 two lactating females had repeatedly been identified prior to these captures. The overall percentage of juveniles (sub-adults and kittens) in the trapped population for both years was 27% and was 18% in 2003 and 32% in 2004.

3.5. Discussion

The history of the pine marten in Ireland and the Killarney valley is one of exploitation, extinction and re-establishment. A fundamental question in the history of this species in KNP is whether the pine marten ever went extinct or did a small remnant population remain undetected for a number of decades? Either could be true. This species is notoriously hard to sight and is often reputed to occur in areas for many years without being seen (NíNeachtain, 1998; Birks, 2002). The two sightings reported prior to the translocation (1982 & 1985), given the experience of the reporter (T. Caruthers, ex-KNP ranger), are suggestive of the presence of pine marten, though they were never confirmed. Genetic evidence has not been able to answer this question unequivocally either but it does point towards the extirpation of pine marten from KNP prior to the translocation (Chapter 6).

Apart from the sightings by Caruthers, pine marten had not been seen in KNP for quite some time, though the exact time frame is unknown (P O'Leary *pers. comm.*). O'Sullivan (1983) refers to an animal being trapped in 1979 in the locality but makes no mention of the reliability of that record or whether it was ever verified. Pine martens are often confused with mink and stoats by members of the public (*pers. obs.*). However within a couple of years of the first release, animals were being sighted up to 10 miles away from the release site. Several sightings of pine martens have been recorded each year in KNP (except for 1998, Appendix 1), some so good they have allowed the reporter to give detailed accounts of the animal's behaviour. This would indicate that at the very least the translocation had the impact of boosting numbers.

Many of the sightings of pine martens in KNP have been restricted to areas where access is possible by jeep; this species is often seen at night time when rangers are on deer patrol duties. The area covered by transect 3, while being the area of the release site, is the ideal place for seeing pine marten. A road (park use only, Dinis road) runs through this area and facilitates ease of access. The compost bin at the Arthur Vincent hostel also in the vicinity attracts pine marten, which scavenge the decomposing remains of the bin (*pers. obs.*; Appendix 1). The areas of the park where no sightings

have occurred (transects 7, 11 & 12) are remote and isolated and not often visited, thus it is not surprising that pine martens have not been recorded there.

One indication of pine marten presence, and a method commonly used in surveys of distribution of this species, is the simple method of looking for scats along transects (O'Sullivan, 1983; Velander, 1983; McDonald, Bright & Harris, 1994; Strachan *et al.*, 1996). Based on scat location I could determine with 100% confidence the presence of pine marten on nine of the twelve transects surveyed for scats (1-5, 7-10) but could not on transects 11 and 12. No scats, potential or otherwise, were ever located on transect 6. Given the ambiguities surrounding the correct identification of pine marten scats (Davison *et al.*, 2002; Chapter 2, section 2.2.2), it was decided that if scats were in an indeterminable state then presence could not be assigned to an area. Given this, I was unable, despite collecting scats I felt were pine marten, to determine with 100% confidence from which species they came from on transects 11 and 12. These transects were only walked fortnightly or monthly (respectively) and therefore scats were too old to correctly identify if encountered. O'Sullivan (1983) suggested that scats can only be determined as pine marten if collected within a maximum of seven days post deposition, but that this is very much dependent on rainfall, season and content. Pine marten presence in the areas covered by transects 11 & 12 was determined with hair traps.

Almost twice the numbers of scats were collected in 2003 compared with 2004. The difference in the two years can primarily be accounted for by the difference in the weather (Appendix 2). The field period of 2003 (April to September) was characterised by mostly long spells of dry sunny weather. In contrast 2004 was typified by heavy rain showers and windy weather a lot of the time. The net result of this was that scats were washed away prior to being collected or were in an indeterminable state when encountered. This has also been found with other Irish studies which have based pine marten presence on location of scats (Warner & O'Sullivan, 1982; O'Sullivan, 1983; NíNeachtain, 1998). NíNeachtain (1998) particularly found this to be a problem in broadleaf scrub woodland compared with the conifer woodland, suggesting that the height of trees and the density of planting in the conifer woodland produced a very sheltered environment in which scats could persist longer than in the scrub woodland.

The number of scats collected in each of the three sessions [April-May, June-July and August- September] decreased from April to September. This has also been found with

other studies (Warner & O'Sullivan, 1982; NíNeachtain, 1998) but not to the same extent. NíNeachtain (1998) attributed the decrease to climatic patterns, scats being washed away most easily in winter and spring. Warner & O'Sullivan (1982) typically found that scats were more abundant between April and October and dramatically decreased over the winter months. Pine marten activity is known to decrease in winter months with peak activity occurring between April and October (Zalewski, 2000). Furthermore, fur-based scats are likely to remain intact much longer in the field than scats composed of invertebrates or fruit (Birks, 2002). As the diet of the pine marten in this study consisted primarily of fruit in autumn (Chapter 5) then it is hardly surprising that the number of scats collected from July or August onwards decreased. Based on scat location I would recommend that the optimal time for surveying pine marten presence is between April and September but areas should only be surveyed if there has been no heavy rain for the previous two days.

The use of hair traps is discussed in detail in Chapter 4. The hair trapping devices used in this study present an alternative method for sampling pine marten presence in an area. Pine marten presence was identified more than once on all transects surveyed using these devices and proved invaluable in detecting presence in the more remotely located areas (transect 11 and 12) where scat location proved problematic and live trapping was not an option. In addition to detecting presence a further five animals (4 males and 1 female) were identified, through DNA extraction from the hair follicle and typing of the SRY gene, using the hair samples retained in the spring of the hair traps (Chapter 7).

Studies on pine marten report them as difficult to live trap, having become wary of traps and people following centuries of persecution (Marchesi, 1989; NíNeachtain, 1998). By contrast the American marten, despite still being a hunted species in many states and in Canada, is reputedly noted for its susceptibility to trapping (Vinkey, 2003). Both Marchesi (1989) and NíNeachtain (1998) also mention that another possibility for their low trapping success may have been caused by traps being lavishly concealed from the public. Other authors have also reported the avoidance of enclosed traps (track plates), with open traps apparently preferred (Foresman & Pearson, 1998). In this study I found that pine martens were easy to trap, despite traps being heavily concealed with moss to give the animal protection from the elements and to keep members of the public from finding them. A new individual was caught every 130 trap nights and an animal was caught approximately every 36 trap nights (every 3.5 days). The low capture rates in

2003 compared with 2004 can be explained by inefficient trapping devices which allowed animals to escape. Once this problem was rectified no more animals escaped (Chapter 2).

The low rates of recapture may be explained by how the animals were handled once captured; they were restrained rather than sedated owing to restrictions on animal sedatives under Irish legislation (Chapter 2). All sedatives are VSO and must be administered in the presence of a veterinary surgeon, who must be in possession of the drugs at all times. Almost half of the recaptures (40%) were from one individual, which was extremely 'trap happy', on transect 4 (N = 16). This female was caught at all ten traps on transect 4 between 2003 and 2004 (Appendix 3). Elsewhere some animals were recaptured but never at more than three points along the transect. SRY analysis showed that of 16 hair samples examined for transect 4 (UL) [a total of 40 were collected for this site, the 16 chosen were done at random] that 11 were male and 3 were female and 2 were excluded from analysis as they failed to amplify with microsatellite primers, but were indicative of females. Thus this may indicate male avoidance of live traps, particularly once trapped, thereby leading to low recapture rates.

It was found that most animals were identified in the first trapping session each year (April-May) and this decreased approaching September. Marchesi (1989) reports a seasonality in capture success, stating that animals are most easily trapped in summer and autumn compared with spring and winter. NíNeachtain (1998) found the opposite to be true and suggests that as the numbers of young increase in the population as autumn progresses then their inexperience of traps leads them to be trapped easily. While this is a possibility her results did not support this hypothesis.

Overall estimated population density was quite high, but similar to that reported previously in Ireland. In broadleaf scrub woodland in County Clare population density was estimated as 0.5 to 2.5 animals per km², the variation being attributed to whether the animals were using farmland outside the scrub habitat, which was never established (NíNeachtain, 1998). Outside of Ireland, estimates of pine marten density for deciduous woodland (Poland: 0.49-0.8, Jedrzejewski *et al.*, 1993) and mixed woodland (Poland: 0.91-1.39, Pelikán & Vackar, 1978; Scotland: 0.58-0.82, Halliwell, 1997) are similar to this study. Coniferous woodlands and particularly one study carried out by Zalewski *et al.* (1995) also found densities (Poland: 0.83-1.74) similar to those found for pine

marten in KNP, while densities for a coniferous woodland in Switzerland were much lower (0.1-0.8, Marchesi, 1989). Marten densities increase in the autumn time when young animals become apparent in the population (Soutiere, 1979). Sub-adults were included in the calculation of densities, but kittens were not and as such their presence may have inflated estimates of density, but when removed the range of estimates had not altered much (Table 3.2).

As traps were not laid out in a grid and radio tracking was not carried out it is impossible to say how small or large home ranges of individuals or male and female pine martens are in KNP. Other studies in Ireland have found Irish pine martens to occupy relatively small home ranges (males: 0.19 - 0.44 km² and females 0.02 – 0.8 km²; Velandar, 1991; NíNeachtain, 1998, based on live trapping and radio telemetry) compared with those estimated for mainland European populations (males: 2.23 – 32.86 km² and females 1.49 – 9.78 km²; Marchesi, 1989; Zalewski *et al.*, 1995; Balharry, 1993b). Despite limited radio telemetry studies, it is becoming apparent that pine martens in Ireland appear able to maintain dense populations with small home ranges. The evidence presented in this thesis would certainly be suggestive of that hypothesis.

The sex ratio of the trapped population of pine marten in KNP tended towards a female bias though was not significantly different to the male bias observed in the RTA population. This is in contrast with all other studies which quote a male bias in trapped populations of pine marten: 2:1 (Yurgenson, 1947; Mal'dzhyunaite, 1957; Marchesi, 1989; Balharry, 1993b), 1.5:1 (NíNeachtain, 1998). A male bias during trapping has also been found for the American marten: 2:1 (Quick, 1956), 1.3 (Thompson & Colgan, 1987) and 1.041:1 (Naylor & Novak, 1994) and for many other species of mustelids (in Buskirk & Lindstedt, 1989). The sex ratio at birth is believed to be 1:1 (NíNeachtain, 1998).

The presence of the male bias has been attributed to various factors. It may be an artifact of the sampling method, with males more likely to be trapped than females (Marchesi, 1989). However the observed female bias in my trapped population may also be as a result of the methodology used. Line transects result in higher density of traps in smaller home ranges compared with larger home ranges and thus in the sexually dimorphic pine marten this is the female (Buskirk & Lindstedt, 1989). Furthermore the spacing between traps may favour the capture of one sex over the other. King (1980)

reported no differences in the sex ratio of trapped population of stoats when traps were spaced at 100m, 400m or 800m intervals but when spaced at 1km a distinct male bias observed.

Males are noted to wander widely and are therefore more likely to come in contact with traps than females (Buskirk & Lindstedt, 1989). It could also be that because of lifestyle, females having to raise kits by themselves, combined with an energy inefficient body shape, die younger owing to extreme physiological stress on their body (Weckwerth & Hawley, 1962). Furthermore, given that this species also exhibits sexual dimorphism, the female pine marten will always be subordinate to males in times of food shortage which might also result in an unbalanced sex ratio (Pulliainen, 1981). In addition within the mustelid group commercial trapping of a population may also affect the sex ratio of that population. The price of the pelt (pelt demand is generally sex dependent, usually males are favoured, and that will be reflected in capture effort), trapping quota, and accurate recording of the sex of the captured animals are dependent upon the trapper and thus will affect sex ratios quoted in the literature and any research carried out within a trapped area (Buskirk & Lindstedt, 1989).

By contrast the sex ratio for the carcasses collected around Ireland (Appendix 4) during this study interestingly shows a male bias (1.3:1). It must be noted that the collection of RTA's maybe biased towards males. As stated above, males tend to wander more widely (Marchesi, 1989) and therefore are more likely to come into contact with roads and vehicles and the methodology used probably favoured the capture of females. However, the female bias observed in the trapped population in KNP may be a direct consequence of the translocation. King (1975) found that in low density populations of weasels that there was a female bias in the trapped population and that when population density was high there was a male bias. Thus it may be that the population of pine marten in KNP are still establishing themselves and are still at a low density and thus the observed female bias in the population. The female bias observed in the trapped population in KNP may be a direct consequence of the translocation. It was unknown how many females and males were translocated but as this is still a recent event (1995) then its effects may well still being seen within the population in KNP. Pine martens are reported to be able to live up to 14 years old (Birks, 2002). However it is more likely that the observed female bias is a direct result of the methodology of the trapping. A comparative study investigating the differences in the sex ratio of the trapped

population using grids and line transects would indicate if the observed female bias was true or an artifact of the trapping procedure.

Pine martens in KNP were found to be reproductive, though it was not always possible to tell if females had reproduced in a given year. The breeding cycle of the pine marten is typical of mustelids in that mating occurs in July and August, when both sexes become fecund, though males testes may enlarge for longer than the above period (June to September). The implantation of the embryo is delayed till the following February or March. Once implanted, gestation is thirty days, following which the kits are born naked and blind. They are solely dependent on the mother for the first eight to ten weeks when they are weaned (Birks, 2002). Thus while I could always tell if an adult male was fecund as my trapping period spanned the time frame during which fecund males should be noticeable, it was not possible to tell if females had bred that year once kits were weaned (lactation being complete by the end of June). For that reason, of the 13 adult females, 6 were found to be lactating while 7 were not. This does not imply that the remaining seven had not had young but rather I could not determine if they had.

Sexual dimorphism was found for the pine marten population in KNP, with adult male pine martens always heavier than females, though there was a certain degree of overlapping. This is typical with what has been reported elsewhere. NíNeachtain (1998) found that males in the Burren were always heavier than females by about 14%. Marchesi (1989) states this difference in Switzerland is about 16%, while in Russia the difference was also approximately 16% (Ryabov, 1962). McDonald & Barrett (1993) refer to males in the region of 12% heavier than females. In this study males were on average 11% heavier than females.

Male pine martens were larger overall than female pine martens on all four measurements (total length, head & body length, tail length & hind foot length). NíNeachtain (1998) found this not to be case with the population she studied in the west of Ireland. Here males were only longer overall and in the body but not in the tail and hind foot. As stated earlier (Chapter 1, section 1.4.1.), in Europe Scottish pine martens are the largest followed by Irish and English pine marten (Velandar, 1991), with animals tending to decrease in size as you move from south-west Europe to north-east Europe (NíNeachtain, 1998).

Game keeping activities and over hunting of the pine marten for fur in the Killarney valley, as elsewhere in Ireland brought this species to the brink of extinction. The loss of habitat in the area also certainly contributed to the demise of this species, though its exact contribution remains undefined. There is no doubt that the pine marten population in KNP has now fully established itself and those animals or their descendants that were translocated there are thriving, having been found throughout the park. It is also apparent that the population is now reproducing and holding its own within the park boundary. Whether the translocation is responsible for the noticeable female bias which was found for the trapped population, or whether this is unique to that population only time will tell. The importance of healthy populations of wildlife in our national parks is only too obvious. As man continues to change the environment, mostly for the worse, then healthy source populations of wildlife within protected areas are essential if we are to maintain Ireland's biodiversity. Though the pine marten has shown itself to be adaptable in the face of a changing environment there is no reason to think that it will be able to adapt to meet all the changes and thus healthy source populations like the pine marten in KNP will be necessary to maintain the longevity of this species on our shore.

CHAPTER 4 has been accepted as a paper for publication in the Journal: *Journal of Zoology, London* and is currently in press. The title of the paper and my co-authors are listed below. Slight modifications have been made to the paper for inclusion into the thesis. As such the material methods section of the paper has been abbreviated (now occurs in chapter 2) and Fig 1 which was a picture of a hair trap appears in Chapter 2 (Fig 2.3 & 2.4). The remainder of the paper appears in this chapter as it will be published in the journal.

FUR SNAGGING AS A METHOD OF EVALUATING PRESENCE AND ABUNDANCE OF A SMALL CARNIVORE, THE PINE MARTEN (*MARTES MARTES*).

Á.B. Lynch, M.J.F. Brown and J.M Rochford.

CHAPTER 4. FUR SNAGGING AS A METHOD OF EVALUATING PRESENCE AND ABUNDANCE OF A SMALL CARNIVORE, THE PINE MARTEN.

Abstract

The pine marten was once considered one of Ireland's rarest carnivores, but is now recolonising many of the areas where it was once extirpated. It is important to monitor its population expansion so conservation management decisions can be made regarding the status of this species. In this study I investigate the use of fur snagging devices as a method of estimating pine marten presence and abundance in lowland and upland broadleaf woodland. I investigated whether these devices reliably detected presence, and how quickly. It was found that hair traps functioned very well, detecting pine martens on all survey transects and being essential in their detection in the most isolated site. A survey length of 6 days was sufficient to detect pine marten in all survey sites, with most detections occurring within the first four days. The hair traps functioned equally as well as scat location for detecting pine marten and had the added advantage of there being no confusion as to the origin of the sample. Live trapping was used to verify the estimates of relative abundance from hair traps. There was a trend for the number of hair samples per site and the number of times animals were trapped per site to be positively correlated, although this was not significant. Hair traps do provide a real alternative for the monitoring of pine martens in both lowland and upland broadleaf woodlands, overcoming many of the disadvantages associated with scat location.

4.1. Introduction

Evaluating small carnivore presence and abundance is essential for their conservation and management, whether populations are considered to be rare or plentiful. In the absence of reliable data on presence and abundance it is impossible to make decisions regarding population status, habitat requirements and anthropogenic impacts (Zielinski & Kucera, 1995). Given limitations on expertise and resources, monitoring schemes should be easily operated (perhaps by a volunteer network), favourable in terms of cost (equipment and labour), apply to a variety of habitats and be workable over a long period of time (Harris & Yalden, 2004).

A variety of techniques exist for monitoring terrestrial carnivores, though in Britain and Ireland it is apparent that for some species [stoat, weasel (*Mustela nivalis*), otter, mink, wildcat] new monitoring techniques are essential prior to the development of any population monitoring programme (Harris & Yalden, 2004). This is also true for pine marten, where no consistent technique exists to reliably detect presence and abundance.

Monitoring techniques can be categorised as direct and indirect. Direct monitoring techniques require contact with the target species. While much information can be gained about the species (sex, weight, breeding status), such techniques are generally too costly. Indirect methods in contrast allow the researcher to gain information without ever having to deliberately come into contact with the target species. Indirect methods can be used to record three aspects of the status of a species: its distribution, its relative abundance and its absolute abundance (Bonesi & Macdonald, 2004). Many indirect techniques have been used for monitoring small terrestrial carnivores; including snow tracking, remote-camera censusing (Bull, Holthausen & Bright, 1992; Zielinski & Kucera, 1995; Foresman & Pearson, 1998; Zalewski, 1999) and track plates (Bull *et al.*, 1992; Zielinski & Kucera, 1995; Foresman & Pearson, 1998; Vinkey, 2003).

For species like the pine marten that are highly mobile, intelligent, alert, nocturnal and with a reputation for being secretive, population monitoring schemes are limited to the use of indirect methods (Messenger & Birks, 2000). In the absence of snow, indirect methods for monitoring pine marten presence and abundance in Ireland and Britain have typically included chance sightings surveys and scat counts along transect lines (O'Sullivan, 1983; Velandar, 1983; Strachan *et al.*, 1996; NiNeachtain, 1998). Sightings surveys, while useful, do require their confirmation and therefore some level of verification in the field (scat, hair or corpse) or through careful questioning of the observer. Pine marten are rarely seen, even where they persist at high density, thus confusion with more common or well known species (stoat and mink) may frequently occur (*pers. obs.*). Aubry & Lewis, (2003) found that results from standardised population monitoring of fishers using photo and trackplate detection devices in the Pacific Northwest strongly contradicted the distribution of fishers from unscreened sighting surveys.

Monitoring pine marten populations by using scat counts along transects, while appearing straight forward, has many associated problems: 1) the ability to locate scats

when pine marten density is low may decrease, 2) the loss of scats from the transect line, through the removal of scats by other species (e.g. slugs, foxes), wet weather or being covered up by leaf litter, 3) the technique is highly reliant on woodland containing pathways/forestry tracks, 4) scat deposition (on prominent features) may be dependent upon position within a pine marten's home range (core or edge) and 5) the ability of a researcher to identify pine marten scats correctly (Messenger & Birks, 2000). Recent work carried out by Davison *et al.* (2002) reported that even in the case of experienced field workers, working in a known pine marten area (Galloway, Scotland), misidentification of scats occurred 20% of the time. The over-riding advantage to scat transects is that they are by far the cheapest monitoring technique available, both in terms of labour and equipment. Consequently Harris & Yalden (2004) propose developing a system for monitoring pine marten distribution based on the reliable identification of scats. However, given the challenges and ambiguities described above this may neither be the best use of resources nor even attainable.

In this study I assess the effectiveness of an alternative method, fur-snagging, for reliably detecting both pine marten presence and abundance in lowland and upland broadleaf woodland. Many designs of hair trap exist for trapping mammalian hair (squirrel: Gurnell *et al.*, 2004; bear (*Ursid spp.*): Romain-Bondi *et al.*, 2004; badger: Frantz *et al.*, 2004). The hair traps used in this study were designed by John Messenger of VWT (Vincent Wildlife Trust) and are described in detail in Messenger & Birks (2000). The traps function by removing a sample of hair from the dorsal surface of the animal (see Fig. 2.3 & Fig. 2.4, Chapter 2). Each trap can only be triggered once, making identification of the hair sample relatively easy and directly comparable with live traps for the assessment of abundance. In tests on captive pine martens the authors found the traps to work well, routinely catching 20-30 hairs and a field test in the west of Ireland confirmed the hair traps to work on wild pine martens. However an extensive study by the same authors in Gwydyr Forest, North Wales over 2,940 trap nights, and in an area where sightings of pine martens are frequent, failed to detect this species (Messenger & Birks, 2000).

4.1.2. Objectives

I had four objectives. First to ensure that the traps worked effectively (retained hair). Second, to compute the time taken for a hair-trap to be located by the target species – latency to detection (LTD), essential in designing how long a survey should run for. Third, to compare the use of hair traps with two other detection methods, scat location and live trapping. Finally, to determine if hair traps could be used reliably to estimate relative abundance of pine martens in the two different habitat types, lowland and upland broadleaf woodland.

4.2. Methods

4.2.1. Study Area

The study took place at 5 woodlands in Killarney National Park (KNP), located in County Kerry, in the south west of Ireland (Irish national grid reference; V9487). Three lowland (below 150m: 1.Tomies Wood (TMW); 2.Muckross Peninsula (MP); 3.Derrycunihy Wood (DC)) and two upland (above 150m: 4.Ullauns Wood (UL); 5.Glaisín na Marbh (GnaM)) broadleaf woodland sites were chosen to carry out the study (Fig. 4.1). For convenience from now on all sites will be referred to by their codes. GnaM is the most remote of all the study sites with no established paths and rarely any human disturbance. This area regularly gets cut off in times of heavy rainfall and is reached by an hour's hike across bog or by crossing the mountains. Thus it was easier to visit once every two weeks than every second day for six days.

4.2.2. Survey Strategy

A full description of the survey strategy can be found in chapter 2 and was applied to the 5 study sites listed above. Two indirect methods were used to evaluate the presence and abundance of pine marten in the 5 study units: hair trapping and scat location. Traditional methods such as live trapping, while not applicable to simple monitoring programmes (as they require expertise beyond the scope of such programmes and are too costly) are necessary in order to standardise the use of indirect methods for quantifying estimates of abundance (Sadler *et al.*, 2004). To evaluate the estimates of relative abundance calculated using hair trapping, I compared number of hair samples, a sample being the total hairs collected from one trap, retrieved per site with results from live trapping (total numbers handled).

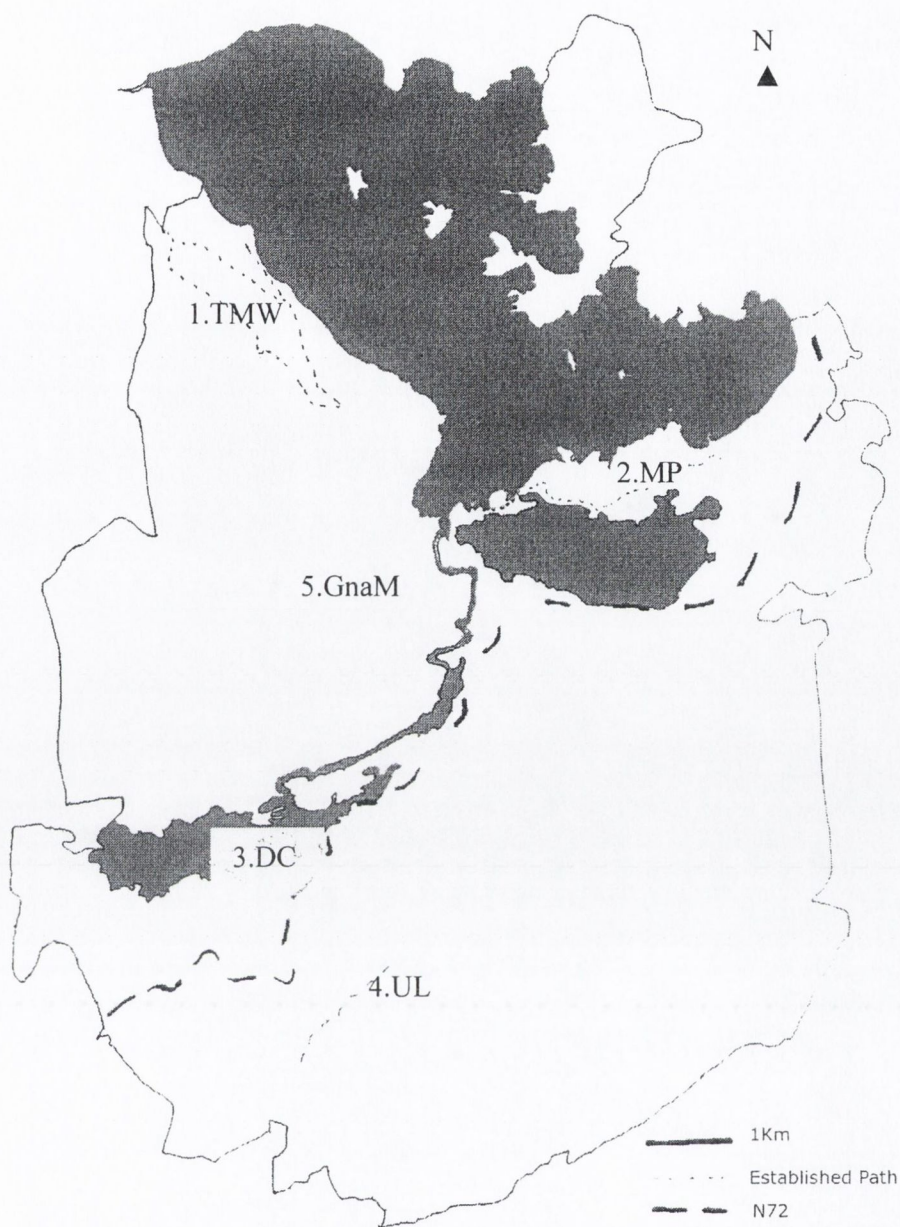


Fig. 4.1. Map of study area (KNP) showing the location of survey transects within the park 1-5 (TMW, MP, DC, UL, GnaM) . Shaded area is the area covered by the lakes of KNP. The N72 (national route) and established paths running through survey sites are also included. Inset the location in Ireland of KNP.

4.3. Statistical Analysis

Chi-square analysis was used to determine whether hair traps varied in success across habitat types and years. In order to include GnaM (where traps were checked every two weeks, rather than every other day) in my analyses the data from TMW, MP, DC and UL were standardised to give a two week estimate such that each positive trap was counted only once for the six day survey period. Thus, the data entered into the analysis was the total number of individual traps that collected hair within a two week period. The analysis was repeated after dividing the data from GnaM by two (to make it strictly comparable to the other data sets) and after removing the GnaM dataset from the analysis. Chi-square tests were also used to test whether the three survey techniques (scat, hair and live trapping) were equally successful and were based on positive sample periods rather than number of samples. Pairwise tests were used to isolate significant effects.

To assess if there was a difference among the three methods and between years in the time taken to detect the target species (Latency to Detection) a two-way ANOVA was used. Year and method were the fixed factors and time taken to detect the target species (measured in days) was the dependent variable. Because live traps were checked daily, whilst scats and hair traps were checked every other day, I repeated the analysis coding the data for these two latter methods to make a positive sample indicate detection on day 1 of the two day survey period, rather than as day 2 (as used in the first analysis). ANOVA's were conducted ignoring sample periods where animals were not detected, and with data sets where such events were set as the maximum length of time of the survey period.

A Pearson's Correlation was used to compare the hair trapping results with live trapping results in order to gauge whether hair traps gave a reliable estimate of pine marten abundance. As the hair traps function by taking a sample from one animal only, the results were compared with the total number of animals live trapped per site for each year.

4.4. Results

4.4.1. Hair Trap Success

A significant difference was found in the success of the hair traps between individual sites (chi-square test: $\chi^2=54.65$, $DF = 4$, $P < 0.0001$). It was apparent from looking at the data that this difference lay in the success of the hair traps between lowland and upland sites (Fig. 4.2). Traps functioned more successfully in upland sites than in lowland sites and this was also significant for both years (combined data: $\chi^2 = 48.7$, $DF = 1$, $P \leq 0.001$; 2003 $\chi^2= 17.59$, $DF = 1$, $P \leq 0.001$; 2004 $\chi^2= 34.42$, $DF = 1$, $P \leq 0.001$). Results when data from GnaM were divided by two, or removed entirely, were qualitatively the same.

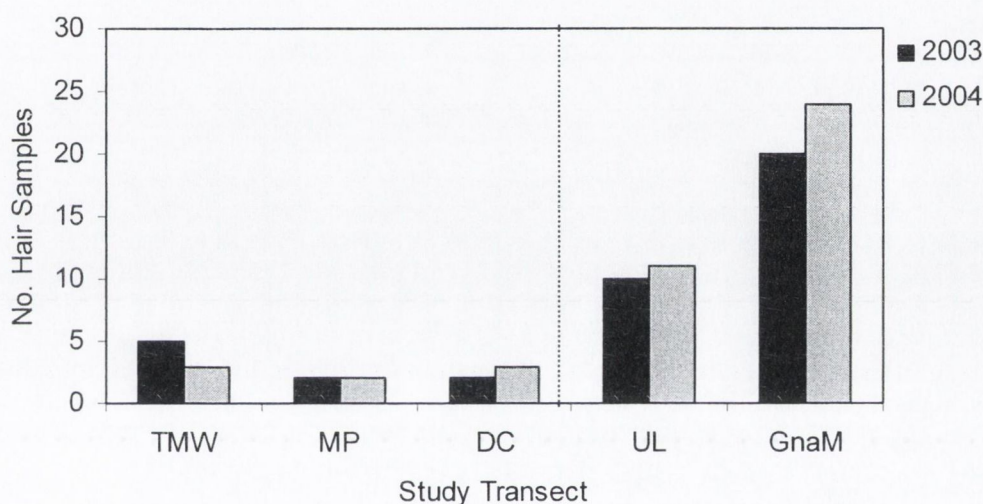


Fig. 4.2. Overall success of hair traps for detecting pine marten presence in each site, grouped into lowland (TMW, MP& DC) and upland sites (UL & GnaM) with a dashed line.

The number of hairs retained in the spring of the trap varied somewhat ($\mu = 10.3$, $SD = 6.64$). However approximately 55% of the samples retrieved (indicated by dashed line Fig. 4.3) held 10 individual hairs or more and in all cases the follicle (hair bulb and the source of DNA) was still present on the hair. The type of hair most commonly caught was the guard hair. The number of hair follicles present plays an important part in any future genetic analysis of the sample, the yield of DNA being directly related to the number of follicles.

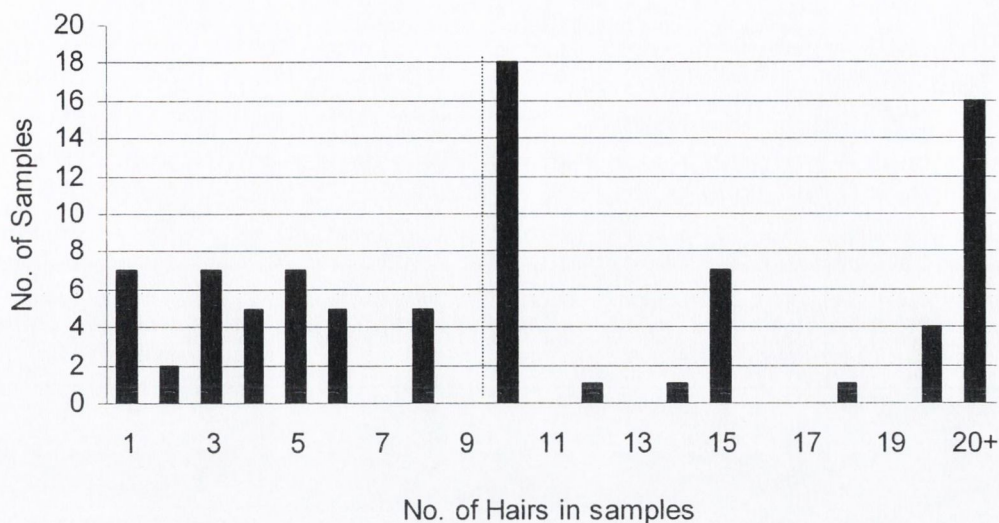


Fig. 4.3. Number of individual hairs caught in each sample. The dashed line indicates samples with greater than 10 individual hairs.

4.4.2. Comparison of techniques for detecting pine marten presence

In 2003, scat location was better than hair trapping, which itself was better than live trapping, at detecting pine marten presence. There was an overall difference among techniques (Table 4.1; chi-square test: $\chi^2 = 12$, $DF = 2$, $P \leq 0.001$) and also in each pairwise test (chi-square tests: χ^2 [hair vs live 03] = 6, $DF = 1$, $P < 0.02$; χ^2 [hair vs scat] 03 = 6, $DF = 1$, $P < 0.02$; χ^2 [live vs scat] = 10, $DF = 1$, $P \leq 0.001$). In 2004 there was no significant difference between the techniques in their detection of pine marten presence; each technique detected this species with equal frequency.

Table 4.1. Number of samples collected in each survey period for each detection method. The figures in parentheses refer to the total number of recaptures in those sampling periods. Survey periods correspond to the following times: 1- April/May, 2- June/July, 3- August/September. The survey unit GnaM was surveyed once every two weeks for six months and is therefore not directly comparable with the other sites. * indicates scats considered as possibly pine marten. N indicates no live trapping was carried out.

Site	Survey Period	Hair Trapping		Scat Location		Live Trapping	
		2003	2004	2003	2004	2003	2004
TMW	1	6	3	23	3	0	1
	2	1	0	8	3	1	2
	3	0	0	2	1	0	1
	Total	7	3	33	7	1	4
MP	1	1	0	23	13	0	3(2)
	2	0	1	10	7	1	2(4)
	3	1	1	5	6	0	1(2)
	Total	2	2	38	26	1	6(8)
DC	1	3	2	6	6	2	1
	2	0	0	3	0	0	0
	3	0	1	2	0	0	2(1)
	Total	3	3	11	6	2	3(1)
UL	1	6	6	9	5	1(1)	3(4)
	2	5	4	6	2	0(5)	1(2)
	3	2	3	4	0	1(5)	1(4)
	Total	13	13	19	7	2(11)	3(12)
GnaM		20	24	9*	0	N	N

4.4.3. Latency to Detection

In the first analyses there was a significant effect of method (2-way ANOVA $F_{2,46} = 8.7$; $P \leq 0.001$), but not of year ($F_{2,46} = 1.5$; $P = 0.2$) or the interaction ($F_{2,46} = 1.7$; $P = 0.2$) (Table 4.2). A significant result was recorded between methods when sample periods in which the target species was not detected at all were included in the analysis (Method: $F_{2,66} = 6.1$; $P = 0.004$). The interaction between year and method was also significant (Year*Method: $F_{2,66} = 6.1$, $P \leq 0.001$). Post hoc tests (LSD) showed that live trapping in 2004 was significantly more effective than hair trapping in 2003 ($P < 0.001$), hair trapping in 2004 ($P = 0.012$) and live trapping in 2003 ($P = 0.00006$). Scat location in 2003 was significantly more successful than hair trapping in 2003 ($P < 0.001$), live trapping in 2003 ($P < 0.001$) and hair trapping in 2004 ($P = 0.009$), while scat location in 2004 was more successful than hair trapping in 2003 ($P = 0.029$).

Owing to the difference in the frequency that hair traps were checked and scats were collected (every second day) compared with live trapping (every day) I standardised the data (see section 4.3), so that the number of checks was the unit measured, and redid the analysis. No significant difference was recorded for method, year or the interaction between year and method (2-way ANOVA $F_{2,46} = 0.41$, $P = 0.66$; $F_{1,46} = 3.37$, $P = 0.07$; $F_{2,46} = 1.51$, $P = 0.23$) respectively. Significant results were recorded for method, year and the interaction between method and year when sample periods in which animals were not recorded were included in the analysis (2-way ANOVA $F_{2,46} = 8$, $P < 0.001$; $F_{1,46} = 5.8$, $P = 0.0183$; $F_{2,46} = 9.4$, $P < 0.001$) respectively. Live trapping in 2003 was solely responsible for the significant differences recorded in the Year*Method interaction, differing significantly from hair trapping 2003 ($P < 0.001$), scat location 2003 ($P < 0.001$), hair trapping 2004 ($P < 0.001$), live trapping 2004 ($P < 0.001$) and scat location 2004 ($P < 0.001$).

Table 4.2. Average latency to detection for each detection method and each year. Mean No. days refers to the time taken to detect a pine marten in a survey unit during sampling periods when they were detected. Total Mean No. Days refer to the average time taken to detect a pine marten on a transect including survey periods when they were not detected. No. Survey Periods refers to the number of survey periods during which a pine marten was detected for each detection method. The figure in parentheses is the total number of survey periods for that year. SD is the standard deviation.

Detection Method	Year	Mean No. days	SD	No. Survey Periods	Total Mean No. Days	SD
Hair	2003	3.75	1.67	8(12)	5.17	2.5
	2004	2.75	1.49	8(12)	4.5	2.84
Live	2003	1.75	0.96	4(12)	4.58	2.15
	2004	1.1	0.32	10(12)	1.92	1.92
Scat	2003	1.83	1.03	12(12)	1.83	1.03
	2004	2.2	1.32	10(12)	3.16	2.55

4.4.4. Correlation between hair traps and live traps

A total of 21 animals were trapped across the 4 study units (TMW, MP, DC and UL), comprising 5 males and 16 females. Of the 21 animals trapped, 7 were captured in 2003 and 14 in 2004 of which two were kittens (MP 2004, 1 ♂ and 1 ♀). There were two trap fatalities in the first session of 2003 (1 male and 1 female) and these were not included in the analysis. Densities were calculated and expressed as number of animals trapped per kilometre squared (km²) (Table 4.3). It is unlikely that these populations are showing a high turnover rate but rather that once an animal is trapped it is difficult to recapture. Dispersal in juvenile pine martens occurs from late August till end of October and most of the animals were trapped before August, with no new animals ever being trapped in September (Table 4.3).

A Pearson’s Correlation was used to assess whether hair traps showed a similar pattern in the number of positive traps as did the data from live trapping. There was a trend for results from hair trapping and live trapping to be positively correlated particularly in the

upland unit, UL (Fig. 4.4), though this was not significant ($r = 0.64$; $P = 0.088$). Overall the number of animals handled in 2004 was greater than in 2003 but this was particularly evident in MP.

Table 4.3. Indicates the number of animals live trapped per transect in both years. Survey periods correspond to the following times: 1- April/May, 2- June/July, 3- August/September. The figures in the columns below refer to the number of animals caught in that period.* refers to animals that were retrapped in the following year. In MP 2004 two of the animals trapped were kittens and where therefore excluded from the analysis for density.

Survey Unit	Year	No. Trapped	Survey Period			Sex	Total Handlings	Est. Density (per km of transect)
			1	2	3			
TMW	2003	1	1			♂	1	0.5
	2004	4	1	3		♂♀♀♂	4	2
DC	2003	2	2			♀♀*	3	1.33
	2004	3	2		1	♀♀♀	4	2
MP	2003	1	1			♀	1	0.5
	2004	6	3	2	1	♀♀♀♀♂♂	14	2
UL	2003	2	1			♀*♂	14	1.33
	2004	3	3			♀♂♀	15	2

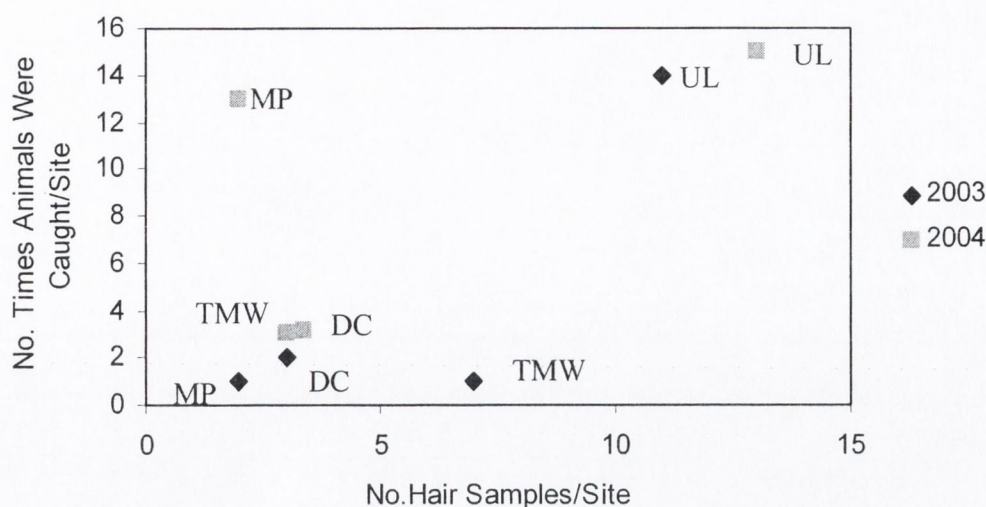


Fig. 4.4. Relationship between the number of hair samples per site and the number of times animals were caught at that site for both years.

4.5. Discussion

In this study I found that hair traps successfully detected the presence of pine marten in both types of habitat – lowland and upland broadleaf woodland. Hair traps functioned better in the upland sites and were essential in the isolated site (GnaM) for detecting pine marten presence in the absence of established paths to walk to look for scats. Based on LTD calculations hair trapping is equally as effective as the other indirect method, scat location, at detecting pine marten presence. Given the ambiguities set out by Davison *et al.* (2002) in correctly identifying from which species scats or faecal matter come from, then it is likely that hair trapping is a real alternative for monitoring pine marten presence. The correct identification of pine marten hair using microscopy is quite easy in an Irish context given the impoverished mammal fauna of similar size (feral cat and mink). I would recommend that where more mustelid species of an equivalent size occur together (in particular the stone marten) that identification is also made using one of the genetic tools available (Domingo-Roura, 2002; Gómez-Moliner, *et al.*, 2004). An evident relationship exists between the number of positive hair traps per transect and the number of positive live traps, however further work needs to be carried out before relative abundance of pine marten can be estimated from hair traps.

The difference in the success of hair traps between the two habitat types may be due to avoidance behaviour towards hair traps in lowland sites compared with upland sites by

pine martens. Foresman & Pearson (1998) described American martens as likely to visit open track plate stations more quickly than closed track plate stations (67% vs 11% after 2 days) and overall open track plates were more successful (92% vs 75% after 12 days). Marchesi (1989) also reported that pine martens avoid traps (live) with human smell. However there was no difference between sites (TMW, MP, DC and UL) in the average time taken to locate hair traps by the target species and on two occasions scats were found on top of hair traps in lowland sites with bait still present (*pers. obs.*). Consequently I believe that the higher number of positive traps in upland sites does partially reflect avoidance behaviour in lowland sites but also the behaviour of the individual animals within an area.

In UL the majority of live trappings were for one animal (88%) which would suggest that this individual showed a liking for traps and probably contributed to the majority of hair samples also. Based on the assumption that home ranges are larger in upland areas, to compensate for decreased amounts of food, the transect in UL is likely to have only covered one home range. The fact that one male was trapped there in 2003 and a different male in 2004, both on one occasion, suggests the transect may have only covered the female's core range which male pine martens may only occasionally visit or mostly during the breeding season. Another female, trapped on this transect in 2004, may simply have been from an adjacent home range and took a foray into the adjoining territory or once trapped avoided being trapped in future. Pine marten hold intrasexually exclusive home ranges, thus while male and females ranges may overlap those of the same sex do not. The boundaries of such territories are quite fluid and are never the same from year to year (DeMonte & Roeder, 1993). This illustrates the importance of knowing the behaviour of the species but also the individuals within the population under investigation. As live trapping was not carried out in GnaM it was not possible to compare with UL. It was also not feasible to verify the data collected from hair samples using genetic analysis as currently the microsatellite markers available for pine marten are not polymorphic enough to discriminate between individuals in Irish populations (Chapter 6).

Securing reliable information on wild mammal populations is an eternal problem for mammal ecologists (Kohn & Wayne, 1995). The fur snagging devices used in this study can be used for non-invasive genetic investigations of populations. Currently work in North America is focusing on developing monitoring methods that incorporate a

presence detection method (e.g. track plate) with the possibility of genetic sampling (Schelexer & Zielinski, 2003). While these hair traps were species specific in this study they could be used to monitor other species: stoat, mink, polecat, stone marten, badger, fox and wildcat.

For genetic studies a large number of hair follicles are desirable in order to ensure a good quantity of DNA for PCR and is particularly important in studies using nuclear DNA. Goosens *et al.* (1998) found that for studies on marmots (*Marmota marmota*) 10 hair follicles per reaction gave better results in terms of fewer errors in identifying individuals correctly than 3 and 1 hair follicles respectively. Furthermore Frantz *et al.* (2004) found that 33% of hair samples used in genetic analysis on badgers came from a single guard hair and advocate that hair trapping can be used for estimating population size even if the target species was rare or patchily distributed. Unlike using sticky paper/substances or barbed wire (Frantz *et al.*, 2004), this design of hair trap results in the sample coming from one individual and thus hairs from a sample can be pooled together without concern of cross contamination.

In 2003 the three detection methods, scat location, hair trapping and live trapping were decreasingly effective in their abilities to detect the presence of pine martens but there was no difference in 2004. Two reasons may account for this. Firstly the number of scats collected in 2003 was nearly twice the number collected in 2004. 2003 was particularly dry with little rainfall, particularly in April and between mid-June to September. Conversely 2004 was typified by heavy rain showers throughout the six month field period, which resulted in fewer scats being located. Heavy rain will easily wash away scats, particularly if the composition is made up primarily of fruit (Messenger & Birks, 2000). Davidson *et al.* (2002) report that slugs can consume an entire scat in 24 hours and the higher precipitation during fieldwork in 2004 would have favoured their presence. Hair traps overcome all these disadvantages. Secondly, in 2003 it was found that though animals were being trapped they were also escaping from cages prior to them being checked at dawn. On two of these occasions (1 male and 1 female) animals were found dead, having broken their backs trying to escape from traps. It was believed that the animals were pulling back the door just enough and trying to escape underneath. Traps used in this study had been used in a previous study where no fatalities occurred (NiNeachtain, 1998). After several attempts to modify the traps in the workshop, two small hooks were fitted to the front of the door and held up with garden

wire. The force of the trap door shutting caused the hooks to fall down and latch under the trap. Once these modifications were made no further fatalities, injuries or escapes occurred and the trapping success rate increased.

It is evident that though hair traps did not detect pine marten presence in all sample periods, they did detect presence of these animals in all survey sites. Furthermore on only one occasion did hair traps not detect the presence of pine marten on the first occasion they were used in a survey site (Table 4.3; MP 2004). The re-sampling of survey sites allowed us to rule out false positive detections or false negatives (Vinkey, 2003). The correct identification of hair may be problematic where stone marten and pine marten occur together, demanding at least 10-20 intact guard hairs (Tóth-Apathy, 2002). DNA analysis could to be used to confirm species identification if this situation arose, however given that the spring routinely traps more than 10 individual hairs then this may not be necessary.

Detection devices with a low LTD are desirable, as shortening survey periods allows for more units to be surveyed during a field season (Vinkey, 2003). Most detections using hair traps were made within the first four days of a survey and were rarely made on the last day a hair trap was checked ($N = 3$ of 16 sample periods). I would suggest that in broadleaf woodlands a survey period of six days is sufficient to positively detect pine marten presence using hair traps. If traps are used during winter time, when activity levels in pine martens are known to decrease (Zalewski, 2000), then I would recommend extending the survey period. Furthermore, if traps cannot be checked daily or every other day then longer interval between checking traps does not pose a problem as hairs can be retained in the spring for up to six months.

The importance of developing indirect techniques for the assessment of relative abundance is paramount for carnivore monitoring, so that reliable data are available to enable informed decisions to be made about species status (Harris & Yalden, 2004). My study shows that the number of hair samples retrieved in a site is correlated with the number of times animals were handled per site. Bonesi & MacDonald (2004) report that trapping mink to estimate abundance is biased by the number of animals present, the time of year and level of activity. Consequently, even density estimates based on live-trapping must be treated carefully. I recommend that caution must be used in

extrapolating my results from hair traps to other sites or habitats to infer relative abundance.

In genetically heterogeneous populations, a combination of hair trapping and DNA analysis could be used to determine relative abundance, but this was not possible in the genetically homogeneous study population (Chapter 6). In contrast, if densities are known for an area then it is likely that hair traps could be used to monitor the population in the long term, but additional work is needed to show this. A further important use of the hair traps may be identifying habitats within a large geographic area that are important for pine marten based on presence and absence data as they represent an easier way to sample mature woodland than locating scats.

As mentioned above, the low rates of recapture in 2003 for live traps are not indicative of a high turnover rate in the population as the field season did not span the entire dispersal period, which tends to be mid August to late October (Soutiere, 1979), but poor trapping success. In any case only three individuals were caught during this time and, while it is possible that these were dispersing young the majority of animals caught were identified before then. It is also probable that the handling of trapped animals resulted in low recapture rates. Under Irish law it is an offence for anyone other than a veterinary surgeon to be in possession of sedatives, therefore all animals were restrained rather than sedated during handling. It was unfeasible to have a vet on site while trapping due to cost and there appears to be no exception in the legislation to govern wildlife research.

Pine martens are reputedly hard to trap. Based on calculations described in previous studies, the number of pine martens trapped per 100 trap nights appears to be in keeping with previous records and is better than that previously recorded for Ireland: Ireland: 0.5-3.3 (this study), 0.303-0.709 (NiNeachtain, 1998); Europe: Switzerland: 0.32-0.48 (Marchesi, 1989) and North America: American marten: 1.1 -2.1 (Weckwerth & Hawley, 1962; Soutiere, 1979; Katnik, Harrison & Hodgman, 1994). It is therefore likely that poor success in catching individuals in 2003 was primarily due to inadequate trapping devices.

Hair traps do provide an alternative for monitoring of pine martens in both lowland and upland broadleaf woodlands. Although this study only covered the period April to

September there is no reason why the traps could not be used during the winter months. This design of hair trap overcomes many of the disadvantages associated with scat counting along transects, live trapping and other designs of hair trap. Hair traps are independent of weather, are not reliant on pathways, positive identification of the hair sample can be made using microscopy or DNA analysis, and in the latter case more readily than scat, the training involved to use them is less than that of scat location and live trapping, they are cost effective compared with live trapping (€6 per unit and €35 per unit respectively) and can be used all year round, even through the breeding season. An added advantage of the trap is that they are ideal for non-invasive genetic studies as the sample retrieved is from one individual and could be modified to remotely sample other species. While I caution the use of the traps for estimation of relative abundance there is no doubt that with further investigation these hair traps could effectively be used to monitor the abundance of pine marten in various habitats particularly if coupled with microsatellite analysis.

CHAPTER 5. THE DIET OF THE PINE MARTEN IN BROADLEAF WOODLAND, WITH SPECIAL REFERENCE TO THE IMPORTANCE OF THE BANK VOLE.

Abstract

The pine marten was once one of Ireland's rarest terrestrial mammals but is now recolonising many of the areas from where it was extirpated. This study examined the annual and seasonal diet of a re-established population of pine marten in the broadleaf woodlands of Killarney National Park between 2003 and 2005. In contrast to previous dietary studies on pine marten populations, fruit not small mammals formed the majority of the diet. Fruit was the most frequently consumed item except in summer (30% Frequency of Occurrence (FO)) and it also contributed the most in terms of % Mass (47.95) and % Fresh Weight Ingested (FWI) (51.4) in the diet. Mammals were the next most important food group overall and were the most important during the summer (20.3% FO and 26.7% FWI). There was no significant difference in the frequency of occurrence of the woodmouse and the recently introduced bank vole (*circa* 1955) in the diet of the pine marten. Birds were the third most notable prey item but only in spring and summer (9.18 % FO & 12.55 % FWI respectively), when nestlings and fledglings were abundant. Frogs were important throughout spring, summer and autumn, while earthworms were only seasonally important in winter. The overall dietary niche breadth (BS) was wide (0.659) and the narrowest niche breadth (α) for the seasons were recorded in autumn (0.39).

5.1. Introduction

A fundamental aim in mammal ecology is to determine what resources a particular species requires to exist and an understanding of the dietary needs of that species is essential in addressing this objective (Livaitis, 2000). Many techniques exist for assessing the diet of a species, which are dependent on its behaviour and what is required of the data set (Litvaitis, 2000). For wild living carnivores, which are typically alert, highly mobile, elusive and which may be predominately nocturnal, the analysis of the components of their diets is restricted to indirect observations. These include the examination of the gut contents of dead animals or through the collection and examination of faecal material left behind by the animal (Powell, York & Fuller, 1997). The use of the former is generally restricted to a short time frame during which the target species can be legally hunted or culled; though this can give additional

information such as sex and age (Goszczyński, 1986), it is obviously destructive in nature. The collection of faecal material, while not as informative, is favoured as it is non-invasive and a large number of samples can be collected. It also allows the investigator to observe seasonal trends in the diet (Marchesi & Mermod, 1989).

Despite being classed as carnivores, marten species [fisher, American marten, stone marten and pine marten] studied so far have a surprisingly catholic diet and are often referred to as opportunistic general feeders (Marchesi & Mermod, 1989; Nagorsen *et al.*, 1991; Genovesi *et al.*, 1996; Powell *et al.*, 1997; NíNeachtain, 1998). The diet of the pine marten has been investigated throughout Europe (Finland, Poland, Russia, Scotland, Spain and Switzerland) and the consensus across studies is that small mammals, particularly voles (*Microtus* and *Clethrionomys spp.*), are the most important prey item throughout the year with other groups (Birds- spring/summer, Insects- summer/autumn, Fruit- autumn/winter, Amphibians/Reptiles- spring/summer) being seasonally important (Goszczyński, 1976 & 1986; Pulliainen, 1981; Lindström, 1989; Marchesi & Mermod, 1989; Jedrzejewski *et al.*, 1993; Clevenger, 1993a; Putman, 2000).

However, studies carried out on islands in the southern part of the pine marten's range (Minorca and Ireland) have shown that mammals are not the back bone of the annual diet, but rather that each of the main food groups (fruit, mammal, bird & arthropods) occurs with equal frequency (though they may differ in seasonal importance), while the other groups (reptiles, amphibians & earthworms) occur throughout the year (Warner & O'Sullivan, 1982; Clevenger, 1993a; Clevenger, 1993c; NíNeachtain, 1998). Clevenger (1993c) found that small mammals, fruit, birds and insects were equally important in the annual diet with all four showing seasonal peaks. Furthermore, in comparing insular and mainland populations in Spain he found that the pine marten had a wider niche breadth on the island compared with the mainland ($BS = 0.754$ *cf* 0.38 respectively), where mammals were the dominant prey items (Clevenger, 1993a). Both NíNeachtain (1998) and Warner & O'Sullivan (1982) found similar patterns of wide niche breadth when investigating the diet of the pine marten in Ireland. It has been suggested that this equal partitioning of prey groups is as a result of a depauperate mammal fauna, commonly associated with islands (Warner & O'Sullivan, 1982). Alternatively the wide niche breadth could also be an indication of food stress (R McDonald *pers comm*), though all

work on pine marten densities in Ireland report similar if not higher densities compared with Europe (see Chapter 3) and as such food would not appear a limiting resource.

By contrast a wide niche breadth may reflect the differential exploitation of the food available to the pine marten, which at lower latitudes includes other prey groups such as fruit, reptiles, amphibians and earthworms present and available due to different climatic patterns. A review of the literature of the geographical variation in genet (*Genetta genetta*) diet reports a similar picture to that observed in pine marten diet indicating that at northern latitudes (Scandinavia) this species is a specialist predator of small mammals but at lower latitudes (Mediterranean) it exploits other food resources during the whole year or during particular seasons (Virgós, Llorente & Cortés, 1999). Thus the authors state that the genet exhibits two patterns in its diet across its range and supports the hypothesis of a generalist or opportunistic predator. A similar argument could be proposed for the pine marten in Ireland, which owing to typically mild winters (Warner & O'Sullivan, 1982; NíNeachtain, 1998) and similarly in the Mediterranean (Clevenger, 1993a), can exploit resources not available to populations at northern latitudes. In the latter study a difference between climatic patterns on islands and those in the mountains where the study was carried out may have caused the observed difference in diet.

Prior to the introduction of bank voles to Ireland, *circa* 1955 (Smal & Fairley, 1984), the small mammal fauna comprised woodmouse, pygmy shrew (*Sorex miniatulus*), house mouse (*Mus musculus*) and rat. Though it has not been investigated it is likely that the bank vole is now a prey item amongst the predator guild in Ireland. Elsewhere the bank vole is consumed by many small to medium sized predators both mammal and avian (Pelikán & Vackar, 1978; Zalewski, 1996). As the bank vole is less active than the woodmouse and is diurnal, as it would be easier to catch and be active at the times most predators are also actively hunting (dusk and dawn) then it would be expected that this species should appear more frequently in the diet of predators where the two are found together. Though it is not clear what level of competition exists between the predator guild in Ireland [badger, fox, pine marten, stoat, mink, owl *spp.*] it is likely that the presence of the bank vole wherever it occurs may reduce competition within this group. As such if the diets of the predator guild in one area were examined simultaneously together with prey availability then it would be possible to examine what role the bank vole plays in their diet despite being absent until recently. Zalewski (1996) examining

the diets of pine marten and tawny owls (*Strix aluco*) found that though the bank vole was a principal prey item of both these predators that they exploited different age classes of bank voles.

Furthermore the presence of the bank vole in the diet of Irish predators may affect populations in several ways. For example the pine marten is considered to be recolonising much of its former range (Smiddy & Berridge, 2002); part of this recolonisation may be due to the presence of the bank vole. It may also allow populations which were food limited to occur at higher densities. Alternatively it may also represent another route through which rodenticides can enter higher trophic levels causing secondary poisoning of the recipient.

Rats and house mice are not common components in the diet of pine marten in Ireland (Warner & O'Sullivan, 1982; NíNeachtain, 1998). The bank vole was absent from all previous dietary studies carried out on pine marten in Ireland except in NíNeachtain (1998). Here the author considered the vole population to be establishing and was unable to calculate vole densities from her small mammal trapping data as trapping success was so low (Total numbers of individuals: N = 78 woodmice and N = 11 bank voles). By comparison, bank voles have been present in Killarney National Park (KNP) since at least 1965 (Smal, 1978) and now have a strong population though their density is restricted by ground cover (Lynch, 2002). Consequently this provided an opportunity to investigate whether the pine marten will incorporate novel mammals into their diet.

5.1.1. Objectives

In this study I assessed the annual and seasonal diet of pine marten in the broadleaf woodlands of KNP over a two year period. I also considered the current role of bank voles in the diet of pine marten within KNP.

5.2. Methods

5.2.1. Study Area

This study took place in KNP (NGRef: V9487) which is located in the southwest of Ireland (Fig. 2.1, Chapter 2).

5.2.2. Survey Strategy

A detailed description of scat collection can be found in Chapter 2 (Section 2.2.2). Here I will present a brief synopsis of the methods used to collect and process data for this chapter. The systematic collection of scats was carried out between April and September 2003 and 2004 on transects 1-12. Scats were also collected during February and March 2003 by myself (transects 1-5, opportunistic collection) and in November and January 2004/05 by Yvonne McCann (transect 4 & 10).

5.3. Statistical Analysis

For comparison with other studies the data were categorised into six main groups: arthropod, fruit, mammal, bird, other (amphibians, reptiles, molluscs and debris) and earthworm for analysis. Also in keeping with the literature the percentage frequency of occurrence (% FO), the number of times a given item occurs over the total number of items, was used to assess the components of the diet. Percentage FO tends to place importance on those items that turn up often in the diet rather than those which contribute the most in terms of mass or energy to the individual. Due to this percentage mass (% M, biomass) and the percentage fresh weight ingested (%FWI), using the total mass of each component and digestibility factors following Lockie (1961), were calculated for comparison.

The fresh weight ingested of earthworms (*Lumbricus terrestris*) was calculated following the method of NiNeachtain (1998, which was based on Reynolds & Aebischer, 1991). This involved removing the excess water from the microscopic fraction and centrifuging each sample for two minutes (1000 rpm). Water was then added so that the volume of the pellet and the water were equal. One cm³ of the mixed solution was added to a petri-dish of known area and the number of chaetae in eight one cm² boxes counted. From this the number of chaetae were estimated and thus the number of segments. The mean fresh weight of an earthworm segment was taken to be 15mg (Reynolds & Aebischer, 1991).

Chi-square analysis was used to investigate whether there was any inter annual variation for months where two years of data were available, prior to assessing whether there was a seasonal difference in diet. For seasonal investigation all data were pooled and divided into the following seasons: spring (February-April), summer (May-July), autumn (August-October) and winter (November-January). A G-Test and Chi-square analysis

was used again to isolate differences in the diet due to season. The Diet Niche Breadth (BS) was calculated using the formula described by Hespeneide (1975). The closer BS is to 1 then the wider the niche (each group being equally represented), while the closer BS is to 0 then the narrower the niche (with one food group becoming more and more important).

$BS = (B - 1) / (n - 1)$ [Where $B = 1 / (\sum p_i^2)$; n = number of groups; p_i = number of items in group i / total number of items].

Dietary Overlap (α) was calculated following Pianka (1975), where $\alpha = 1$ indicates identical diets and $\alpha = 0$ indicates no overlap between diets. The significance level was $p = 0.05$.

$\alpha_{xy} = (\sum p_{ix} * p_{iy}) / ((\sum p_{ix}^2) * (\sum p_{iy}^2))^{0.5}$ [where p_{ix} or p_{iy} = number of items in group i for habitat x or y / total number of items].

5.4. Results

5.4.1. Overall Diet

From the analysis of the total sample of scats ($N = 387$) the diet of the pine marten in KNP consists of a wide variety of food items ($N = 30$) (Table 5.1; Appendix 5). In total there were 883 components and each scat had on average approximately 2 components ($\mu = 2.28$, $SD = 0.99$). For the overall description of diet the data will be presented in decreasing importance of % FO. The % Mass and % FWI are discussed directly afterwards for each group to indicate whether items that were consumed frequently actually contributed the same in terms of energy. Only % FO will be used to illustrate the differences in the diet for each group across the four seasons.

Fruit was the most frequently consumed component in the diet (30.01 %FO; Table 5.1), of which ivy (*Hedera ilex*) was the most frequently consumed item followed by yew (*Taxus baccata*) and blackberry (*Rubus spp*). None of the remaining fruit species consumed by the pine marten contributed significantly to the diet. Fruit was also the largest group consumed in terms of % Mass (47.95) and % FWI (51.44) (Fig. 5.1). Arthropods were the next most frequently consumed item (29.22% FO) but contributed little overall in terms of mass (5.07% M) or FWI (1.97% FWI). Amongst the arthropods the order Coleoptera was the most frequently consumed group (22.9% FO).

Mammals were the next most frequently consumed group (20.27% FO) in the diet of the pine marten with woodmouse and bank vole (5.77% & 4.42% FO respectively) being

the most notable prey items in this group. There was no significant difference in the occurrence of these two species in the diet of the pine marten (Chi-square: $X^2 = 2.14$, $DF = 1$, $P = 0.15$). In terms of % Mass (15.29) and % FWI (26.73), mammals were the second most important group in the diet, after fruit (Fig. 5.2). Birds were the next most frequently consumed group, with passeriformes being the most noteworthy order amongst the birds (3.17% FO) though there was a large number of birds that remained unidentified (5.32% FO; Table 5.1). Based on % Mass and % FWI, Birds were the third most important prey group in the diet of the pine marten (Fig. 5.1). Finally frogs (*Rana temporaria*) were the main component of the Other Group (5.44 % FO), while earthworms were present in the diet albeit in small amounts (3.05 % FO and 1.67 % FWI).

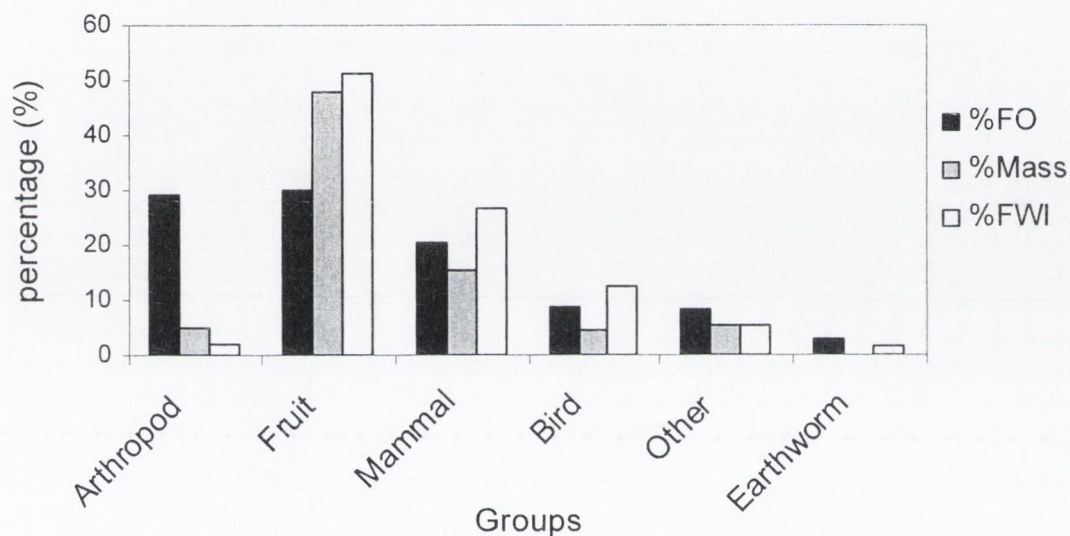


Fig. 5.1. % FO (frequency of occurrence), % M (Mass) and % FWI (fresh weight ingested) for each group in the diet of the pine marten in KNP.

5.4.2. Overall Dietary Niche Breadth

Overall there was a trend for one or two items within each group to dominate the majority of prey consumed within that group. On the whole the pine marten showed a wide dietary niche breadth, with the BS value = 0.659 indicating that four groups (fruit, mammals, birds and insects) were more important than the remaining two (other and earthworm).

Table 5.1. Dietary components found in the scats of pine marten (N = 387) in KNP expressed as the percentage frequency of occurrence (%FO), percentage mass (% Mass) and percentage fresh weight ingested (% FWI). No value was computed for % M for earthworm as it was not possible to weigh chaetae with the balance available.

Group	Component	% FO	% M	% FWI
Arthropoda	Beetles (Coleoptera)	22.99	4.19	1.61
	Bees & Wasps (Hymenoptera)	0.79	0.16	0.06
	Butterflies (Lepidoptera)	0.68	0.06	0.05
	Wood Louse (Isopoda)	0.91	0.06	0.02
	Larvae	0.11	0.03	0.01
	Not identified	3.74	0.56	0.22
	Total	29.22	5.07	1.97
Fruit	Blackberry (<i>Rubus sp.</i>)	2.26	2.92	0.03
	Bilberry (<i>Vaccinium myrtilius</i>)	1.47	2.08	2.23
	Ivy (<i>Hedera ilex</i>)	16.65	26.18	28.12
	Rowan (<i>Sorbus accuparia</i>)	0.91	0.3	0.3
	Yew (<i>Taxus baccata</i>)	6.12	15.65	16.8
	Apple (<i>Mallus sp.</i>)	0.57	0.14	0.15
	Hawthorn (<i>Crategus monyгна</i>)	0.11	0.09	0.09
	Pine (<i>Pinus sp.</i>)	0.45	0.03	0.03
	Not identified	1.47	0.56	0.6
	Total	30.01	47.95	51.44
Mammal	Woodmouse (<i>Apodemus sylvaticus</i>)	5.77	4.44	7.49
	Bank vole (<i>Cleithrionomys glareolus</i>)	4.42	3.85	6.49
	Pygmy shrew (<i>Sorex minutus</i>)	4.42	0.99	0.02
	Rat (<i>Rattus norvegicus</i>)	0.11	0.43	1.41
	Red squirrel (<i>Sciurus vulgaris</i>)	0.45	0.07	0.12
	Rabbit or Hare (<i>Lagomorph spp.</i>)	0.45	0.18	0.3
	Deer (<i>Cervus spp.</i>)	0.23	0.09	0.43
	Not identified	7.59	5.24	8.85
	Total	20.27	15.29	26.73
Bird	Song birds (Passeriformes)	3.17	1.02	2.89
	Falcons (Falconiformes)	0.11	0.29	0.83
	Egg Shell	0.34	0.008	0.001
	Not identified	5.32	3.14	8.94
	Total	8.95	4.47	12.66
Other	Snail (Mollusca)	0.90	0.03	0.037
	Common frog (<i>Rana temporaria</i>)	5.44	3.89	5.38
	Debris	2.15	1.49	0.11
	Total	8.49	5.42	5.41
Earthworm	Chaetae (<i>Lumbricus terrestris</i>)	3.05	-	1.67

5.4.3. Seasonal Diet

No significant inter annual variation was found for the diet of the pine marten for any of the months for which there was two years of data available (Chi-square: April: $X^2 = 5.9$, DF = 5, $P = 0.3$; May: $X^2 = 8.7$, DF = 5, $P = 0.1$; June: $X^2 = 6.6$, DF = 4, $P = 0.19$; July: $X^2 = 7.2$, DF = 4, $P = 0.1$; August: $X^2 = 0.9$, DF = 4, $P = 0.9$).

There was a significant difference in the diet of the pine marten in KNP across the four seasons (Fig. 5.2; G-Test: Likelihood Ratio = 95.6, DF = 15, $P \leq 0.001$). Fruit was the most important dietary group in spring time, while earthworms were the least ($X^2 = 216.9$, DF = 5, $P < 0.001$). During the summer months arthropods and mammals were the chief prey items in the diet while earthworms were the least ($X^2 = 114.8$, DF = 5, $P < 0.001$). Once again fruit was by far the most notable food group in autumn followed by arthropods; earthworms were absent from the diet ($X^2 = 97.9$, DF = 5, $P < 0.001$). Fruit was also the most important group in winter with the groups of bird and other contributing little to the diet at this time ($X^2 = 73.9$, DF = 5, $P < 0.001$).

Overall the % FO of arthropods in the diet did not vary among seasons with between 25 to 34% of the diet comprising of this group throughout the year (Fig. 5.2; $X^2 = 5$, DF = 3, $P = 0.13$). In contrast a significant result was recorded for fruit with peak intake occurring during autumn, though this group was also important in the diet during winter and spring ($X^2 = 47.7$, DF = 3, $P < 0.005$). Mammals were consumed significantly more in spring and summer than they were in autumn and winter (Fig. 5.2; $X^2 = 20.8$, DF = 3, $P < 0.005$). Birds were primarily taken in the diet during spring and summer and hardly ever during autumn and winter (Fig. 5.2; $X^2 = 11.4$, DF = 3, $P = 0.01$). There was no difference observed between the seasons in the % FO of the Other group, comprising frogs, mollusca and debris ($X^2 = 4.5$, DF = 3, $P = 0.2$). Earthworms were largely taken during the winter with small occurrences in the diet during spring and summer with no intake during autumn (Fig. 5.2; $X^2 = 41.2$, DF = 3, $P < 0.005$).

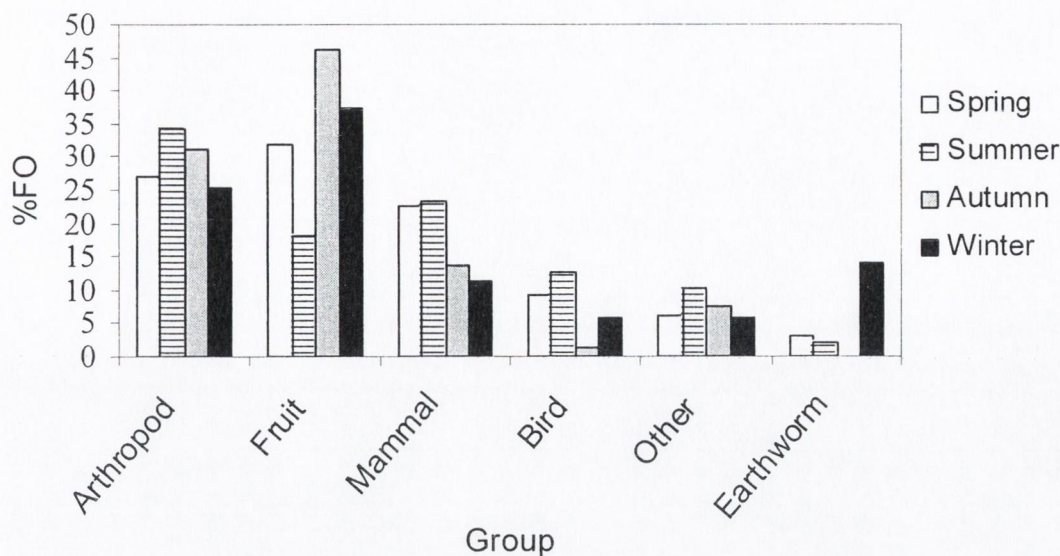


Fig. 5.2. Seasonal variation in the % FO (frequency of occurrence) of each dietary group for the four seasons.

5.4.4. Seasonal Dietary Niche Breadth and Overlap

A similar dietary niche breadth (BS) was observed for spring, summer and winter (BS = 0.63, BS = 0.67 & BS = 0.62 respectively). For these seasons the diet, while dominated by one or two groups, contained all six. The dietary niche breadth for autumn was much narrower than that recorded for the other three seasons indicating that one food group, fruit (46.25 % FO) dominated the diet (BS = 0.39). A high degree of diet niche overlap (α) was observed in the diet of the pine marten in KNP across all seasons ($\alpha_{\text{spring-summer}} = 0.94$, $\alpha_{\text{spring-autumn}} = 0.95$, $\alpha_{\text{spring-winter}} = 0.95$, $\alpha_{\text{summer-autumn}} = 0.83$, $\alpha_{\text{summer-winter}} = 0.83$ & $\alpha_{\text{autumn-winter}} = 0.95$).

5.5. Discussion

The diet composition of pine marten in this study does differ from what has been reported for the pine marten throughout Europe. Most studies refer to small mammals as the backbone of the diet, with other groups being seasonally important (Goszczyński, 1976; Marchesi & Mermod, 1989; Balharry, 1993b; Jedrzejewski *et al.*, 1993; Haliwell, 1997). In this study, fruit was the most important diet item to the pine marten followed closely by mammals, particularly woodmouse and bank vole. Birds were the next most important item followed by frogs and earthworms respectively. Arthropods and particularly the order Coleoptera were ingested frequently, as has been found with all

dietary studies on pine marten, but they contributed very little in terms of overall mass and fresh weight ingested. The pine marten's diet was characterised by a wide niche breadth, indicative of an opportunistic and general feeder. Values for % FO, % FWI and BS (where given) recorded in the literature, together with a synopsis of some of the studies carried out on the diet of the pine marten are given in Appendix 5.

Fruit was found to be the most commonly ingested component and the item that contributed most to the diet of the pine marten in KNP in terms of % M and % FWI. While KNP has many fruiting species (Kelly, 1975), it is evident that the pine marten focused on three main fruits: ivy, yew and blackberry and largely ignored the rest. Ivy was a principal prey item in the diet of the pine marten throughout winter and spring as noted previously by Warner & O'Sullivan (1982). Yew is only locally abundant in one area of the park but it was evident that wherever yew fruit occurred it was consumed, while blackberries available throughout the park were taken when ripe (September) and was also found by Fairley (1970). Other authors have also found fruit to be an important resource during winter and spring, with the berries consumed being those abundant to that area (Goszczyński, 1976; Marchesi & Mermod, 1989).

Most authors cite small mammals as the backbone of the diet of pine marten and that other groups like fruit, while important, are taken seasonally (Goszczyński, 1976; Marchesi & Mermod, 1989; Balharry, 1993b; Jedrzejewski *et al.*, 1993; Halliwell, 1997). While there was a seasonal pattern to the intake of fruit in the diet (peaking in autumn with smaller peaks in winter and spring), this was not obvious. Fruit was always more frequently consumed and contributed more to the diet of the pine marten in KNP than mammals, except in summer, a pattern similar to that found by NíNeachtain (1998) for broadleaf scrub woodland in the west of Ireland. In contrast Warner & O'Sullivan (1982) found that overall the intake of birds was marginally greater than fruit, which was the main prey item throughout autumn and winter. Similarly Clevenger (1993c) investigating an island population in Minorca found that likewise while one group may be the most important overall (highest % FO for the overall diet) that no one group dominated throughout the year, each being seasonally important.

Mammals were the most important food item after fruit with peak intake occurring in the spring and summer, the latter season being dominated by mammals. A pattern observed in many other studies but which was absent from this study, is the dominance

of the vole species in the mammal prey category over others (e.g. woodmouse, shrews, rats, squirrels) (Lockie, 1961; Storch, Lindström & de Jonge, 1990; Balharry, 1993b; Jedrezejewski & Jedrezejewska, 1993; Gurnell *et al.*, 1994; Zalewski, 1996; Putman, 2000). In the KNP pine marten population, the woodmouse was the most frequently consumed mammal with bank voles a close second. The bank vole has only recently occurred in Ireland (*circa* 1955) and was absent or establishing in study areas where the diet of pine marten had previously been examined (Warner & O'Sullivan, 1982; NíNeachtain, 1998). NíNeachtain (1998) classed the bank vole as a relatively unimportant food item indicating the low abundance of this species as a possible reason ($N = 11$ bank voles trapped *cf* $N = 78$ woodmouse). By contrast the bank vole is an integral part of the diet of the pine marten in KNP.

It is probable that this small difference in the diet of the pine marten between woodmouse and bank vole is a reflection of the lower population density of the latter. In KNP it has been found that only in one area did bank vole numbers exceed woodmouse in population density estimates (Reenadinna Yew Wood, transect 3, Fig 2.1; Lynch, 2002). The average estimate of bank vole density for each month based on minimum number alive (July, August & September) was approximately 26 compared with 19 per 0.002 km² for the wood mouse ($\mu_{\text{bankvole}} = 26.33$, $SD = 6.43$; $\mu_{\text{woodmouse}} = 19.66$, $SD = 7.3$). By comparison, in the neighbouring oak wood woodmouse density was higher than bank voles and, as the majority of woodland sampled in this current study was oakwood, this reflects a truer picture of the dynamics of these two small mammal species ($\mu_{\text{bankvole}} = 14.33$, $SD = 4.0$; $\mu_{\text{woodmouse}} = 28$, $SD = 7.55$). The difference in density in the yew wood can be accounted for by the presence of karst limestone pavement providing additional three dimensional spaces thereby allowing for decreased interactions between individuals, which are known to regulate bank vole populations, and protection from predation (Alibhai & Gipps, 1985). Elsewhere bank vole density would appear to be limited by the amount of groundcover (Lynch, 2002). Whether the lack of a difference between the woodmouse and bank vole in their %FO in the diet of the pine marten is due to the pine marten actively selecting one species over the other remains speculative and to prove so would require a more in-depth study of prey availability and selection. Nevertheless it is evident that the bank vole is an important prey item for the pine marten in KNP.

Birds accounted for 20% of the diet of pine marten in Warner & O'Sullivan's study (1982), which the authors hypothesised was a result of the depauperate mammal fauna in their study area (only woodmouse, house mouse and rat were present). A similar pattern was observed in Minorca, another island population, where birds accounted for approximately 20% (FO) of the overall diet (Clevenger, 1993c). Most authors cite an intake of about 10-15% (Lockie, 1961; Goszczyński, 1976; Balharry, 1993b; Jedrezejewski & Jedrezejewska, 1993; NíNeachtain, 1998). In this study birds accounted for 8.95 % FO and 12.65 %FWI. As is the case with all studies on pine marten, passerines made up the bulk of the avifauna taken and were primarily consumed in spring and summer. Most studies report birds as similarly consumed in spring and summer when naive juveniles, nestlings and fledglings, are present in the environment (Goszczyński, 1976; Marchesi & Mermod, 1989; Balharry, 1993b; NíNeachtain, 1998). Some studies report them as chiefly a winter food, easily taken when weakened by the winter weather (Warner & O'Sullivan, 1982).

A high percentage of bird remains were left unidentified owing to the lack of diagnostic features with which to identify them. In faecal analysis bird remains are generally identified to order using the barbules of the feathers (Day, 1966). These tended to be destroyed in many cases. NíNeachtain (1998) suggests looking for barbules amongst the microscopic fraction but this was not found to be useful, as I often found no barbules present or these were also destroyed. However Reynolds & Aebischer (1991) report that micro and macro fragments of birds do not generally occur in the same scat of foxes, so that though there may be feathers in the macroscopic component this does not necessarily mean that they will be in the microscopic fraction or vice versa, or that they correspond to the same individual (prey item).

Arthropods did not contribute much to the diet in terms of % Mass or % FWI, but after fruit were the most frequently consumed item. Within the Arthropod group, the order Coleoptera was the most important food item with Hymenoptera, Isopoda and Lepidoptera all consumed in equal but low amounts. Other authors have found that though Coleoptera always dominate, the order Hymenoptera is also important (Marchesi & Mermod, 1989; Balharry, 1993b). In these studies animals had been reported robbing hives for honey, particularly at night when the inhabitants were immobile, but no incidents of hive robbing were found in KNP.

Interestingly there was no distinguishing seasonal peak for arthropods, this group being consumed in equal amounts throughout the year (Appendix 5). NíNeachtain (1998) reported such a pattern for pine martens in County Clare (Ireland), though Warner & O'Sullivan (1982) found that this group peaked in summer and autumn in the same county. Clevenger (1993c) reports a similar peak in autumn for Minorcan pine martens but once again it is not profoundly distinguished from other seasons. Other studies show dramatic peaks for summer and autumn with little or no consumption in the spring and winter (Marchesi & Mermod, 1989; Balharry, 1993b). It is likely that the relatively mild climate in the south-west of Ireland is conducive to arthropod activity throughout the year.

The main component consumed of the Other group was the common frog (Ireland has only one frog species and toads are absent from the area). Though frogs are not taken in large amounts at any time of the year, they are consistently eaten throughout the spring summer and autumn. Balharry (1993b) and Marchesi & Mermod (1989) also report a similar pattern, thus indicating that they are a resource which the pine marten exploits regularly.

Earthworms were only found to be important during the winter months. This is in stark contrast with the two previous Irish studies which report them as an important part of the diet of the pine marten throughout the year (Warner & O'Sullivan, 1982; NíNeachtain, 1998). Marchesi & Mermod (1989) also found many remains of earthworms in their samples and also report the earthworm to be important in the diet. In other studies the importance of earthworm in the diet has been overlooked as it is thought chaetae can be ingested when the animal is preying upon birds or through the accidental ingestion of soil when consuming berries (Warner & O'Sullivan, 1982).

In this study earthworms were only important in the winter, but chaetae were also found in scats in spring and summer but not in autumn. While I could attribute chaetae found in samples during the spring and summer (none were found in autumn) as indirect ingestions through the consumption of birds (birds were most frequently taken in spring and summer) I cannot reconcile this with the data for winter. Though chaetae may be ingested accidentally when foraging for other food stuffs, namely bird and berries (shed chaetae are present in the soil or in the guts of birds having preyed on earthworms themselves), this could not account for all the occurrences of chaetae in the samples

especially in winter. Thus earthworms are part of the diet of the pine marten in KNP, particularly in winter when other food items are less abundant.

The diet niche breadth ($BS = 0.659$) of the pine marten population investigated in this study is the lowest recorded for Ireland thus far. NíNeachtain (1998) reports a BS of 0.798 (based on the six categories) for her population of pine marten in hazel-scrub woodland while Warner & O'Sullivan (1982) report a BS of 0.803 (based on six categories) for pine martens in lowland broadleaf woodland. The higher BS in both these studies is as a result of a more even intake of all prey items in contrast to this study where fruit, mammals, birds and insects made up the bulk of the diet and the other two groups were very much secondary items (other and earthworms). The observed reduction in the niche breadth compared with other Irish studies, though speculative, may be due to the presence of the bank vole in the diet which was absent from the previous studies. Thus the two groups other and earthworm are not as important. A comparative study investigating the diet of the pine marten in woodlands with or without the bank vole may give weight to this argument.

Similarly Clevenger (1993a) reports a wide niche breadth for the island population of Minorca ($BS = 0.754$ based on five categories, no earthworm *cf* 0.829 this study excluding the earthworm category) but a much narrower niche breadth for the mainland population studied ($BS = 0.38$, based on same five categories). This he attributes to a poorer mammal prey base and reduced interspecific competition on the island as compared with the mainland population. Though not an island population Marchesi & Mermod (1989) recorded an equivalent BS to this study ($BS = 0.658$, based on five categories, no earthworm category) fruit, mammals birds and arthropods were the main components of the diet on that occasion.

A similar BS (0.62 - 0.67) was recorded for all seasons except for autumn (0.39) which was dominated by the fruit group; approximately 50% of the autumn diet was fruit. NíNeachtain (1998) also recorded a seasonal dip in the BS during summer and autumn but this was not as dramatic as observed in our study. The dietary niche overlap was similar for all seasons except for summer with autumn and winter. This was as a result of mammals being the dominant prey item in summer while fruit was for the remainder of the seasons. NíNeachtain (1998) recorded a more variable dietary niche overlap particularly for spring and summer with autumn. This is due to the interchangeability in

the dominant food group (earthworms in spring, birds & insects in summer & fruit in winter). Overall the dietary breadth and dietary niche overlap found in this and other studies indicate that the pine marten is a generalist, opportunistic feeder.

With the exception of the summer months, when mammals are the most important food group, fruit is the most notable prey group consumed throughout the year. Though mammals are not the backbone of the diet as noted in many other studies, they are consistently taken. The bank vole, firmly established within KNP, is now an integral and important component of the mammal prey consumed by the pine marten. This species is also likely to be important in the diet of pine marten where ever it occurs within Ireland. Insects are important all year round and birds are important when at their most vulnerable, spring and summer. The wide niche breadth found in this study is consistent with what has been reported for other island populations. The implications of these findings for the conservation of the pine marten lie with woodland management. As a generalist feeder with a wide niche breadth the maintenance of woodlands with good fruiting species diversity, which themselves then provide the habitat for the wide range of prey items that the pine marten consumes, is essential to meet the dietary requirements of this species. Many of the woodlands within KNP are infested with rhododendron and are over grazed, thereby showing little sign of regeneration. Thus the inhibition of woodland regeneration may have consequences for the long term viability of the pine marten in the park.

CHAPTER 6. EFFECTS OF TRANSLOCATION AND RANGE FRAGMENTATION ON THE GENETIC DIVERSITY AND STRUCTURE OF THE IRISH PINE MARTEN POPULATION.

ABSTRACT

Conservation genetic studies on pine marten have shown that the Irish population is both genetically less diverse than, and isolated from the rest of Europe. These findings were, however, based on a small sample size ($N = 9$) which may not have adequately described the current population of pine marten in Ireland. I aimed to expand the knowledge of the current genetic status of the pine marten through increased sampling effort and to explore the impact translocation has had on the genetic diversity of the pine marten population in KNP and whether this population had gone fully extinct prior to being repatriated. It was found that despite increased sampling effort a similar pattern of low diversity was observed (genetic variation was 33.8 %) and is indicative of a smaller founding population post glaciation. The population in KNP shows significantly reduced genetic diversity possibly due to translocation compared with the Source population (the populations from which the animals were translocated from Co. Clare and Co. Mayo) and the rest of Ireland (24.9 % *cf* 34.7 % and 33.8% respectively) probably resulting from a combination of small founder effect due to translocation and inbreeding. No unique alleles were found in KNP, those found being a subset of the source population which were themselves a subset of alleles found within Ireland. This may be indicative of full extirpation of this species from the park prior to re-establishment, as it might be expected that unique alleles should have been observed had the population been truly present prior to reestablishment and isolated for an extended period. Only one haplotype was found in Ireland for the control region and one for the cytochrome *b* gene further suggesting complete extirpation. However complete separation of the KNP population may not have been long enough for sequence divergence to occur. Though the pine marten population in Ireland is genetically less diverse compared to other island populations studied within its range (Scotland ~ 40%) and shows a further reduction in genetic variation within the population at KNP, there does not appear to be any outward evidence that this is affecting the population, with a female biased sex ratio attributed to trapping methodology rather than a natural dynamic of the population (Chapter 3).

6.1. Introduction

The study of molecular ecology, specifically the use of DNA, and the associated tools within this discipline, is now an integral part of the conservation of wildlife and provides an important avenue through which information can be gathered about species and their populations. Conservation genetics takes the methods and theories of molecular ecology and uses them to interpret the natural history and current status of wildlife populations, the eventual outcome of which when combined with demographic, ecological, behavioural and physiological characteristics is to provide valuable knowledge to aid wildlife managers to develop effective management strategies for species (O'Brien, 1994).

Studies using molecular markers have been carried out to explore the origins of populations (Davison *et al.*, 2001, Stone *et al.*, 2002). Given the historic and present day persecution of many mammal species, high levels of habitat loss and habitat fragmentation worldwide, molecular tools have also been used to examine the problems associated with small populations, isolation of populations, relationships between fragmented populations and reintroductions (Paetkau *et al.*, 1998a; Kyle *et al.*, 2000; Kyle *et al.*, 2001; Waldick, *et al.*, 2002; Drew *et al.*, 2003; Vinkey, 2003). In addition, molecular techniques have been used in more practical ways to gain information about the specific dynamics of individual populations such as dispersal (Broquet *et al.*, 2005), sex ratios (Dallas *et al.*, 2003; Chapter 7 this study), population estimates (Mowat & Paetkau, 2002), parentage (Pemberton *et al.*, 1995; Schröpfer *et al.*, 1997) and to identify species by the traces (e.g. hair, scats) they leave behind (Davison *et al.*, 2002; Domingo-Roura, 2002; Palomares *et al.*, 2002; Frantz *et al.*, 2004).

6.1.2. Microsatellites

Microsatellites, the genetic markers used in this study, have become the most popular of all molecular markers since their discovery over a decade ago (Schlötterer, 2000) and have many advantages over alternative polymorphic systems (Pemberton *et al.*, 1995). This is due to the fact that these markers occur ubiquitously throughout the nuclear genome, can be highly polymorphic, evolve quickly, small amounts of test DNA are required, absolute sizing of alleles is possible, screening is fast, and they are adaptable to automated systems (Pemberton *et al.*, 1995; Hancock, 1999). Also, as codominant markers (both alleles show up in analysis) expected heterozygosity (H_e) can be calculated, a key parameter used for evaluating the level of genetic diversity observed

within the population under investigation. Populations with values of H_e approximating 1 are considered to exhibit high levels of genetic diversity, while those closer 0 show depleted levels of diversity. Typically low levels of diversity are observed in populations which are highly fragmented, have gone through bottlenecks, have been heavily persecuted or have been reduced to a small number of breeding individuals (Table 6.1). A low level of genetic variation is thought to be a factor which increases a population's susceptibility to disease and makes it more vulnerable to stochastic events and certainly many populations which are reduced to small numbers or excluded from receiving immigrants (populations on islands or geographic or man made barrier) exhibit low levels of variation primarily due to inbreeding.

Microsatellites are sequences, widely dispersed throughout the eukaryotic genome, made up of a single sequence motif (between 2-6 bp in length, tandemly repeated) and are arranged head-to-tail without interruption by any other base or motif (Bruford & Wayne, 1993). Microsatellites are classed into three families based on the type of repeat. Pure microsatellites are those in which the core repetitive unit is maintained throughout the region while imperfect microsatellites have the core repetitive unit interrupted by non-repeat sequences. Compound microsatellites have two or more different but repetitive units adjacent to each other. The flanking sequence on either side is non-repetitive and may also contain base substitutions, insertions or deletions that also affect allelic size (Rosenbaum & Deinard, 1994). Though dinucleotide repeats (e.g. AT/TA or GA/CT) are the most commonly availed of microsatellite type, tri and tetra nucleotides are also used. Generally microsatellite loci are very polymorphic and can contain from 1-50 alleles (*cf* Allozymes (genetic variations of the same enzyme): 1-5; RAPDs (Randomly Amplified Polymorphic DNA): 2) at a given locus, with between 5-20 polymorphic loci (*cf* Allozymes: 10-50; RAPDs: 10-100) being discovered per species examined (Jarne & Lagoda, 1996).

Table 6.1. Reported levels of % expected heterozgosities (H_e) for 12 different species from the literature, including available data on the pine marten. % H_e is given as the absolute or average calculated for the population indicated unless a range was reported in the case of many areas sampled. * refers to populations which were on located on islands. ^(PB) refers to pre-bottleneck, while ^(DB) refers to during bottleneck.

N	Species	Country or Region	Population Status	Number of Loci	H_e (%)	Source
1	Pine marten	Ireland	Increasing	8	34.0*	Kyle <i>et al.</i> (2003)
		Scotland	Increasing	8	42.3*	
		England	Rare	8	66.1	
		Germany		8	56.2	
		Sweden		8	57.3	
		Finland		8	57.2	
		Netherland		8	53.8	
		Latvia		8	63.8	
		Italy		8	61.0	
2	American marten	Yukon & North West Territories	Healthy & Harvested	11	65.6	Kyle <i>et al.</i> (2000)
		Canada	Healthy & Harvested	11	62.6	Kyle & Strobeck (2003)
					40.2 *	
		Pacific Northwest USA	Threatened	14	6*-68	Small <i>et al.</i> (2003)
3	Fisher	North America	Threatened	13	62.25	Kyle <i>et al.</i> (2001)

N	Species	Country or Region	Population Status	Number of Loci	H _e (%)	Source
4	Wolverine	North America	Threatened	12	42.09-68.48	Kyle & Strobeck (2001)
		Northern Norway	Increasing	15	35.0-43.6	Walker <i>et al.</i> (2001)
		Southern Norway			30.4-38.6	
		Sweden			32.4-41.8	
		Pre 1970			26.9	
5	Black footed ferret (<i>Mustela nigripes</i>)	Wyoming, USA	Endangered	14	10.0 ^(PB) 8.0 ^(DB)	Wisely <i>et al.</i> (2002)
6	Sea otter (<i>Enhydra lutris</i>)	Pacific coast, USA	Endangered	4	82.3 ^(PB) 37.7-53.2 ^(DB)	Larson <i>et al.</i> (2002)
7	Lynx	Scandinavia	Endangered	11	51.0	Hellborg <i>et al.</i> (2002)
		Finland			62.0	
		Baltic			60.0	
9	Brown bears (<i>Ursus arctos</i>)	Coastal & interior Alaska	Declining	17	49.6-77.9	Paetkau <i>et al.</i> (1998a)
		North America	Declining	8	29.8-78.8	Paetkau <i>et al.</i> (1998b)
10	Black Rhino (<i>Diceros bicornis</i>)	Africa	Endangered	9	45.9 (<i>minor</i>) 50.5 (<i>bicornis</i>) 73.1 (<i>michaeli</i>)	Harley <i>et al.</i> (2005)
12	N.Atlantic right whale (<i>Eubalaena glacialis</i>)	North Atlantic	Endangered	11	46.3	Waldick <i>et al.</i> (2002)

A correct appreciation of the rate and model of mutation in microsatellites is essential for the analyses of population structure as it is the major determinant in the variability between populations (Jarne & Lagoda, 1996). The two most commonly proposed mutation models to explain evolution in microsatellites are the Infinite Allele Model (IAM) and Stepwise Mutation Model (SMM). Both models are based on the premise that the central driving force behind mutation is DNA slippage (Rosenbaum & Deinard, 1994; Beebee & Rowe, 2004). IAM proposes that mutation may involve any number of repeats and always results in an allele state not seen previously in the population. By contrast SMM suggests that alleles are derived by the loss or gain of a single repeat and thus may mutate towards allelic states already present in the population (in Estoup & Cornuet, 1999).

A third model based on IAM but which is considered more applicable is the k Allele Model (KAM), which permits newly generated alleles to occupy any k pre-existing allelic states (in Rosenbaum & Deinard, 1994). A fourth model, based on the SMM, known as the Two-Phase Model (TPM), suggests that X repeats can be lost or gained through mutation. Where $X = 1$ this is SMM (in Estoup & Cornuet, 1999). Studies on mutations at microsatellite loci have shown that this process usually involves the loss or gain of one repeat and, less frequently, more than one. Furthermore they have also indicated 1) that alleles with larger number of repeat units have higher mutation rates, 2) perfect microsatellites have higher mutation rates than imperfect or compound microsatellites as interruptions appear to stabilise the motif, 3) mutations at microsatellite loci involve more gains than losses and 4) there is a mechanism (selection or mutation) operating to constrain the size of the repeat motif (in Estoup & Cornuet, 1999). It is generally believed that SMM or some derivation is the correct model for the evolution of microsatellites.

An added advantage of using microsatellites is that markers, or more specifically the primers developed to recognise those markers, for one species have been shown to work in other species. For example microsatellites currently available for the pine marten are derived from the American marten, wolverine and otter (Chapter 2). The isolation of new microsatellites for a species is both expensive in terms of cost and labour, thus when microsatellite primers from one species can be used in another (commonly referred to as degenerate primers or imperfect primers) then this will ultimately cut down on the overall cost of the project.

There are however, a number of limitations associated with microsatellite markers. Firstly, the length of the microsatellite typed is determined by the length of the entire sequence, to include the flanking sequence. Insertions and deletions both within an individual repetitive unit and / or in the flanking sequence may lead to product sizes which are not consistent with incremental increases or decreases of the repetitive unit (Schlötterer & Pemberton, 1998). Insertions and deletions are more common when markers are used in cross species amplifications (degenerate primers) and lead to a second problem associated with microsatellites and by far the most important- null alleles. Null alleles are non-amplifying alleles, caused by insertions and deletions in the flanking sequence, which cause an obstruction in the primer binding site. Significant deviations from Hardy Weinberg equilibrium (HWE) in terms of heterozygote deficiency (or homozygote excess) can be diagnostic of null alleles. However, high numbers of homozygotes may also be due to genetic drift, particularly in isolated populations and also in inbred populations.

Many studies choose to ignore the presence of null alleles in the data set or in given populations within the data set (Kyle *et al.*, 2003) while others would urge their recognition as it can lead to wrong conclusions being drawn about population status (Pemberton *et al.*, 1995). Null alleles have also been found to be associated with badly degraded or low quantities of DNA (such DNA is commonly associated with non-invasive samples), even when microsatellite primers are species specific (Taberlet *et al.*, 1996; Gagneux *et al.*, 1997). Thirdly despite microsatellites occurring across many taxa the number of microsatellites discovered has been found to differ significantly between species and more importantly in the number of loci suitable for population studies (such studies require loci that are at least moderately polymorphic) (Schlötterer & Pemberton, 1998).

6.1.3. Mitochondrial DNA (mtDNA)

In addition to the nuclear markers described above, mtDNA has been used extensively in the last three decades to compile pictures of the evolutionary and demographic past of many species (Davison *et al.*, 2001; Kyle & Strobeck, 2001; Stone *et al.*, 2002). In contrast to microsatellites, which as part of the nuclear genome exist as only one copy per cell, the number of copies of the mitochondrial genome per cell is dependent on the number of mitochondria within a given cell. The mammalian mitochondrial genome is a

circular double stranded haploid entity which is approximately 15,000 to 17,000 bp in length (Ballard & Whitlock, 2004).

Four main differences separate the mitochondrial and nuclear genome. MtDNA is believed to be maternally inherited, though some studies have shown that this may not always be the case (in Ballard & Whitlock, 2004). Beebee & Rowe (2004) suggest that because parental leakage occurs at such a low frequency and the vast excess of the mitochondrial genome is inherited from the mother's side then analyses can proceed as if it is solely maternally inherited. The second difference is that it is assumed that the mitochondrial genome does not undergo recombination. Once again this assumption has been challenged with rare events of recombination thought to occur (Beebee & Rowe, 2004). A third difference between the two genomes is the pattern of inheritance of genetic information. The mitochondrial genome, being haploid and maternally inherited, transfers one copy of the genome to the next generation. The nuclear genome, being diploid and bi-parentally inherited, transfers 4. As the effective population size (N_e) is usually expressed in terms of individuals, then the N_e for mtDNA is approximately half of nuDNA and is further reduced to a quarter when polyploidy is taken into account. Thus mtDNA would fix new alleles faster than nuDNA (Ballard & Whitlock, 2004). Fourthly mitochondrial DNA has been found to generally have a higher mutation rate than nuDNA.

Two areas commonly studied within the mitochondrial genome are the control region, more specifically the D-Loop (displacement loop which is the only non-coding area of the genome) and the cytochrome *b* gene. The control region is considered to be the most variable and hence most useful in population studies, while thought less useful the cytochrome *b* gene is also used due to the availability of universal primers for it (Kocher *et al.*, 1989; Beebee & Rowe, 2004). The use of mtDNA in studies investigating threatened or managed species falls into two categories. Firstly those studies concerned with gene conservation, i.e., the identification and management of genetic diversity, and secondly molecular ecology, i.e., the use of mtDNA variation to guide and assist demographic studies of populations (Moritz, 1994). The mitochondrial genome has been used in three ways to increase our knowledge of wildlife populations, 1) for the measurement of genetic diversity, particularly in species which have recently declined, 2) to segregate evolutionary divergent populations and perhaps establish Evolutionary Significant Units for conservation and 3) to assess the conservation values

of populations from an evolutionary perspective (Moritz, 1994; Davison *et al.*, 2001; Stone *et al.*, 2002; Drew *et al.*, 2003).

One potential problem with using mtDNA is the occurrence of nuclear copies of DNA (numts). Numts are nuclear copies of the mitochondrial genome which reside within the nucleus of the cell. These copies may be amplified instead of the actual copy of mtDNA leading to the misinterpretation of results (Beebee & Rowe, 2004). It has also been found that data from mtDNA analysis and nuclear markers do not always reach the same conclusion. This can occur when males disperse but females remain philopatric or due to the lower number of effective genes under consideration in the mitochondrial genome (Moritz, 1994).

6.1.4. Conservation genetics of the European pine marten

Little work on the molecular ecology of the pine marten has been carried out to date. One European wide study, using mtDNA examined the postglacial colonisation of Europe by the pine marten (Davison *et al.*, 2001) and reported an absence of ancient lineages suggesting that the present day distribution of martens is made up of animals that have colonised from a single refugium. As sample sizes were limited from many of the populations examined ($N = 9$ for Ireland), and phylogenetic trees were poorly supported by bootstrapping, the authors suggest that the full picture may not have been presented and that ancient lineages may well exist. It did, however, reveal a connection between the Irish pine marten and martens found on the Iberian Peninsula (all belonging to the same sub-group 1b), suggesting that this may have been where the pine marten colonised Ireland from. By contrast those haplotypes found in the UK belonged to the other subgroup 1a, with the exception of two English martens whose haplotypes point towards introgression with the American marten. Whether the Irish pine marten arrived on our shores of its own accord or was brought here by early settlers is still open to debate.

Another study using microsatellite analysis revealed a relatively high degree of structuring within the European pine marten, compared with American marten, primarily due to anthropogenic impacts and isolation by distance through fragmentation of preferable habitat (Kyle & Strobeck, 2003; Kyle *et al.*, 2003). Kyle *et al.* (2003) report similar levels of genetic variation for continental populations (Table 6.1.; average expected heterozygosity (H_e) across eight loci = 58.2%) but significantly reduced levels

for island populations sampled, Scotland ($H_e = 42.3\%$) and especially Ireland ($H_e = 34\%$). Once again uneven sample sizes between populations and low sample sizes for some populations (average number of samples per population based on 9 populations: $\mu = 17.77$, $\min = 7$, $\max = 59$) have made the data hard to interpret accurately. Population assignment tests did not assign available genotypes for the Irish population to any other population in the study, however there were no available data for Spain and France, which may have confirmed the evidence of a link with the Iberian peninsula presented in the study using mtDNA, (Davison *et al.*, 2001).

Other researchers have focused on developing techniques for monitoring pine marten populations by the identification to species level from non-invasive samples, in order to allow positive determination of the pine marten from other species (fox, stoat, mink etc.) which may leave similar traces behind (Davison *et al.*, 2002; Domingo-Roura, 2002; Statham *et al.*, 2005). In chapter 7 of this study I used sex primers available for otters to determine sex of non-invasive hair samples from pine martens.

The above studies by Davison *et al.* (2001) and Kyle *et al.* (2003) are the sole studies to have investigated the population structure and diversity of the European pine marten. Though both studies indicate that the Irish population is isolated from the rest of Europe and has decreased levels of genetic diversity as a result, probably due to a bottleneck, neither go into depth on the subject. The historic origins of the pine marten population in Ireland (Chapter 3), possibly established since the end of the last post glacial period or introduced for its fur, and the impact the decline of this species across much of its range in the last century and the translocation of animals within that range on the genetic structure and diversity of this population merits further investigation. In this study I aimed to address the latter of these two points.

6.1.5. Objectives

The objectives of this study were threefold. Firstly to expand on the work of Davison *et al.* (2001) and Kyle *et al.* (2003) through increased sampling effort to see if similar patterns of genetic diversity and structure previously documented were still evident. Secondly, to assess whether the translocation of pine marten to Killarney National Park has led to a decrease in genetic diversity of the population. Thirdly, to explore whether pine marten were present in Killarney National Park prior to the translocation.

6.2. Methods

A full description of the methods used in this chapter can be found in Chapter 2 (section 2.3.2). In brief, tissue samples (Fig. 6.1; N = 27; ear biopsies; 2mm circle) were collected from live captured animals during field work. Additional samples were also collected throughout Ireland (Fig. 6.1; N = 28; leg muscle and ear biopsies; Appendix 4) from animals killed on the roads (road traffic accidents: RTA's). All DNA was extracted using a Qiagen DNeasy tissue Kit (Appendix 11) and checked for quality and quantity by visualisation on an agarose gel.

PCR conditions for microsatellite work followed Davis & Strobeck (1998) and contained 1 x PCR Buffer [200mM Tris-HCl (Ph 8.4), 500mM KCl], 2.5mM MgCl₂, dNTPs [120 µM each of dATP, dTTP, dCTP, dGTP], 0.16µM Primer, 0.3 units Taq Polymerase and 2µl of DNA template with final reaction volume of 25µl. The PCR program was 1 min @ 94°C, [30 s @ 94°C, 20 s @ 54°C and 5 s 72°C] x 3 cycles, [15 s @ 94°C, 20 s @ 54°C and 1 s @ 72°C] x 33 cycles, 30 s @ 72°C and then ramping to 4°C, with a runtime of 46 min 38 s. PCR conditions for MtDNA followed Davison *et al.* (1999) and contained 1 x PCR Buffer [200mM Tris-HCl (Ph 8.4), 500mM KCl], 1.5mM MgCl₂, dNTPs [200 µM each of dATP, dTTP, dCTP, dGTP], 0.14µM of each primer, 0.5 units Taq Polymerase and 2µl of DNA template with a final reaction volume of 50 µl. The PCR program for MtDNA was 5 min @ 96°C, [1 min @ 94°C, 1 min @ 55°C and 1 min @ 72°C] x 35 cycles and the ramping to 4°C with a runtime of 2 hr 11 min 5 s.

All microsatellite PCR products were run on Spreadex gels, visualised using the Alpha Imager 3300 and scored by eye. Two regions of the mtDNA genome were sequenced, control region (320 bp) and cytochrome *b* (364 bp). PCR products for mtDNA were sent to Lark Technologies to be sequenced commercially. Owing to budget constraints the following samples were sequenced for the control region (N = 25: Ky 1-4; Ce 1-2, 6-8; G 1-6; Oy 1-2; L 2-3; M1, S1, Ww1, Wm1 & Ke1) and for the cytochrome *b* region (N = 19: Ky 1-4; Ce 1-2, 6-7; G 1-6; Oy 1-2; M1; Ww1; Wm1 & Ke1). Sequence alignment and editing were carried out using Mac Vector.



Fig. 6.1. Map of Ireland showing the distribution of samples. The square indicates samples that came from Killarney National Park while the dots indicate road traffic accidents (RTAs). Samples Ky 1 and 4 were RTAs within the park boundary and were not marked animals (Appendix 4).

6.3. Statistical Analysis

In this study statistical analysis was carried out on three sample groups: KNP (N = 27), Source (N = 9) and IRE (N = 28). Samples within the KNP grouping were from animals live trapped during the study (Appendix 3, with the exception of K1 which was included in the IRE grouping). The Source grouping included animals from County Clare (N = 8) and County Mayo (N = 1) as these were the counties from which animals were taken to re-establish the KNP population (see Chapter 3). The IRE group were the samples taken from around Ireland and included the Source group (Appendix 4). In addition to these sample groups, the Kyle *et al.* (2003) data set has also been reanalysed using the statistics in this chapter for direct comparison and also to see if the increased sample size alters the current description of genetic diversity and structure. Country abbreviations for the Kyle *et al.* (2003) data set are as follows: England (Eng), Scotland (Sco), Ireland (Ire), Germany (Ger), Netherlands (Net), Italy (Ita), Sweden (Swe), Finland (Fin), Slovenia (Slo) and Latvia (Lat). Where programs for analysis were used or found free to use on the web or free to download from the web, they are either referenced in the text (if published) or the web address can be found in Appendix 12.

Tests of disequilibrium and heterogeneity

Departure from Hardy Weinberg equilibrium (HWE) was tested for at each locus and overall using the program GENEPOP version 3.1 (Raymond & Rousset, 1995; Appendix 12). Loci showing significant departures ($\alpha = 0.05$) from HWE were examined to see whether this was a result of heterozygote deficiency or heterozygote excess. For loci found to be significantly heterozygote deficient, the frequency of null alleles was calculated using two separate estimators. The first estimator (P_n ; Chakraborty *et al.*, 1992; Appendix 12) does not take into account that missing data may be indicative of the individual being homozygous for the null allele and is calculated using observed and expected heterozygosities. The second estimator (ML-NULLESTIMATOR; Appendix 12) is a maximum likelihood estimator which calculates the frequency of null alleles from genotype counts in a sample that may or may not have missing data. As such where samples failed to amplify due to DNA problems these can be excluded prior to analysis. Genotypic disequilibrium, using GENEPOP, was used to test for independence amongst loci. Significant deviations indicate that loci are not neutral.

Genetic Variation

Genetic variation was assessed in each of the sample groups using the average number of alleles per locus, expected heterozygosity (H_e , Nei & Roychoudhury, 1974) and the occurrence and frequency of alleles in each of the sample groups. The computer program Microsatellite Analyser (MSA) was used to generate the data required for the above summations (Dieringer & Schlötterer, 2003; Appendix 12). Wilcoxon's signed-ranks tests were used to examine significant differences in heterozygosity between sample groups (Sokal & Rohlf, 1997). The effect of increasing sample size was also investigated for the Kyle *et al.* (2003) data set.

Genetic Distances

Pairwise F_{ST} values (estimated by a weighted analysis of variance) were recalculated for the Kyle *et al.* (2003) dataset and then again for this data set with the extra Irish samples found in this study included. F_{ST} values were calculated following Weir & Cockerham (1984) using GENEPOP and assume HWE and genotypic equilibrium. F_{ST} values are measures of genetic distance between populations, with values closer to 1 indicating populations are genetically distinct and values close to 0 suggestive of high migration rates between populations. Weir & Cockerham (1984) also advocate the sample sizes of populations should be equal. It is also assumed that loci which are fixed for the one allele make no contribution to the estimation.

Assignment Test

The assignment test used in this study was calculated using the program WHICHRUN (Appendix 12) and estimates which population within your dataset a given genotype has the highest probability of occurring in. A detailed discussion of the calculations upon which this test is based can be found in Paetkau *et al.* (1995). High values of self assignment are symptomatic of populations with little mixing or gene flow. The assignment test assumes that populations are in HWE and there is no linkage disequilibrium (Waser & Strobeck, 1998).

6.4. Results

The results of this chapter will be presented as follows. Departures from HWE will be assessed, along with presence of null alleles and departure from genotypic equilibrium. It is essential that these criteria are assessed correctly as the following tests assume that the populations under observation conform to HWE and genotypic equilibrium. The parameters used to assess genetic variation will then follow and will explore the impacts translocation and reestablishment have had on the genetic diversity of the population in KNP. Data from Kyle *et al.* (2003) was also reassessed in the light of increased sample size to examine if patterns of genetic distances between populations and population assignment remain the same. Finally I will examine the mtDNA evidence for animals being present in KNP prior to translocation.

6.4.1. Tests of disequilibrium and heterogeneity

Overall five of the eight loci under investigation in this study failed to conform to HWE (Table 6.2). The remaining three loci (1, 6 & 7) are monomorphic (only one allele; Appendix 6 & 7) and therefore departures from HWE could not be investigated. All significant deviations were as of a result of heterozygote deficiency (Table 6.3), with only Loc8 showing heterozygote excess (Table 6.4). Within the KNP grouping Loc8 deviated from HWE, while the only other two polymorphic loci found in this sample (2 & 5) conformed to HWE (Table 6.2). Deviations from HWE in the KNP sample resulted from heterozygote excess at Loc8. In the Source population only Loc8 conformed to HWE (Table 6.2) while loci 2, 3 and 5 showed significant deviations, caused solely by heterozygote deficiency (Table 6.3). In the IRE group all polymorphic loci showed significant deviations from HWE with the exception of Loc8 (Table 6.2), deviations were due to excess homozygotes at these loci (Table 6.3).

Table 6.2. Results of the probability test of deviation from Hardy Weinberg Equilibrium (HWE). Significant results are recorded in italics. – indicates a monomorphic locus.

	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
	MA1	MA2	MA18	MA19	GG7	GG14	Lut604	Ggu454
KNP	-	0.1938	-	-	0.203	-	-	<i>0.0327</i>
Source	-	<i>0.05</i>	<i>0.01</i>	-	0.21	-	-	<i>0.05</i>
IRE	-	<i>0.0129</i>	<i>0.0000</i>	<i>0.0011</i>	<i>0.0058</i>	-	-	0.2829
Overall	-	<i>0.0000</i>	<i>0.0000</i>	<i>0.0005</i>	<i>0.0255</i>	-	-	<i>0.0052</i>

Table 6.3. Results of the heterozygote deficiency test of deviation from Hardy Weinberg Equilibrium (HWE). Significant results are recorded in italics. – indicates a monomorphic locus.

	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
	MA1	MA2	MA18	MA19	GG7	GG14	Lut604	Ggu454
KNP	-	0.0513	-	-	0.5678	-	-	1
Source	-	0.1	<i>0.01</i>	-	0.9	-	-	1
IRE	-	<i>0.0357</i>	<i>0.0000</i>	<i>0.0014</i>	0.4629	-	-	1
Overall	-	<i>0.0129</i>	<i>0.0000</i>	<i>0.0004</i>	0.1129	-	-	1

Table 6.4. Results of the heterozygote excess test of deviation from Hardy Weinberg Equilibrium (HWE). Significant results are recorded in italics. – indicates a monomorphic locus.

	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
	MA1	MA2	MA18	MA19	GG7	GG14	Lut604	Ggu454
KNP	-	0.9644	-	-	0.5387	-	-	<i>0.0267</i>
Source	-	0.8	1	-	0.35	-	-	0.05
IRE	-	0.9576	1	1	0.5027	-	-	0.1488
Overall	-	0.9739	1	1	0.08729	-	-	<i>0.0042</i>

As much of the work in this chapter was based on the Kyle *et al.* (2003) paper, which recorded no significant deviations from HWE for any of the populations sampled with the exception of Scotland (Loc3) and Finland (Loc6) (both deviations were heterozygote deficiency), I reran their data set as my result was unexpected. I found several of the populations were found to deviate from HWE (heterozygote deficiency), but no longer do when corrected for experiment wise error except for the above, as stated in the paper.

Estimates of null alleles, though both estimators gave slightly different results, indicate that there are null alleles present and they occur at some loci with high frequency. Amongst my sampled areas based on the estimate in Chakraborty *et al.* (1992), Loc2 and Loc3 were found to have a high frequency of null alleles present. From the Kyle *et al.* (2003) data set with the exception of Loc5, a high frequency of null alleles were present at the remaining loci for one or more of the populations sampled (Table 6.6). The second estimator showed a similar pattern to that observed by the first with deviations between the two being accounted for by the different methods of estimation (Table 6.7). Using the second estimator more loci were found to have null alleles, albeit some at very low frequency.

Within my sampled areas, Loc5 was found to deviate from genotypic equilibrium with the exception of KNP where all loci were in equilibrium. In the Source grouping association was found between Loc2 and Loc5 ($\chi^2 = 6.41$, DF = 2, P = 0.03). In the IRE samples Loc2 and Loc5 ($\chi^2 = 14.529$, DF = 2, P = 0.001) as well as Loc4 and Loc5 ($\chi^2 = 7.807$, DF = 2, P = 0.02) were found to deviate from genotypic equilibrium. When all 55 samples were examined it was found that Loc2 and Loc5 ($\chi^2 = 6.502$, DF = 2, P = 0.039) showed association and also Loc4 and Loc5 ($\chi^2 = 6.6$, DF = 2, P = 0.037). As Loc5 shows obvious signs of linkage, it would be appropriate to exclude this locus from future analysis. As the above shows, many of the markers are not in HWE and are linked, thus caution should be taken when interpreting the results from the proceeding analysis.

Table 6.5. Comparison of the (1) Kyle *et al.* (2003) dataset and the (2) Kyle *et al.* (2003) with extra Irish samples added. Significant values are highlighted by being italicised. – indicates that only one allele was present at that locus for that population and therefore Hardy Weinberg (HW) probabilities could not be calculated. Hs is highly significant. These have not been corrected for experiment wise error.

	Loc1		Loc2		Loc3		Loc4		Loc5		Loc6		Loc7		Loc8	
Probability Test																
Pop	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>
Eng	0.01	0.01	0.34	0.32	0.147	0.15	0.009	0.009	0.69	0.7	1	1	0.034	0.03	0.027	0.02
Sco	0.36	0.38	0.01	0.01	0.0000	0.000	0.003	0.004	0.0269	0.02	0.09	0.09	-	-	1	1
Ire	-	-	0.63	0.0009	0.0369	0.0000	-	0.0000	1	0.1	0.22	0.26	-	-	1	0.03
Ger	0.59	0.58	0.59	0.61	0.2244	0.21	1	1	0.82	0.81	0.04	0.04	-	-	0.2098	0.2
Net	1	1	0.89	0.88	0.7301	0.71	1	1	1	1	0.0671	0.06	1	1	0.784	0.78
Ita	0.007	0.01	0.03	0.027	0.0017	0.006	1	1	0.48	0.48	0.0027	0.009	0.2	0.19	0.0792	0.07
Swe	0.004	0.003	0.23	0.25	0.0022	0.004	0.008	0.008	0.4355	0.4	0.46	0.46	0.46	-	0.5	0.49
Fin	0.17	0.21	0.61	0.61	0.0045	0.004	0.001	0.0007	0.04	0.04	0.000	0.000	0.007	0.004	0.008	0.0004
Lat	0.54	0.54	0.07	0.09	1	1	0.54	0.57	0.45	0.45	0.0671	0.06	0.54	0.54	0.794	0.79
Slo	0.36	0.36	0.45	0.46	1	1	0.2991	0.3	0.6434	0.66	-	-	0.1097	0.1	0.46	0.46
Over	0.002	0.002	0.055	0.001	Hs	Hs	0.0007	0.0001	0.21	0.07	Hs	Hs	0.01	Hs	0.03	0.002
Heterozygote Deficiency																
Pop	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>
Eng	0.08	0.05	1	1	0.14	0.15	0.01	0.01	0.4	0.42	0.51	0.51	0.1	0.11	0.18	0.18
Sco	0.65	0.63	0.008	0.007	0.000	0.000	0.01	0.02	0.7	0.76	0.01	0.01	-	-	0.64	0.66
Ire	-	-	0.95	0.02	0.02	0.000	-	0.00	0.95	0.15	0.99	0.27	-	-	1	1
Ger	0.58	0.59	0.477	0.49	0.2	0.19	0.63	0.63	0.4	0.38	0.04	0.04	-	-	0.31	0.33
Net	0.84	0.85	0.52	0.53	0.5834	0.571	0.54	0.56	0.61	0.61	0.05	0.06	1	1	1	1
Ita	0.06	0.05	0.001	0.0018	0.0019	0.004	0.72	0.7	0.37	0.38	0.007	0.006	0.2	0.2	0.005	0.006
Swe	0.00	0.009	0.456	0.44	0.0001	0.0002	0.09	0.09	0.37	0.35	0.46	0.46	-	-	0.68	0.68
Fin	0.47	0.47	0.79	0.78	0.01	0.01	0.71	0.73	0.36	0.34	0.0001	0.0000	0.0001	0.0001	0.002	0.004
Lat	0.59	0.58	0.54	0.55	0.3896	0.39	0.62	0.6	0.56	0.57	0.06	0.06	0.6	0.59	0.31	0.3
Slo	0.2	0.2	0.265	0.23	0.72	0.77	0.48	0.49	1	1	-	-	0.1	0.11	0.26	0.25

	Loc1		Loc2		Loc3		Loc4		Loc5		Loc6		Loc7		Loc8	
Heterozygote Excess																
Pop	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>
Eng	0.75	0.76	0.45	0.43	1	1	1	1	0.66	0.65	0.96	0.96	0.89	0.9	0.82	0.83
Sco	0.43	0.43	0.98	0.98	1	1	0.98	0.98	0.26	0.25	0.98	0.98	-	-	0.56	0.55
Ire	-	-	0.12	0.98	1	1	-	1	0.24	0.84	0.20	0.97	-	-	0.82	0.002
Ger	0.57	0.57	0.61	0.66	0.92	0.92	0.56	0.56	0.72	0.7	1	1	-	-	0.79	0.78
Net	0.38	0.38	0.64	0.64	0.65	0.64	0.69	0.7	0.53	0.51	1	1	0.66	0.65	0.14	0.14
Ita	0.95	0.95	0.99	0.99	1	1	0.4	0.42	0.68	0.68	1	1	1	1	0.99	0.99
Swe	0.99	1	0.55	0.55	0.99	1	0.92	0.92	0.64	0.63	0.67	0.67	-	-	0.38	0.4
Fin	0.97	0.96	0.25	0.24	0.98	0.98	0.31	0.27	0.64	0.63	1	1	1	0.99	0.99	0.99
Lat	0.66	0.65	0.53	0.53	0.84	0.82	0.88	0.58	0.58	0.56	1	1	0.66	0.66	0.84	0.85
Slo	0.91	0.9	0.97	0.96	0.58	0.56	0.6	0.6	0.51	0.5	-	-	1	1	0.96	0.96

Table 6.6. Frequency of null alleles at each locus based on the estimate provided in Chakraborty *et al.* (1992). Loci which were found to be significantly heterozygote deficient (Tables 6.3 & 6.5) are indicated in bold. Data for my sampled areas are listed first followed by Kyle *et al.* (2003).

Locus	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
Pop	MA1	MA2	MA18	Ma19	GG7	Gg14	Lut604	Gg454
KNP	0	0.13	0.18	0.03	0.38	0	0	0.3
Source	0	0.15	0.43	0.3	-0.07	0	0	-0.25
Ire	0	0.19	0	0	0.11	0	0	0.24
Eng	0.577	-0.06	0.52	0.54	0	0.029	0.025	0.17
Sco	-0.03	0.09	0.42	0.17	-0.006	-0.02	0	-0.008
Ire	0	-0.12	0.65	0	-0.11	-0.19	0	-0.06
	0	0.098	0.17	1	0.025	0.09	0	-0.18
Ger	0.018	0.029	0.17	0	0.04	0.59	0	0.06
Net	-0.07	0.016	0.01	0.01	-0.03	0.32	-0.096	-0.13
Ita	0.26	0.08	0.29	-0.09	0.011	0.39	0	0.24
Swe	0.42	-0.01	0.18	0.15	0.047	0.02	0	-0.03
Fin	0.21	-0.04	-0.06	-0.07	-0.06	0.65	0.23	0.16
Slo	0.11	0.14	-0.05	0.084	-0.07	0		0.14
Lat	0.42	-0.01	0.18	0.15	0.047	0.02	0	0.07

Table 6.7. Frequency of null alleles at each locus based on the estimate provided in ML-Null Allele Estimator (Appendix 12). Loci which were found to be significantly heterozygote deficient are indicated in bold (Table 6.3 & 6.5). Data for my sampled areas are listed first followed by Kyle *et al.* (2003). The second row of Ire in Kyle *et al.* (2003) includes all the Irish samples.

Locus	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
Pop	MA1	MA2	MA18	Ma19	GG7	Gg14	Lut604	Gg454
KNP	0.0001	0.15	0.0001	0.0001	-	0.0001	0.0001	-
Source	-	0.21	0.42	0.31	-	0.33	0.0001	-
IRE	0.11	0.09	0.43	0.31	-	0.26	0.25	-
Eng	-	-	0.21	0.22	0.08	-	-	-
Scot	-	0.06	0.23	0.05	-	0.03	0.06	-
Ire	0.0001	-	0.25	0.0001	-	-	0.0001	-
	0.26	0.1	0.36	0.31	0.04	0.19	0.13	0.07
Ger	-	-	-	-	-	0.21	0	-
Net	-	-	-	-	-	0.0001	-	-
Ita	0.23	0.16	0.32	-	-	0.39	0.41	0.16
Swe	0.22	-	0.22	-	-	-	0	-
Fin	0.07	-	0.03	-	-	0.5	0.28	0.13
Slo	-	0.03	-	-	-	0.77	-	0.37
Lat	-	-	-	-	-	0.18	-	-

6.4.2. Genetic Variation

Overall good levels of heterozygosity were observed at loci which were polymorphic for my sample groups (Table 6.8) but when averaged across the eight loci examined the %He was found to be low (Table 6.9). Significant differences were found for %H_e between KNP and the Source and IRE groupings ($P \leq 0.0001$), while no significant difference was found between the Source and IRE grouping. The number of alleles found at an individual locus ranged from 1 to 7 (Table 6.8) and within the three sample groups the average number of alleles across all eight loci ranged from 1.75 to 2.875 (Table 6.9). The %He was

recalculated for the Kyle *et al.* (2003) data set and was found that with the increased sample size that %He in the Ire sample decreased slightly from 33.41 to 31.42 (Table 6.10).

Table 6.8. The percentage expected heterozygosity (%H_e) and number alleles (#A, in parentheses) calculated using Microsatellite Analyser (MSA) at each locus for both sample sets. 0 indicate loci which had only one allele and therefore expected heterozygosity could not be calculated.

	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
Pop	MA1	MA2	MA18	MA19	GG7	GG14	Lut604	Ggu454
KNP	0(1)	76.8(3)	0(1)	0(1)	73.8(4)	0(1)	0(1)	48.4(2)
Source	0(1)	78(4)	43.9(2)	30.3(2)	73.6(6)	0(1)	0(1)	52.3(2)
IRE	0(1)	89.4(5)	28.9(2)	15.9(2)	83.3(7)	0(1)	0(1)	51.6(2)

Table 6.9. The average number of alleles per locus (#A) and percentage expected heterozygosity (%H_e) for each sample group.

Population	Code	n	# A		He (%)	
			Average	SD	Average	SD
KNP	K	27	1.75	1.16	24.9	35
Source	S	9	2.375	1.77	34.77	32.55
IRE	Ire	28	2.875	2.59	33.8	37

Table 6.10. Percentage Expected Heterozygosity (%H_e) for Kyle *et al.* (2003) (paper), my recalculation of it (H_e re-run) and also with my data added (H_e extra data). The data for Slovenia was not included in the published paper. The % H_e for England appears high but is presumed to be as a result of introgression with the American marten.

N	Population	H _e (paper)		H _e (re-run)		H _e (extra data)	
		%	n	%	n	%	n
1	Eng	66.1	6	64.92	6	64.92	6
2	Sco	42.3	59	42.77	59	42.77	59
3	Ire	34	9	33.41	9	31.42	64
4	Ger	56.2	10	57.69	10	57.69	10
5	Net	53.8	9	55.31	9	55.31	9
6	Ita	61	15	59.82	15	59.82	15
7	Swe	57.3	16	59.44	16	59.44	16
8	Fin	57.2	26	58.58	26	58.58	26
9	Slo			63.92	5	63.92	5
10	Lat	63.8	8	66.24	8	66.24	8

Alleles found in KNP were a subset of those in the Source population which in themselves were a subset of the alleles in the IRE sample group (Fig. 6.2). 14 alleles were common to all three groupings, while 5 which did not appear in KNP occurred in the Source and IRE grouping. Only two alleles were found to occur in the remainder of the Irish samples (Fig. 6.2). A similar pattern is observed when the relative frequencies of alleles within each grouping are compared at each of the eight loci examined (Fig 6.3). When the combinations of alleles were examined at each locus, it was found that KNP had a number of combinations which did not occur in the Source grouping or in the Ire grouping either (Table 6.11).

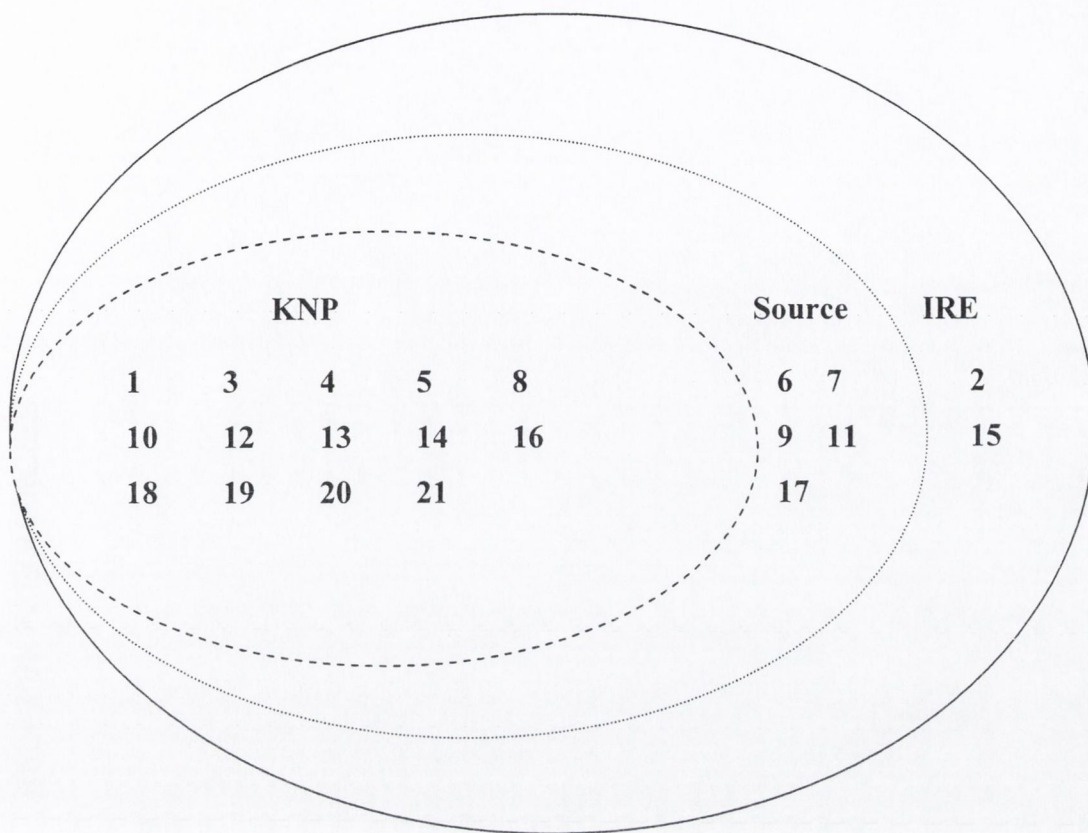
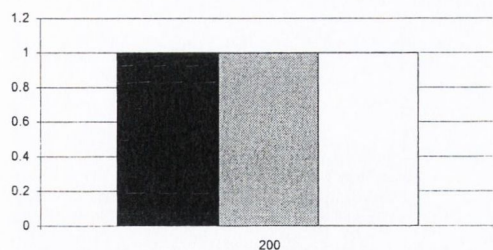
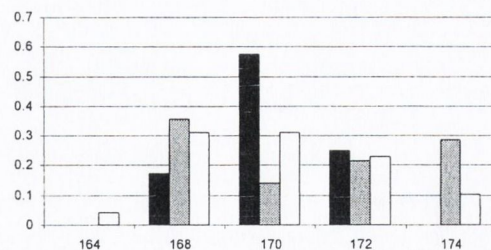


Fig. 6.2. Venn-diagram showing the nesting of alleles from KNP, Source (population from which KNP translocated animals originated: Clare and Mayo) and IRE across all eight loci. Alleles have been noted as 1-21, in order of their appearance at loci 1-8 (See Fig. 6.3). Alleles common to all three samples appear in the first oval which is also KNP. Alleles in common to the Source population and total Ireland samples appear in the second oval. Alleles found elsewhere in Ireland appear in the third oval.

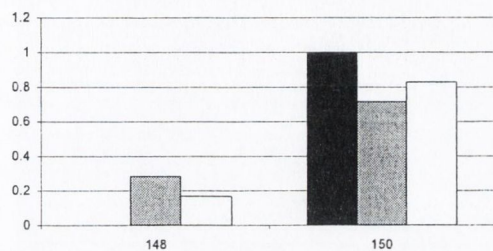
RELATIVE FREQUENCIES



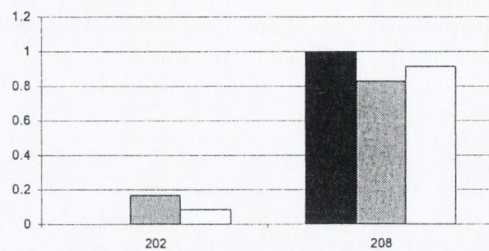
Loc1: MA1



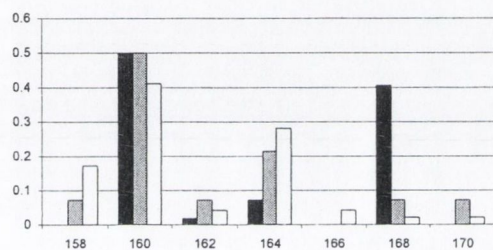
Loc2: MA2



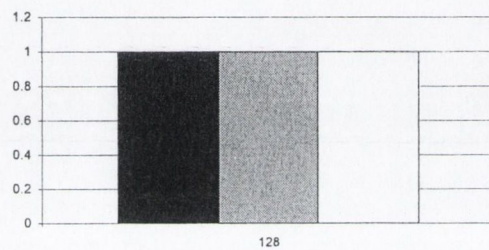
Loc3: MA18



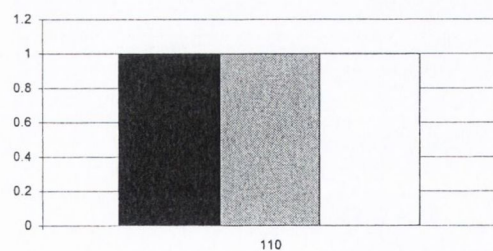
Loc4: MA19



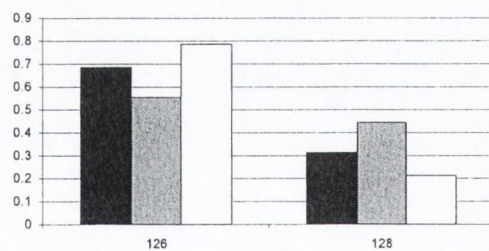
Loc5: GG7



Loc6: GG14



Loc7: Lut604



Loc8: Ggu454

ALLELES

Fig. 6.3. The relative frequencies of alleles occurring at each locus in the three sampled areas KNP  Source  and IRE .

Table 6.11. Number of combinations of alleles at each locus. N is the number of allele combinations found at a locus. n is the number of samples in which those combination of alleles occurred. Missing is the number of samples which never produced a PCR product.

N	Loc1: MA1			Loc2: MA2			Loc5: GG7			Loc6: GG14		
	KNP	Source	IRE	KNP	Source	IRE	KNP	Source	IRE	KNP	Source	IRE
1	200/200	200/200	200/200	168/168	168/172	164/168	160/160	158/160	158/160*	128/128	128/128	128/128
n =	27	7	25	1	3	2	6	2	8	27	8	26
2				168/170	168/174	168/168	160/164	160/160	160/160			
n =				3	2	2	1	1	2			
3				168/172	170/170	168/170	160/168	160/164	160/162*			
n =				2	2	2	12	3	1			
4				170/170	172/174	168/172	162/164	168/170	160/164			
n =				9	1	6	1	1	4			
5				170/172	174/174	168/174	164/168		160/166*			
n =				3	1	1	5		2			
6				172/172		170/170			162/164			
n =				2		5			1			
7						170/172			164/164*			
n =						2			4			
8						170/174			168/170*			
n =						1			1			
9						172/172						
n =						1						
10						172/174						
n =						1						
11						174/174						
n =						1						
Missing	0	2	3	8	1	4	0	2	4	0	1	4
N	Loc3: MA18			Loc4: MA19			Loc7: Lut604			Loc8: Ggu454		
	KNP	Source	IRE	KNP	Source	IRE	KNP	Source	IRE	KNP	Source	IRE
1	150/150	148/148	148/148*	208/208	202/202	202/202	110/110	110/110	110/110	126/126	126/126	126/126
n =	27	2	4	28	1	1	27	8	25	10	1	15
2		150/150	150/150		208/208	208/208				126/128	126/128	126/128
n =		6	20		6	22				17	8	13
Missing	0	1	4	0	3	4	0	1	3	0	0	0

6.4.3. Genetic Distances

Genetic distances range from 0 to 1 with those values closest to 0 indicating populations that are genetically close, while those nearer 1 point to populations that are genetically distinct. The closest population found to the Irish sample group, taking into account an increased sample size, was found to be Sweden (0.2573), while the furthest population was found to be Finland (0.3935; Table 6.12). In the Kyle *et al.* (2003) dataset the closest is found to be England (0.1922) but Sweden is only marginally more distant (0.1974). Again the most distant population is Finland (0.3233; Table 6.12). In neither datasets were any of the populations examined considered to be genetically close to the Irish population, relative to values observed between populations from continental Europe. For example a genetic distance of 0.0097 was found for Latvia and Italy (Table 6.12).

Table 6.12. Pairwise Fst values between populations of pine marten at 8 microsatellite loci. For Kyle *et al.* (2003) (lower diagonal) and updated with my data (upper diagonal). Values near to 1 indicate populations which are genetically distant while values near to 0 point to genetically close populations.

Pops	Eng	Sco	Ire	Ger	Net	Ita	Swe	Fin	Lat
Eng	0	0.1155	0.3366	0.0541	0.1172	0.0918	0.0575	0.1802	0.0843
Sco	0.115	0	0.3474	0.2039	0.2491	0.2134	0.1514	0.3185	0.2507
Ire	0.1992	0.2268	0	0.3234	0.3410	0.3734	0.2573	0.3935	0.3605
Ger	0.0541	0.2039	0.2347	0	0.0602	0.0421	0.0116	0.1229	0.0558
Net	0.1172	0.2491	0.2784	0.0602	0	0.1152	0.1122	0.1836	0.1135
Ita	0.0918	0.2134	0.2519	0.0421	0.1152	0	0.0398	0.0833	0.0097
Swe	0.0575	0.1154	0.1974	0.011	0.1122	0.0398	0	0.1184	0.0636
Fin	0.1802	0.3185	0.3233	0.1229	0.1836	0.0883	0.1184	0	0.0481
Lat	0.0843	0.2507	0.3021	0.0558	0.1135	0.0097	0.0636	0.0461	0

6.4.4. Population Assignment Test

The assignment test used in this study was calculated using the program WHICHRUN (Appendix 12) and estimates which population within your dataset a given genotype has the highest probability of occurring. A similar pattern was observed in the assignment of genotypes to populations compared with that found for genetic distances. Most of the

genotypes for the Irish sample were assigned to Ireland (87%), with the few that weren't being assigned to Scotland (6%) Sweden (3%), and the Netherlands (3%) (Table 6.13). Overall the effect of increasing the sample size resulted in a reduction of self assignment within the Irish population from 100% to 87% (Table 6.13). Once again continental countries showed the lowest percentage assignment ($\leq 50\%$) indicating mixing between populations, while insular populations, with the exception of the Netherlands (88%) and Finland (73%), showed high percentage self assignment (Table 6.13).

Table 6.13. Assignment test results for Kyle *et al.* 2003 data (top row) and updated with Irish populations collected in this study (bottom row). All assignments were based on genotypes from 8 microsatellite loci. N is the number of individual genotypes for that population sample. % refers to the number of samples within each population that are self assigned. Where the % is different on the second this indicates that the number of self assignments has altered due to the increased sample size for Ireland.

POP	N	Eng	Scot	Ire	Ger	Net	Ita	Swe	Fin	Lat	%
Eng	6	3	2	1	0	0	0	0	0	0	50
		3	1	1	1	0	0	0	0	0	
Sco	59	1	52	2	0	0	1	2	0	1	88
		1	53	1	0	0	1	2	0	1	90
Ire	9	0	0	9	0	0	0	0	0	0	100
		0	4	56	0	2	0	2	0	0	87
Ger	10	1	0	0	3	0	1	4	0	1	30
		1	0	0	3	0	1	4	0	1	
Net	9	0	0	0	1	8	0	0	0	0	88
		0	0	0	1	8	0	0	0	0	
Ita	15	0	0	0	0	1	6	1	2	5	40
		0	0	0	0	1	6	1	2	5	
Swe	16	0	0	2	2	0	0	9	1	2	37
		1	0	1	2	0	0	9	1	2	
Fin	26	1	0	0	0	0	0	2	19	4	73
		1	0	0	0	0	0	2	19	4	
Lat	8	0	0	0	1	0	1	1	1	4	50
		0	0	0	1	0	1	1	1	4	

6.4.5. Mitochondrial DNA Analysis

There was only one haplotype for the control region and one haplotype for the cytochrome *b* gene and these were identical to those already published in Davidson *et al.* (2001). As such no further analysis was carried out. Any variation that occurred between sequences was due to error during the sequencing process. All sequences can be found in Appendix 8.

6.5. Discussion

Hardy Weinberg, null alleles and linkage disequilibrium

Testing for conformity of HWE, therefore random mating, is important in order to exclude the presence of null alleles (or at high frequencies at any rate) and to ensure that study areas from which samples were drawn were not so large so as to contain sufficient internal structure to cause a Wahlund effect (Paetkau *et al.*, 1997). The Wahlund effect arises from inappropriate population subdivision (Brookfield, 1996). Conversely such deviations may be due to the presence of null alleles, so individuals who should be heterozygote are classed as homozygote as one of the alleles at the loci failed to amplify (null allele). Significant deviations were recorded in all three sample groups and within the Kyle *et al.* (2003) data set, though in the later case such deviations only occurred in two populations once the data had been corrected for multiple testing. However the non-documentation of null alleles for each population individually may lead, as in this case, to researchers inappropriately selecting microsatellite markers to carry out population genetic studies on the basis of conformity to HWE and genotypic equilibrium.

Significant deviations from HWE, in terms of heterozygote deficiency as stated above, can be symptomatic of null alleles being present at loci. Though both the estimators used in this study returned different frequencies of null alleles for each locus, the difference may be accounted for in the method of estimation and in all cases similar patterns were observed. The null allele frequency estimator proposed by Chakraborty *et al.* (1992) does not take into account loci which have data missing. Missing data may be due to technical problems with PCR, such as degraded DNA causing allelic dropout (one allele fails to amplify) or contaminants in the DNA inhibiting PCR (both alleles fail to amplify). This is frequently seen in non-invasive samples such as bone, hair or scats (Taberlet *et al.*, 1996; Larson *et al.*, 2002) but may also be due to the presence of 'true' null alleles.

By contrast the second estimator assumes that missing data is homozygous for the null allele. This is likely to give a better estimate of null allele frequency at loci but is dependent on knowing whether samples failed to amplify due to technical difficulties or not. In this study the history of all samples was known. Thus it was assumed that given all samples amplified for at least 4 or more microsatellite loci that failure to amplify was not due to DNA degradation or contamination (Appendix 9). Furthermore those alleles which showed the highest frequency of null alleles where the polymorphic loci, with the exception of loci 6 and 7 in the Source and IRE groups. The observed frequency of occurrence of null alleles at these loci was due to missing data, i.e. no PCR product was detected after nine rounds of PCR. Consequently when the history of the data set is unknown, then Chakraborty *et al.*'s (1992) estimator would be preferable as it makes no assumptions on missing data. For example, a data set like Kyle *et al.* (2003) where the history of all the samples was unknown.

Pemberton *et al.* (1995) state that null alleles are common in microsatellites, and that this must be accommodated in any analysis of microsatellite data. The same authors found that of sixteen microsatellites screened for red deer, three were found to have non-amplifying alleles. Callen *et al.* (1993) found that similarly microsatellites in humans exhibited a high number of null alleles (approximately 30%). Yet many authors simply state the presence of null alleles in their data set and continue with analysis putting forward the argument that as null alleles were only likely to be present (based on significant heterozygote deficiencies) in one or two of the populations under investigation, then this is of no consequence (Kyle *et al.*, 2001; Kyle & Strobeck, 2001; Kyle *et al.*, 2001; Waldick *et al.*, 2002; Kyle & Strobeck, 2003; Kyle *et al.*, 2003; Small *et al.*, 2003). Pemberton *et al.* (1995) however demonstrate that even with the exclusion of a small number of null alleles that the effects on the interpretation of results can be striking, resulting in misleading inferences about inbreeding in populations. Two ways to reveal and correct for the presence of null alleles is to altering primer annealing temperature or redesign primers (Pemberton *et al.*, 1995).

Two other causes, apart from the 'natural' occurrence of null alleles at the markers may account for the high number of homozygotes observed. Firstly, as the primers in this study are not species specific then this may have caused the presence of null alleles as mentioned above. The high number of individual populations exhibiting homozygote excess in the

Kyle *et al.* (2003) dataset would certainly seem suggestive of this. Furthermore Locus 3 was still found to exhibit heterozygote excess for Scotland after correction for multiple testing as was Locus (6) for Finland. Paetkau & Strobeck (1995) found on sequencing the alleles at a dinucleotide locus (G10P) with excess homozygotes, that alleles were identical except at one position which in amplifying alleles had a C residue and in those that didn't a G residue. Furthermore their results indicated that amplification occurred in some species of *Ursids* but not others, suggesting that cross species amplifications as stated in section 6.1.2 may give rise to null alleles. Though this may not always be the case with null alleles being detected using primers, which are both species specific and degenerate, in equal measures (Callen *et al.*, 1993; Pemberton *et al.*, 1995).

Secondly, given the history of the Irish population (see Chapter 3), and the fact that three of the loci in the Source and IRE grouping and five of the loci in the KNP were fixed for one allele, then it is also likely that inbreeding has played a large part in the observed high numbers of homozygotes. Inbreeding, while not altering the frequency of alleles in the population does redistribute the frequency of genotypes thereby increasing the number of homozygotes and decreasing the number of heterozygotes (Keller & Waller, 2002). Thus it may be a combination of null alleles (occurring naturally and due to cross species amplification) and genetic drift due to inbreeding which are causing the observed high number of homozygotes.

The observed heterozygote excess at locus 8 in the KNP group is mostly likely due to a founder effect, as most of the animals from the Source population were found to have the combination 126/128 for this locus (Table 6.11). Thus enough time may not have elapsed since the re-establishment of pine marten in KNP for this Locus to have reached equilibrium. Therefore given more time this may change. Locus 5 was found to be linked with locus 2 and 4 in the Source and IRE grouping. However linkage disequilibria, non-random associations of alleles, are expected in recently bottleneck-and-expanding populations (Hansson & Westerberg, 2002). An assumption of population genetics analysis is that markers should be neutral and should the above eight markers be in HWE and polymorphic enough to allow complete analysis of population structure then it would be appropriate to exclude locus 5 from further analysis. As my results indicated, this was not the case and locus 5 was retained in the analysis. Given the above problems associated with

the markers used in this study, I caution any interpretation of the following results as they are all based on the assumption that the loci used conform to HWE and genetic equilibrium.

Genetic Variation

Similar to that found by Kyle *et al.* (2003), my data also revealed that the Irish population of pine marten show depleted levels of genetic diversity (approximately 33%). Though not strictly comparable with other studies as different microsatellites were used, this would appear to be analogous to populations of American marten occurring on islands with comparable histories though in some cases the H_e for Ireland was higher (Table 6.1). Small *et al.* (2003) found that H_e ranged from 6 to 59% for populations of martens studied on the islands in the Pacific northwest of America and Canada. The lowest H_e recorded was for Admiralty Island where the effective population is thought to be about 300 individuals, brought to such low levels by overharvesting and persecution. Other populations on islands showed H_e similar to that observed for Ireland (24% Queen Charlotte Islands and 32% Vancouver Island). By contrast red squirrel populations in Ireland, known to have been reintroduced into Ireland a hundred years ago (it is unknown if the current population is an admixture of native and reintroduced individuals), shows levels of genetic diversity similar to that observed to continental pine martens (L. Finnegan *pers. comm.*). This may indicate that Irish martens have gone through a severe bottleneck in the last 100 years (as evidenced historically) or have always shown reduced diversity due to founder effect. A comparative study of genetic diversity from samples pre 1850 and current pine martens would help clear this matter up. Additionally the presence of a single haplotype for two regions of the mitochondria may support a small founding population post glaciation as the Scottish pine marten shows a similar pattern and it is known to be an early post glaciation coloniser (Strachan *et al.*, 1996; Davison *et al.*, 2001).

One plausible answer to account for the difference in genetic variability observed between Ireland and Scotland (the two island populations investigated in Kyle *et al.*, 2003) given that these two islands have similar histories of exploitation, may be found in the timing and cessation of that persecution. Scottish pine martens were thought to have reached their nadir in the 1920's and from that time shown population expansion (Langley & Yalden, 1977). By comparison the Irish pine marten population was at its lowest in the mid 1940's (see Fig. 1.2) but in all probability only began to show expansion in its range post 1980

when it became protected under the Irish Wildlife Act (1976) and following the banning of strychnine poison (1991) (O'Sullivan, 1983). It is most likely a combination of both these factors, the challenge lies in segregating low diversity due to natural and anthropogenic causes. Further sampling in Europe, particularly to include the Balearic Islands, may be illuminating and aid in elucidating the entire picture of the genetic diversity of pine marten in Europe.

The reduction in H_e observed between Kyle *et al.* (2003) and when the extra Irish samples had been added is mostly due to the fact that the KNP group showed reduced heterozygosity due to translocation. This reduction in H_e illustrates nicely how small founding populations have lower genetic diversity and gives weight to the argument presented in the previous paragraph. The KNP grouping in this study showed significantly lower levels of genetic diversity compared with the Source group, from which it was derived, and the rest of the Ireland samples. Both the Source and IRE groups had similar levels of diversity. Williams, Rhodes & Serfass (2000) investigating source and reintroduced populations of fishers in America found no difference in the levels of genetic variance observed between the two groupings. Though the molecular markers used in their study were allozymes so direct comparison is difficult, this may be due to the fact that some of the reintroduced populations were + 50 years old at the time of the study.

Williams *et al.* (2000) did however note differences in allele frequencies between the source and reintroduced populations, with large increases occurring in the frequency of rare alleles in the reintroduced populations. This they suggest is evidence of genetic drift and sampling errors during the initial founding events. My data would indicate some difference in the frequency of alleles amongst the three groupings with seven alleles occurring at a greater frequency in the KNP grouping than observed in the Source or IRE grouping. However no unique or private alleles were found in the KNP grouping and coupled with depleted genetic diversity and no verifiable record during the national census (O'Sullivan, 1983) may indicate that there were no animals present in KNP prior to the translocation. Two sightings, though never verified, suggest otherwise (Chapter 3, section 3.1.1). Additionally no unique mitochondrial haplotypes were found for the Irish pine marten. By contrast Vinkey (2003) investigating extirpated and repatriated populations of fishers found unique and common haplotypes and common to suggest both native and reintroduced stock

respectively and amongst populations that had been translocated as recently as this one. Thus it does seem likely that pine martens were extirpated from KNP prior to re-establishment.

Genetic Distances and Assignment Tests

The observed pattern in genetic differences was maintained despite the inclusion of 55 new samples for the Irish population, suggesting that genetic structure of the European pine marten is primarily due to persecution and habitat fragmentation (Kyle *et al.*, 2003). By contrast genetic distances between populations of American marten in mainland North America and Canada suggest high levels of movement between populations and that distant populations are only so due to isolation by geographic distance (Kyle *et al.*, 2000). Irish pine martens were genetically the most distant of all groups sampled. This is hardly surprising given that Ireland is an island, but may support the suggestion that the pine marten colonised Ireland by itself as the evidence does not suggest multiple introductions, which would have conceivably occurred had man introduced this species given the many arrivals by man from very many different European countries to this shore and continued trade with Europe (Chapter 3). Furthermore the assignment test also indicates that Irish pine martens are one of the most isolated groups in Europe. Those populations in Europe showing high levels of self assignment also had the lowest number of mitochondrial haplotypes, with both Scotland and Ireland having only one haplotype each for the control region (Davison *et al.*, 2001). By comparison the Scottish population of pine marten, considered one of the earliest post glacial colonisers (Strachan *et al.*, 1996), have a similar mitochondrial and microsatellite history to the Irish population and such similarity may also mean the Irish pine marten was one of the early arrivals. Davison *et al.* (2001) point to a putative link between the Irish pine marten and martens on the Iberian Peninsula, one which due to the lack of available samples was not examined using microsatellite analysis in Kyle *et al.* (2003). Thus should such samples become available then this may help in deciphering the origins of the pine marten and whether their absence from the current microsatellite data set is masking an interesting story.

MtDNA

All animals examined in this study for both the control region (N = 25) and cytochrome *b* (N = 21) gene showed no new haplotypes to that already published, having one haplotype

at each region (Davison *et al.*, 2001). It was hoped that if populations had been isolated long enough then it would be possible using mitochondrial analysis to determine whether pine martens were present in Killarney prior to translocation. Vinkey (2003) used this technique to show that fisher populations in Montana were primarily from re-established populations but two unique haplotypes also point to (located in west-central Montana) native animals that were present prior to translocation. My inability to locate new haplotypes is a result of the isolation of pine marten populations not occurring for any great length of time and thus sequence divergence did not occur. This is the most likely answer as similar results were found by Davison *et al.* (2001) for the Scottish population of pine marten, which as stated previously has a similar history to the Irish pine marten (see Chapter 3).

Conservation Implications

Conservation genetic studies of the Irish pine marten have repeatedly shown that overall this population exhibits low levels of genetic diversity, which in this study I have shown translocation has caused this to decrease further. As mentioned previously, reduced genetic variation may result in a decrease in the fitness of a population putting them at risk of going extinct again. Though there was an observed bias in the trapped population of pine marten this was believed as a result of the trapping methodology used (Chapter 3) and not a natural dynamic of the translocated population. However further translocations should not occur without prior knowledge of the genetics of the populations concerned so the newly established population could be selected for good diversity. Historical evidence would suggest that the decline of the pine marten in Ireland did not coincide with large scale deforestation but rather the advent of gamekeeping and increased exploitation post famine, 1850 (Chapter 3). A similar pattern has been observed in Scotland (Langley & Yalden, 1977). The current expansion of the range of the pine marten is also evidence of this, but the speed of the apparent recovery has probably been facilitated by commercial forestry plantations (O'Sullivan, 1983). Moderate to high rates of gene flow between populations will be required to maintain the observed levels of genetic diversity and avoid further inbreeding and thereby a further loss in genetic diversity and as such this species will need continued protection from persecution.

A number of important questions were raised during this study. Firstly, whether the current set of markers are appropriate for studying the conservation genetics of the pine marten. It may be that microsatellite markers developed and specifically designed for pine marten would be less problematic. As it stands the current set of microsatellite markers are not sufficient to infer anything of significance about the conservation status of the pine marten in Ireland as they do not conform to Hardy Weinberg Equilibrium which is a key assumption in many of the tests subsequently carried out. Additionally the evidence that one of the microsatellite markers was linked also contributes to their unsuitability as mutation would not be neutral. Thus this limits their usefulness in more practical aspects of pine marten conservation such as individual identification. The ability to use microsatellite markers to that end would be an excellent advancement in pine marten population monitoring as non-invasive samples could be used and thus prevent further trap accidents occurring as documented in Chapter 3. Secondly, should better markers become available then it may be possible to test whether the Irish pine marten has always exhibited such low levels of genetic diversity or whether those observed are a result of the recent bottleneck this species has been through. Thirdly, it would be interesting to test for association between Irish pine martens and those on the Iberian Peninsula to corroborate the evidence presented in Davison *et al.* (2001).

CHAPTER 7 has been accepted for publication in the Journal: *Molecular Ecology Notes* and is currently in press. The title of the paper and my co-author are listed below. Slight modifications have been made to the paper for inclusion in the thesis, which include the addition of two tables (Table 7.1 and Table 7.2) and one figure (Fig 7.2). These were omitted from the paper owing to word and space constraints. The remainder of this chapter appears as it will in the journal.

Molecular sexing of pine marten (*Martes martes*): How many replicates?

ÁB Lynch and MJF Brown

CHAPTER 7. MOLECULAR SEXING OF PINE MARTEN: HOW MANY REPLICATES?

Abstract

I used primers developed for the SRY gene in otters to determine sex in pine marten. The otter SRY primers work accurately for pine marten and assigned sex correctly in most replicates. They can be used on tissue and non-invasively collected hair samples for the identification of sex. I found that, based on five sets of replicates, DNA extracted from leg muscle and hair gave significantly better results than DNA extracted from ear tissue. Finally results indicate the optimum number of PCR replicates to accurately assign sex using this technique for tissue samples are leg muscle (2), ear biopsy (5) and hair sample (2).

7. 1. Introduction

Pine marten are widespread but not necessarily abundant throughout most of western Europe and are considered rare or endangered in many southern European countries (Davison *et al.*, 2001). Knowing the sex of an individual or the sex ratio of a population is important for long term conservation and development of management plans for species. Advances in molecular sexing of individuals have enabled researchers to separate the sexes in non-sexually dimorphic species and can also be used on non-invasive samples (hair or scat) or where the dead animal's body is too badly damaged or decomposed to identify sex (Dallas *et al.*, 2000; Matsubara *et al.*, 2001; Pilgrim *et al.*, 2005). Though it may be possible to separate sexes apart by individual parts of the body unique to one sex (e.g. the baccula in pine martens) this will only be possible if the full or at least the relevant parts of the carcass are received. Any molecular technique used to identify sex should be accurate, reliable, sensitive, straightforward and cheap (Griffiths, 2000).

Several different techniques have been developed for the identification of sex in mammals based on genes conserved on the mammalian Y chromosome e.g. SRY, ZFY & AMGY. Only male DNA produces a band using the SRY system but sex specific products are produced when using ZFY and AMGY. Pilgrim *et al.* (2005) found that neither of the Y-specific genes targeted (ZFY or AMGY) produced a sex specific product for American marten, mink, fisher or wolverine. It is likely this test would be inconclusive for the closely related pine marten as microsatellites developed for the

American marten and wolverine have been successfully used for studying pine marten (Kyle *et al.*, 2003). Woods *et al.* (1999) state that in the absence of a system which amplifies a section of the X and Y chromosome simultaneously with the same primers, that the use of the SRY gene is an accurate method for sexing. Amplification of the SRY gene during PCR results in a product being produced for males but not for females, thus absence of a product could indicate a female or PCR failure. Few authors report on errors associated with using this technique or how many replicates should be carried out prior to a sample being confirmed as either male or female (Dallas *et al.*, 2003; Ortega *et al.*, 2004).

7.1.1. Objectives

Here I consider the feasibility of using PCR primers developed for the SRY gene of otter for sex identification in pine marten and calculate the probability of identifying males with a single round of PCR.

7.2. Methods

Total DNA was extracted from 56 individuals (N = 35 ear tissue and N = 21 leg muscle) and 79 non-invasively sampled hair samples using a Qiagen DNeasy Tissue Kit. All samples were extracted with negative controls (no sample material added) to control for contamination. Tissue samples were taken from live captured pine martens (Samples 1-28; ear biopsy: 2mm diameter) and road traffic accidents (RTA) (Samples 29-56; leg muscle (n = 21; 25mg) or ear biopsy (n = 7; 2mm diameter)). Ear tissue samples were taken from RTA animals when the body was too badly damaged to obtain leg muscle or when only ear tissue was available. The sex of 47 of the animals or carcasses was known, while 9 was unknown owing to the carcass or sample being too badly damaged or decomposed to sex.

Of the 79 hair samples (average number of hairs per extraction: $\mu = 12.51$, SD = 8.17, min = 1 & max = 40 & average number of days before collection: $\mu = 6.47$, SD = 7.3, min = 1, max = 28) extracted, 55 produced DNA (average number of hairs per extraction: $\mu = 14.85$, SD = 8.26, min = 3, max = 40 & average number of days before collection: $\mu = 5.53$, SD = 7.18, min = 2, max = 28) and of these 44 were used for the study (amplified with microsatellite on first occasion). Hair samples were obtained from the same population as the live trapped animals above using the hair trap design

described in Messenger & Birks (2000). All samples had previously amplified during microsatellite analysis (ÁBL *unpublished data*) using the primers available for pine marten which are not species specific (Kyle *et al.* 2003).

The primers, Lut-SRY F and Lut-SRY R, and PCR protocol have previously been described in Dallas *et al.* (2000) (90°C/1 min 45 s, [90°C/15s, 60°C-0.5°C per cycle/15 s] x 20 cycles, [90°C/15 s, 50°C/15 s] x 15 cycles, 72°C/1 min). The reaction volume (10µl), contained 1 x PCR buffer, 0.25 units Taq (Invitrogen, recombinant), 2.5mM MgCl₂, 200µM dATP, dCTP, dGTP, dTTP and 1µM of each primer. A hot start PCR was performed. A 70 bp product is produced for male otters. Five replications were carried out for each set of samples (except 26 & 27, 4 replicates). Products were electrophoresed on a 1 x TBE agarose gel (2% w/v) containing 0.625mg/ml ethidium bromide and visualised with UV. All work was carried out by the principal author (ÁBL; female).

7.3. Statistical Analysis

Chi square analysis (χ^2) was used to test for the disparity in sample types (ear biopsy, leg muscle and hair) for correctly identifying sex in males. Probabilities were used to describe the number of replications for 1% missassignment and were calculated by multiplying the probabilities for each replication round by the next. Once a value < 0.001 was reached this was the number of replications needed to for 1% missassignment.

7.4. Results

A 70 bp band was observed for all known males at least once (N = 22) (Table 7.1). No band was ever observed for any of the known females (N = 25) including the positive control (Fig. 7.1). In ear samples 1-28, 62.5% of the PCR reactions were correctly identified as males (Table 7.1). For samples 29-56 (known males N = 12), 96.66% of PCR reactions were correctly identified during the 5 replicates (Table 7.1). Of the unknown males (N = 4) 75% of reactions proved positive and the remaining 5 unknown animals were classified as females as no bands were ever observed for them (Table 7.1).

Table 7.1. Comparison of results of 5 PCR runs for sex identification in pine marten. Sex is the sex of the animal: ♀: female, ♂: male & U: unknown. T is the tissue type used: E: ear tissue & L: leg muscle. Columns 1-5 indicate the result for each sample for the 5 PCR runs. – indicates no PCR product and **n** no PCR carried out.

ID	Sex	T	1	2	3	4	5	ID	Sex	T	1	2	3	4	5
1	♀	E	-	-	-	-	-	29	U	E	-	-	-	-	-
2	♀	E	-	-	-	-	-	30	U	E	-	-	-	-	-
3	♀	E	-	-	-	-	-	31	U	E	-	♂	♂	♂	-
4	♂	E	♂	♂	♂	-	♂	32	U	E	-	♂	♂	♂	♂
5	♀	E	-	-	-	-	-	33	U	E	-	-	-	-	-
6	♂	E	♂	♂	♂	♂	♂	34	U	E	-	-	♂	♂	♂
7	♀	E	-	-	-	-	-	35	♂	L	♂	♂	♂	♂	♂
8	♀	E	-	-	-	-	-	36	♂	L	-	♂	♂	♂	♂
9	♀	E	-	-	-	-	-	37	U	L	-	-	-	-	-
10	♀	E	-	-	-	-	-	38	♀	L	-	-	-	-	-
11	♀	E	-	-	-	-	-	39	♂	L	♂	♂	♂	♂	♂
12	♀	E	-	-	-	-	-	40	♀	L	-	-	-	-	-
13	♂	E	♂	♂	♂	♂	-	41	♀	L	-	-	-	-	-
14	♂	E	♂	♂	♂	-	-	42	♀	L	-	-	-	-	-
15	♀	E	-	-	-	-	-	43	♂	L	♂	♂	♂	♂	♂
16	♂	E	♂	♂	♂	-	-	44	♂	L	♂	-	♂	♂	♂
17	♀	E	-	-	-	-	-	45	♀	L	-	-	-	-	-
18	♀	E	-	-	-	-	-	46	♂	L	♂	♂	♂	♂	♂
19	♀	E	-	-	-	-	-	47	U	L	♂	♂	♂	♂	♂
20	♀	E	-	-	-	-	-	48	U	L	-	-	-	-	-
21	♀	E	-	-	-	-	-	49	♂	E	♂	♂	♂	♂	♂
22	♂	E	♂	♂	♂	-	-	50	♀	L	-	-	-	-	-
23	♂	E	♂	♂	♂	-	-	51	♂	L	♂	♂	♂	♂	♂
24	♀	E	-	-	-	-	-	52	♀	L	-	-	-	-	-
25	♂	E	♂	♂	♂	-	-	53	♂	L	♂	♂	♂	♂	♂
26	♂	E	n	-	♂	-	-	54	♂	L	♂	♂	♂	♂	♂
27	♂	E	n	-	♂	-	-	55	♂	L	♂	♂	♂	♂	♂
28	♀	E	n	-	-	-	-	56	♂	L	♂	♂	♂	♂	♂
+ve	♀	L	-	-	-	-	-	+ve	♀	L	-	-	-	-	-

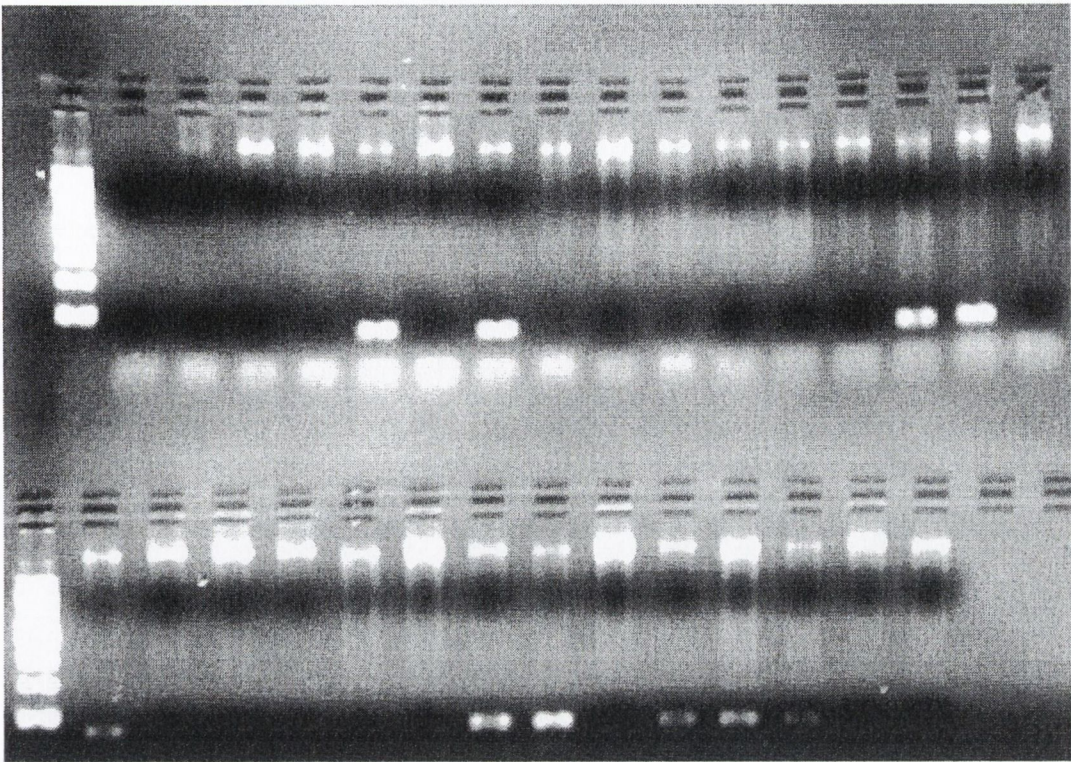


Fig. 7.1. Agarose gel for samples 1-28 showing DNA ladder (lanes 1 & 18, first band is 100bp), lane 2 is the negative control and lane 32 the positive control (female). Males are shown in lanes 6, 8, 15, 16, 19, 25, 26, 28, 29 & 30. The samples in the rest of the lanes are from females.

32 of the 44 hair samples were designated as male and the remaining 12 as female. Of the 160 reactions performed on the male hair samples only 5 failed to show a band and all samples showed bands on at least four occasions (Fig. 7.2 & Table 7.2). DNA extracted from leg muscle and hair was significantly more successful for sexing males than ear tissue (Chi-Square: $X^2 = 49.72$, $DF = 2$, $P < 0.00001$). The number of replications for 1% missassignment for samples 1-28 was 5, while it was 2 for samples 29-100 (Table 7.3).

Table 7.2. Results for each sample for each of the five replicates (1-5) for each of the hair samples. Sex was unknown in all cases (U) except for the two positive controls which were female (♀) and male (♂) respectively. – indicates no PCR product (band).

ID	Sex	T	1	2	3	4	5	ID	Sex	T	1	2	3	4	5
1	U	♂	♂	♂	♂	♂	♂	29	U	-	♂	♂	♂	♂	♂
2	U	-	-	-	-	-	-	30	U	♂	♂	♂	♂	♂	♂
3	U	-	-	-	-	-	-	31	U	♂	♂	♂	♂	♂	♂
4	U	-	-	-	-	-	-	32	U	♂	♂	♂	♂	♂	♂
5	U	-	-	-	-	-	-	33	U	-	-	-	-	-	-
6	U	♂	♂	♂	♂	♂	♂	34	U	♂	♂	♂	♂	♂	♂
7	U	♂	♂	♂	♂	♂	♂	35	U	♂	♂	♂	♂	♂	♂
8	U	♂	♂	♂	♂	♂	♂	36	U	♂	♂	♂	♂	♂	♂
9	U	♂	♂	♂	♂	♂	♂	37	U	♂	♂	♂	♂	♂	♂
10	U	♂	♂	♂	♂	♂	♂	38	U	♂	♂	♂	♂	♂	♂
11	U	-	-	-	-	-	-	39	U	♂	♂	♂	♂	♂	♂
12	U	♂	-	♂	♂	♂	♂	40	U	♂	♂	♂	♂	♂	♂
13	U	♂	-	♂	♂	♂	♂	41	U	♂	♂	♂	♂	♂	♂
14	U	♂	-	♂	♂	♂	♂	42	U	♂	♂	♂	♂	♂	♂
15	U	-	-	-	-	-	-	43	U	♂	♂	♂	♂	♂	♂
16	U	♂	♂	♂	♂	♂	♂	44	U	♂	♂	♂	♂	♂	♂
17	U	♂	♂	♂	♂	♂	♂	+ve	♀	-	-	-	-	-	-
18	U	♂	♂	♂	♂	♂	♂	+ve	♂	♂	♂	♂	♂	♂	♂
19	U	-	-	-	-	-	-								
20	U	♂	♂	♂	♂	♂	♂								
21	U	♂	♂	♂	♂	♂	♂								
22	U	-	-	-	-	-	-								
23	U	-	-	-	-	-	-								
24	U	♂	♂	♂	♂	♂	♂								
25	U	-	-	-	-	-	-								
26	U	♂	♂	♂	♂	♂	♂								
27	U	♂	♂	♂	♂	♂	♂								
28	U	-	-	-	-	-	-								

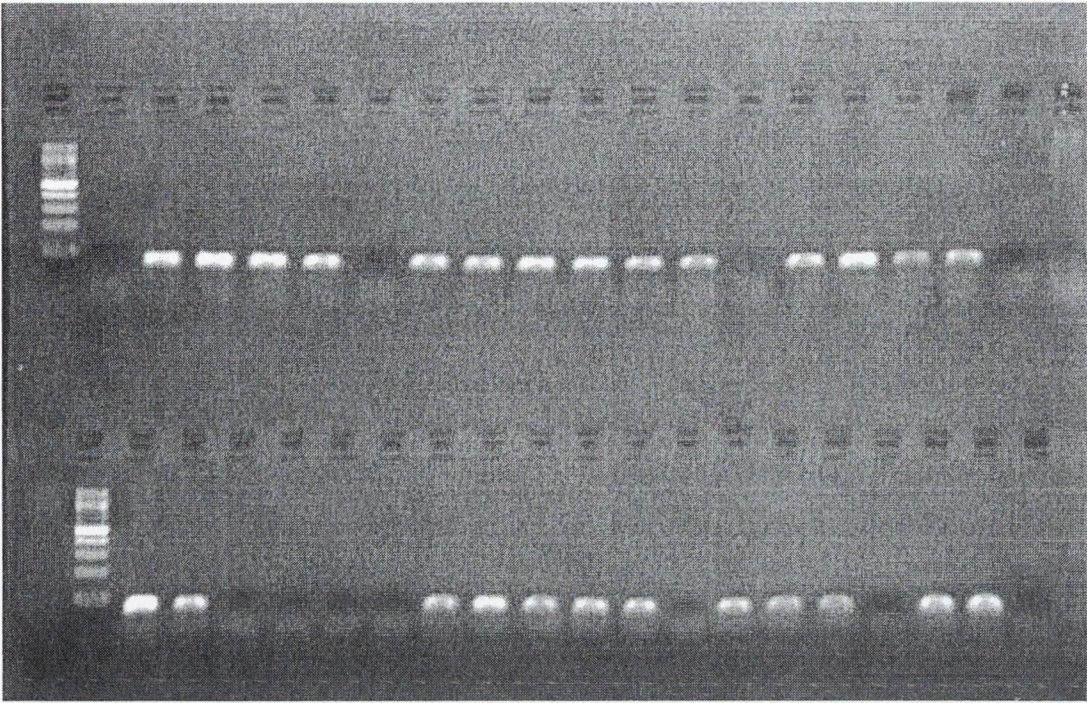


Fig. 7.2. The first lane on each row is the 100bp ladder. Lane 2 on the first row is the negative. Lanes with bands (70bp) indicate males. Lanes without bands were females.

Table 7.3. Indicates the sex of each sample after five replicates of PCR. M and F refer to male and female respectively. RTA is referring to road traffic accidents.

N	Sample	Sex		Sex		No. of replications for 1% missassignment
		Known		Unknown		
		<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	
1-28	Live trapped	10	18	-	-	5
29-56	RTA	12	5	4	5	2
57-100	Hair	-	-	32	12	2

7.5. Discussion

The proof of positive PCR amplification and the number of times a sample is typed are important in determining whether an animal will be assigned as a male or not when using the SRY system, as absence of a band may be indicative of either a female or PCR failure. Dallas *et al.* (2000) suggest incorporating another marker into the PCR assay to indicate a successful PCR. However, even if another marker is incorporated into the PCR assay and co-amplification occurs this is still not indicative of a female as PCR failure can occur for many reasons (Ortega *et al.*, 2004). In this study at least one male always amplified in each PCR replicate indicating positive PCR success.

I found that DNA extracted from ear biopsies were not as successful during amplification as leg muscle or hair samples, though this may have been as a result of sampling technique. Biopsies were taken from animals while they were restrained and while working solo and as such the optimum sample may not have been obtained in all cases hence lower quantities and/or quality of DNA. Ortega *et al.* (2004) found using primers for the ZFY gene that 85% of scats from animals of known sex and 86% of scats of unknown origin produced the correct banding pattern while the rest either produced unsatisfactory results (9.3% & 12% respectively) or failed to produce PCR products (0.5% & 2% respectively). Although I would have expected my non-invasively collected hair samples that are considered of poorer quality (Pilgrim *et al.*, 2005) to produce more errors than ear biopsies, this was not the case, and this result stresses the importance of replication for all sample types. I would suggest that a minimum of two replicates be carried out for all tissue and hair samples before characterisation as male or female and only after prior amplification with a microsatellite.

Knowledge of the sex ratio of a population or populations is important for making management decisions. The data generated from the non-invasive hair samples during this study allowed the identification of a minimum 5 new animals, 2 (both males) of whom were located on transects where live trapping had been carried out but no males were trapped (Chapter 3). The remaining three animals (2 males and 1 female) were located in remote regions of the study area (transects 11 & 12) where live trapping was not feasible and were far enough away from other transects and each other to be classed as new animals.

When dealing with highly mobile and elusive carnivores like the pine marten, where the cost of live trapping is expensive or prohibited or restricted for whatever reason (legal protection or accessibility of the terrain), the use of molecular techniques and non-invasive samples provide beneficial tools for aiding conservation and management. I have shown here that primers developed for identifying sex in otters can be used for the same purpose in pine martens on both tissue and non-invasively collected hair samples.

8.1. General Discussion

This thesis sought to give an appraisal of the translocation of pine marten to KNP while increasing the knowledge of the dynamics of pine marten populations in Ireland. I also investigated two novel methods for monitoring pine martens populations: hair traps and the use of the SRY gene to identify sex from non-invasively collected samples and to explore the impact translocation has had on the genetic diversity of the population in KNP.

Ten years on the translocation of pine marten to KNP would appear to have been successful. During this study presence of pine marten was detected on all transects using one or more of the three detection methods used: scat location, hair trapping and live trapping. As has been found by others, scat location was very much dependent upon weather (O'Sullivan, 1983; Velandar, 1983; McDonald *et al.*, 1994; Strachan *et al.*, 1996; NíNeachtain, 1998), with wet or showery weather not conducive to the location of scats in a determinable state. Furthermore, the location of scats in areas with no established pathways was virtually impossible. While on occasion in these areas scats may have been found at or around hair traps, none were fresh enough to determine by odour which is a vital characteristic in separating pine marten faeces from foxes (O'Sullivan, 1983; Davison *et al.*, 2002).

By contrast hair traps were not weather dependent and presented a real alternative for monitoring pine marten populations, particularly in sites where there were no established pathways. In this study, hair traps had a similar LTD to scat location and live trapping, and presence of pine marten was detectable within 6 days. There was an observable difference in the success of hair traps between upland and lowland sites, which was partially attributed to avoidance behaviour and also to the individual behaviour of animals. American martens have been shown to avoid enclosed traps, preferentially choosing to enter open track plates first (Foresman & Pearson, 1998). Though this may explain to some extent the difference in trap success between upland and lowland sites, it was more likely to be explained by the behaviour of one trap happy animal (female) which made up the bulk of the captures in one of the upland sites and that current handling of live captured animals is not favorable to retrapping animals once caught. SRY analysis showed that of 16 hair samples examined for transect 4 (UL)

that 11 were male and 3 were female and 2 were excluded from analysis as they failed to amplify with microsatellite primers, but were indicative of females. Thus this may suggest male avoidance of live traps particularly once trapped as recapture rates, particularly male, was low.

There was a trend for the number of positive hair traps to be correlated with the number of positive live traps. Bonesi & Macdonald (2004) report that trapping mink to estimate abundance is biased by the number of animals present, the time of year and level of activity. Consequently, even density estimates based on live-trapping must be treated carefully. I recommend that caution be used in extrapolating my results to other sites or habitats to infer relative abundance from hair traps. However the importance of developing indirect techniques for the assessment of relative abundance is paramount for carnivore monitoring, so that reliable data are available to enable informed decisions to be made about species status (Harris & Yalden, 2004). Therefore though interpretation of the results from the hair traps do need to be treated carefully, if coupled with microsatellite analysis to allow for individual identification this may enable future researchers to standardise their use for measuring relative abundance. Such work would be dependent upon suitable markers, which I have demonstrated are not available.

The use of primers developed for the SRY gene in otters was used to identify male and female pine martens from non-invasively collected hair samples using the above hair traps. The results of this analysis indicated another five animals were present in KNP, two of which were males I never caught during live trapping and the remaining three (2 males and 1 female) occurred in areas where live trapping had not been carried out. An important point raised in this study was the repeatability of the result. Despite being considered of higher quality (Pilgrim *et al.*, 2005), DNA from ear biopsies was found to work less well when compared to non-invasively collected hair samples, thereby stressing the importance of the replication for all sample types. This technique could be used as a tool to investigate the difference in diet of male and female pine martens, however as amplification could be biased by the remains of male prey items in the diet then some initial work investigating the errors associated with this technique would be necessary.

Despite being reputedly hard to catch (Marchesi, 1989; NíNeachtain, 1998), animals were caught on all transects that live trapping was carried out on but recapture rates

were low. As mentioned above this was most likely due to the way animals were handled; they were restrained rather than sedated. Such measures were taken because it was impossible to carry the sedatives necessary to anaesthetise the animals without a veterinary surgeon being present. Therefore, due to low recapture rates, densities of animals were expressed as the minimum number of animals alive per kilometer squared. Though densities estimated in this study appeared high compared with some studies (Pelikán & Vackar, 1978; Jedrzejewski *et al.*, 1993; Halliwell, 1997), they were typical of others (Zalewski *et al.*, 1995). The limited amount of research carried out on pine marten home range in Ireland indicates that pine martens live at relatively high density and consequently occupy small home ranges (Velandar, 1991; NíNeachtain, 1998; Birks 2002). Further research is needed to test this hypothesis and to investigate the reasons behind it.

Live trapping did reveal a female bias in the live-trapped population at KNP (overall 1.5), which was reduced to 1.18 when the hair trapped population was included. All previous studies on martens, both pine marten and American marten show a male bias in the trapped populations (pine marten: Yurgenson, 1947; Mal'dzhyunaite, 1957; Marchesi, 1989; Balharry, 1993a; NíNeachtain, 1998; American marten: Quick, 1956; Thompson & Colgan, 1987; Naylor & Novak, 1994). The presence of the male bias has been attributed to various factors. It may be an artifact of the sampling method, with males being more likely to be trapped than females (Marchesi, 1989). Males are noted to wander widely and are therefore more likely to come in contact with traps than females (Marchesi, 1989). It may be that, because of lifestyle, females having to raise kits by themselves, combined with an energy inefficient body shape, may die younger owing to extreme physiological stress on their body (Weckwerth & Hawley, 1962). Furthermore, given that this species also exhibits sexual dimorphism, the female pine marten will always be subordinate to males in times of food shortage which may also result in an unbalanced sex ratio (Pulliainen, 1981).

By contrast the sex ratio of the RTA's is biased towards males which might be because, as stated above, males wander more widely and are therefore more likely to come into contact with vehicles. However, several other plausible reasons may explain this. It is most likely due to the trapping methodology used, with line transects favoring the trapping of individuals with the smallest home ranges (Buskirk & Lindstedt, 1989). Alternatively it could be that pine martens are known to live for several years in the

wild (Birks, 2002) but it is unlikely that the first generation of the translocation still occur in KNP, rather it is their offspring. The sex ratio at birth is believed to be one (NíNeachtain, 1998). However, it may be that this changes in newly colonised areas, which are optimal habitat. It could also be that many of the females trapped were not actually the resident owner of the territory in which they were caught. Perhaps resident females are highly tolerant of daughters when food is not a limiting factor, thereby allowing them to remain within their territory. Though I have attributed the high numbers of non-breeding adult females to my inability to detect signs of breeding status based on time of capture, it may also be that these females had not bred yet and were still present within their mother's home range. Conversely, males may be holding larger territories encompassing more females than the one or two normally observed thus there are fewer males than females in an area to be trapped. As each transect only covered approximately 2.5 km in length then this could also be true.

The overall percentage of juveniles (sub-adults and kittens) in the trapped population for both years was 26.66% and was 18% in 2003 and 31.8% in 2004, and was in keeping with other studies (Marchesi, 1989; NíNeachtain, 1998). The higher percentage of juveniles trapped in 2004 is most likely a reflection of the higher total number of individuals trapped in 2004 compared with 2003. Though females were found to be lactating in 2003, no kittens were caught. However, in 2004 two kittens were trapped. The remaining juvenile animals trapped were sub-adults and were considered to be either hunting within their mother's home range or beginning to disperse. Should microsatellite markers, which are polymorphic enough, become available then it would be possible to investigate dispersal distances and philopatry by young pine martens. As expected biometric measurements showed that pine martens were sexually dimorphic with adult males always being larger and heavier than adult females and also to the same extent which has been recorded elsewhere (Ryabov, 1962; Marchesi, 1989; McDonald & Barrett, 1993; NíNeachtain, 1998).

The diet of the pine marten in KNP was similar to that recorded by other studies in Ireland (Fairley & O'Gorman, 1974; Fairley, 1975a; Warner & O'Sullivan, 1982; NíNeachtain, 1998) and on other islands (Minorca: Clevenger, 1993c), with all components being consumed more or less equally. However it was different to studies from continental Europe and Scandinavia where small mammals particularly vole *spp.* are the backbone of the diet throughout the year with the remaining components of the

diet taken seasonally (Goszczyński, 1976; Marchesi & Mermod, 1989; Balharry, 1993b; Jedrzejewski *et al.*, 1993; Haliwell, 1997; Putman, 2000). The bank vole, a previously unrecorded item in the diet of pine marten in Ireland, was consumed as frequently as the woodmouse, with lower consumption more likely a result of bank vole population density than preferential selection of the woodmouse by the pine marten. This diet also confirms the importance of the earthworm as a winter component in the diet of the pine marten. Generally earthworms are considered as items which are ingested secondarily (Warner & O'Sullivan, 1982), but evidence in this thesis points towards them being actively preyed upon. Little is known about the foraging patterns of pine marten. On one hand it could be assumed that this species has no dietary competitors and occupies a niche which if present in Ireland would also be filled by weasels, stone marten and polecats to a certain extent. Conversely it could be argued that foxes, badgers and stoats and perhaps mink and feral cats fill this niche in Ireland. Much research needs to be carried out to investigate the relationships between the different predators in Ireland and the impact each species has on the other.

The failure of the microsatellite markers used in this study to conform to HWE lessens their value but raises a number of interesting points regarding the markers themselves and their suitability, and the degree of inbreeding in the Irish pine marten. The observed deviations from HWE in terms of heterozygote deficiency are indicative of both null alleles at microsatellite loci and inbreeding. The frequency of occurrence of null alleles may be increased by cross species amplifications (Pemberton *et al.*, 1995) but may also be due to inbreeding which, while not altering the frequency of alleles in the population does redistribute the frequency of genotypes thereby increasing the number of homozygotes and decreasing the number of heterozygotes (Keller & Waller, 2002). Though I could not segregate heterozygote deficiency due to null alleles and inbreeding, this is something that markers specific to *Martes martes* may be able to do.

Conservation genetic studies of the Irish pine marten have shown that overall this population exhibits low levels of genetic diversity (Kyle *et al.*, 2003), which in this study I have confirmed and also shown translocation has caused it to decrease further. Scottish pine martens also show lower levels of genetic diversity compared with Europe (Kyle *et al.*, 2003), but not as low. The history of the decline of pine marten in Scotland is a mirror image of the decline in Ireland in terms of exploitation, yet the level of genetic diversity found in Scottish pine martens is significantly higher. Furthermore

both populations show very similar results in terms of F_{ST} and Assignment values. Although speculative, this difference may be due to the timing and cessation of the decline in the Scottish pine marten population, being earlier in Scotland than in Ireland (Langley & Yalden, 1977). It may also be entwined in the history of colonisation by pine marten to this island; Ireland perhaps having a smaller founding population compared with Scotland has reduced genetic variability. The translocation of animals to KNP from counties Clare and Mayo has further reduced this genetic variability, which may impact on the fitness of the population at a later stage.

Evidence to suggest pine martens were present in KNP prior to the translocation is contradictory. On one hand two sightings were reported prior to the re-establishment, which given the experience of the reporter would appear believable. Furthermore this species is notoriously hard to sight and is often reputed to occur in areas for many years without being seen (NiNeachtain, 1998; Birks, 2002). On the other hand pine martens are often confused with mink and stoats (*pers. obs.*) and may be especially so if only fleeting glimpses are obtained. The genetic evidence, microsatellite or mtDNA, does not support the presence of pine marten in KNP prior to translocation. All the alleles found for KNP were a subset of those from the source sample which were a subset of the IRE group. Furthermore there were no new haplotypes from the mtDNA to support a persistent population of pine martens at KNP. Though this may be due to the fact that populations were either not isolated from each other or the duration of the isolation was not long enough to allow for sequence divergence. The latter is most likely true as similar results were found by Davison *et al.* (2001) for the Scottish population of pine marten, which as stated previously has a similar history to the Irish pine marten.

In conclusion much of the work presented here supports and compliments previous investigations into pine marten population ecology but have highlighted a number of interesting questions. Is the bias in favour of females observed in the trapped population in KNP a real dynamic of the population, a by-product of the translocation effort or an artifact of the trapping methodology? Will the decrease in genetic diversity observed in the KNP pine marten population have an effect on the fitness and the long term viability of this population? That aside, the decrease in genetic variability observed within the population in KNP should however serve as a warning to wildlife managers considering translocation as a tool for re-establishing wildlife populations. Despite the pine marten showing itself to be adaptable in the face of a changing environment there is no reason

to think that it will be able to adapt to meet all the changes. Thus healthy source populations within protected areas, like the pine marten population in KNP, will be necessary to maintain the longevity of this species on our shores. Both of the novel techniques for monitoring pine marten populations in this study, hair-trapping and sex identification, will compliment existing techniques for monitoring this rare and elusive species.

8.2. Future Work

Several potential areas of research became apparent during the course of this study and relate to the population biology and distribution of the pine marten in Ireland. These areas arose out of work carried out in this study and also from lack of information to support some aspects of the work discussed. Firstly it needs to be determined whether the observed female bias in the trapped population in KNP is an artifact of the trapping methodology or whether it is a true reflection of the population and if so is that linked to a reduction in genetic diversity. It would be possible to determine this by carrying out a line versus grid trapping methodology in the same area and compare whether there is a male or female bias. Furthermore the hair trapping work together with the sex primers revealed a further 4 males in KNP, though only two of these were on live trapped transects, which may suggest that male pine martens were avoiding live traps. This may be because they escaped from live traps and were reluctant to reenter or the traps were too heavily concealed. As hair traps and live traps were positioned in the same places, I can rule out not finding the traps.

However if a female bias is still observed then it would be important to ask what is causing this bias. It has been shown previously in weasel populations that at low density females dominate the population while at high density males do (King, 1975). It may be that the population in KNP is still at low density, not having reached carrying capacity and a similar mechanism is operating. It may also be a direct result of the translocation, which is only ten years old. If however it is a result of the trapping methodology, then such results would be important if a study was targeting a particular sex to gather information on a particular aspect of the population biology of this species for example neo-natal denning requirements or male dispersal.

Secondly it was shown that the current suite of microsatellite markers available for pine marten is not useful for population genetics studies on individual populations. Prior to

determining whether the observed reduction in heterozygosity is linked with the observed female bias, it would be wise to develop microsatellite markers specifically for the European pine marten. Currently some of the markers are monomorphic, while others were either out of Hardy Weinberg equilibrium or show linkage disequilibria. Thus if specific markers were available it may allow researchers to carry out detailed investigations into the population dynamics of this species and perhaps identifying individuals. Furthermore it would allow population genetic studies together with radio telemetry to assess dispersal capabilities and patterns for the pine marten and more importantly, given the observed low levels of genetic diversity, barriers to that dispersal. Such studies would also allow us to assess whether the pine marten population in KNP is a source population, which theoretically it should be as it occurs within a protected area and is rarely disturbed by man. If on the other hand immigration into the area was high then it would be possible to start investigating reasons for that.

Thirdly, little is known in Ireland about home range size in pine martens, while nothing is known of the denning requirements of this species. The little work that has been carried out on pine marten populations in Ireland suggests that martens in Ireland may live at higher density and occupy smaller home ranges compared with other marten populations studied in Europe. Why exactly that is the case has not been determined. If for example we consider the wide niche breadth of the pine marten population investigated in this study as an indicator of ecological stress, then it is possible to hypothesise that there should be an equivalent increase in home range territory. It is likely that food is not the limiting factor for pine martens but that lack of suitable den sites is a limiting factor. As such it is also imperative to have an understanding of the structures pine martens use to make their dens in, particularly natal dens, and equally important the driving force behind that selection – predation, protection from the elements etc.. Information on both these population parameters will aid in avoiding the observed conflicts that are currently taking place between pine martens and owners of buildings in which they are taking up residence in and facilitate our understanding of their movements within their home ranges. Such work will have important implications in forestry practices and may help identify key factors that promote persistence of pine marten populations within commercial forestry. As commercial woodland enterprises strive towards biodiverse commercial woodlands then certainly such information would help to develop best codes of practice for forestry operations such as clear felling and thinning operations.

Though the diet of the pine marten has been explored in this thesis, much work still needs to be carried out to examine the relationship between prey availability and prey selection by this species. Such studies will be imperative in understanding the dynamics of pine marten populations in sub-optimal habitats, like conifer plantations for example. Furthermore, a parallel study investigating the diet of this species in areas with and without the bank vole may be interesting in revealing different resource utilisation patterns by the pine marten. Also by investigating the diet of the predator guild in the area together with prey availability and then comparing it with published literature or with areas that have a larger number of predators, the United Kingdom for example, then it would be possible to infer how the predators partition resources amongst them and whether this has any bearing on population biology of individual species. A similar comparative study investigating predator guilds with and without the bank vole present would allow the role of this recently introduced species be determined in Ireland.

It has been shown in this study that there are now primers available to accurately sex pine marten. This technique could be used to isolate differences in the diet due to sex. This may help elucidate, or perhaps rule out differential prey exploitation by males and females, as an evolutionary mechanism behind sexual dimorphism in this species or as evidence of niche expansion in the absence of the polecat. The female pine marten would theoretically prey upon smaller body prey items, while the larger male would prey upon bigger prey items. Given that pine martens are protected in this country and it is highly unlikely that enough carcasses from RTA's can be collected to carry out a study with enough power to say anything meaningful, this may represent the only way this hypothesis could be tested. However one potential flaw with that theory may relate to the wide niche breadth observed for the Irish pine marten. As Ireland has a typically mild climate throughout the year then other prey items such as fruit, amphibians and passerine birds are available year round. Thus it may be such a niche expansion would never be observed in Ireland, as a driving force such as cold winters (rarely get snow cover) for example means that the pine marten in this country does not prey primarily on small mammals but exploits all the food resources available to it. By comparison Scotland, the stronghold of the pine marten in the United Kingdom, experiences much more severe winters than here and hence the main food available to the pine marten it are small mammals particularly in the winter. This merits careful investigation.

Finally a comprehensive national survey of pine marten in Ireland is required to set a baseline for the distribution of this species. The previous census is both out of date and squares surveyed were based on historical and recent sightings (at that time) of pine marten. Thus it would be beneficial to carry out a complete national census to map the current distribution of pine marten which would not be biased by historical accounts. Such a survey should be based on 10 x 10km randomly selected squares and should cover the entire island. The work above has shown how unreliable scat and sightings of pine marten can be. I would recommend that the positive presence of pine marten should be confirmed and where scat location is being used as a method to determine presence, it should be backed up by hair trap work. It is possible to conclude that within Ireland research on pine martens is only in its infancy.

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APPENDIX 1. Sightings of pine marten in Killarney National Park between 20/06/1985 and 01/08/2004. Blank spaces indicate no information was available.

Date	Number	Transect	Remarks	Observer
20.06.1985	1	1	On forestry road	KNP ranger
23.04.1988	1	3	In a tree	KNP ranger
07.12.1988	1	3	Chasing two squirrels	KNP ranger
17.03.1991	1	6	Crossing road	KNP ranger
14.07.1992	1	1	Crossing forestry road	KNP ranger
03.11.1995	1	8	Juvenile	KNP ranger
22.01.1996	1	9	Crossing the road	KNP ranger
20.06.1996	1	3	Crossing forestry road	KNP ranger
30.07.1996	1	10		KNP ranger
27.06.1997	1	10		KNP ranger
12.09.1997	1	8		KNP ranger
28.09.1997	1	3		KNP ranger
14.01.1999	1	5		KNP ranger
20.05.1999	1	3		KNP ranger
20.06.1999	1	9	Crossing the road	KNP ranger
22.06.1999	1	2	Crossing road	KNP ranger
25.06.1999	1	8	In Torc waterfall car park	KNP ranger
25.07.1999	1	2	Crossed road at 16.45	KNP ranger
07.11.1999	1	3	Sitting in oak tree	KNP ranger
30.12.1999	1	5	Killed on the road	KNP ranger
10.06.2000	1	10	Killed on the road	KNP ranger
27.06.2000	1	3	On the Dinis road	KNP ranger
24.07.2000	1	2	On forestry road	KNP ranger
14.08.2000	1	3		KNP ranger
30.11.2000	1	4	Dead on road	Ian Hester
23.04.2001	1	6	In an oak tree	KNP ranger
06.05.2001	1	3	On the wall of Brickeen Bridge	KNP ranger

Date	Number	Transect	Remarks	Observer
08.07.2001	1	1	On forestry road	KNP ranger
15.07.2001	1	3/9		KNP ranger
16.07.2001	2	2	Seen at 15.30	KNP ranger
06.01.2001	1	6	Near the road	KNP ranger
16.07.2002	2	3	2 animals fighting at 16.30h	KNP ranger
28.04.2003	1	3		KNP ranger
26.05.2003	1	3	Foraging at compost bin	Á.Lynch
07.06.2003	1	2	Crossing the road	KNP ranger
23.06.2003	1	3		KNP ranger
03.08.2003	1	3	23.00 hours	KNP ranger
15.08.2003	1	3	12.45	KNP ranger
15.02.2003	1	3	20.30	KNP ranger
04.04.2004	1	3		KNP ranger
06.04.2004	1	3	09.45	KNP ranger
19.06.2004	1	3	13.00	KNP ranger
01.08.2004	1	3	22.00	KNP ranger

APPENDIX 2. Monthly weather data for Killarney National Park for 2003 and 2004, expressed in hours. μ is the average, SD is the standard deviation, Mx is the maximum and Mn is the minimum. Data is from the Valentia Weather Observatory (National Grid Reference: V457788) and which is 11 meters high. Data was provided by Met Eireann.

Month	Year	Air Temp. (°C)				Rainfall (mm)				Wind (Knots)			
		μ	SD	Mx	Mn	μ	SD	Mx	Mn	μ	SD	Mx	Mn
Jan	03	7.2	3.2	11.7	-2.5	0.2	0.6	6.1	0	11.5	6.5	28	1
	04	8.2	3.3	13.9	-1.2	0.2	0.7	7.7	0	10.8	6.6	35	1
Feb	03	7.9	2.2	12.2	-0.1	0.2	0.8	7	0	11.3	6.2	27	0
	04	7.4	3.3	13.9	-1.2	0.1	0.5	5.6	0	9.1	6.1	30	1
Mar	03	9.4	2.5	18.2	0.5	0.1	0.4	6	0	9.5	5.8	27	0
	04	8.5	2.3	13.8	0.7	0.2	0.8	8.7	0	11.4	6.7	34	0
Apr	03	10.8	3.2	22.5	3	0.2	0.8	11.2	0	8.9	4.9	27	0
	04	9.6	1.9	14.4	2.7	0.1	0.4	6.2	0	10.2	5.0	30	1
May	03	11.2	2.2	22.5	3	0.2	0.5	7	0	10.2	4.7	24	0
	04	11.6	2.6	18.5	4.4	0.05	0.2	3.9	0	8.1	5.4	29	1
Jun	03	13.8	1.9	21.5	7.8	0.2	0.9	8.8	0	8.8	4.3	20	1
	04	14.0	2.0	20.2	6.5	0.1	0.6	7.2	0	8.4	4.7	23	0
Jul	03	15.0	1.4	20	9.1	0.2	0.6	7.2	0	8.3	3.2	18	0
	04	14.5	1.9	20.9	7.4	0.1	0.6	8.1	0	7.5	4.1	19	0
Aug	03	16.5	2.9	25.5	8.4	0.1	0.9	2.4	0	6.2	3.6	17	0
	04	15.6	2.0	20.9	7.5	0.1	0.6	10.2	0	8.3	4.3	27	0
Sep	03	14.6	2.6	21.4	6.5	0.1	0.4	4.3	0	6.8	3.9	19	0
	04	14.9	2.5	24.4	7.7	0.2	0.7	6.3	0	9.9	5.8	26	0
Oct	03	11.5	3.4	18.2	10.6	0.1	0.3	4.6	0	8.4	4.4	21	1
	04	10.2	2.2	14.6	0.6	0.3	0.7	5.2	0	9.2	5.4	25	0
Nov	03	10.1	2.9	16.3	1.6	0.4	1.0	10	0	10.8	6.5	38	1
	04	10.4	1.8	13.5	3.8	0.1	0.4	3.5	0	7.6	4.4	21	0
Dec	03	8.6	2.4	13.2	0.6	0.2	0.6	7.1	0	9.2	5.0	24	0
	04	9.4	2.2	12.7	1.4	0.2	0.5	5	0	10.9	6.0	30	0

APPENDIX 2. Fig. A showing the total monthly rainfall for 2003 and 2004 recorded at the Valentia Observatory. Weather patterns tend to be localised (due to the presence of the mountains) and therefore this may not be the actual total rainfall which occurred in Killarney National Park. Fig. B shows the number of wet days recorded each month for 2003 and 2004.

Fig. A.

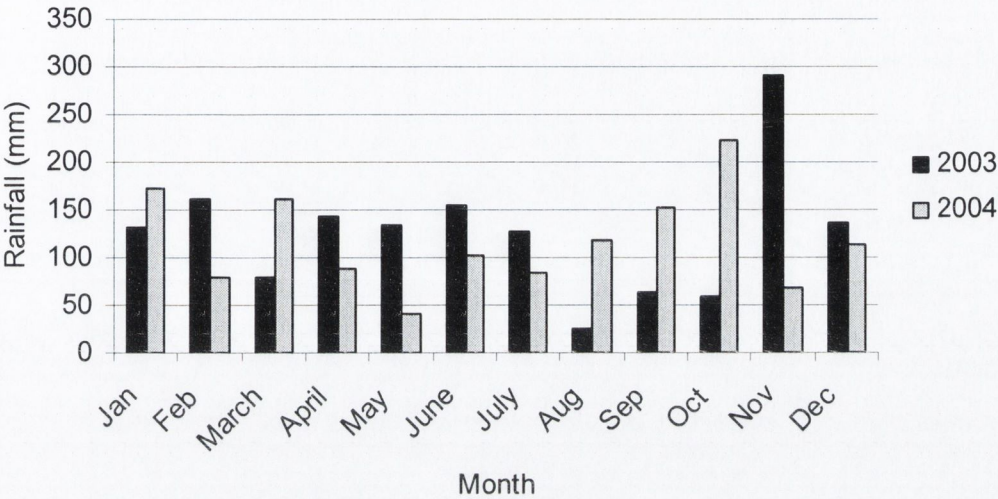
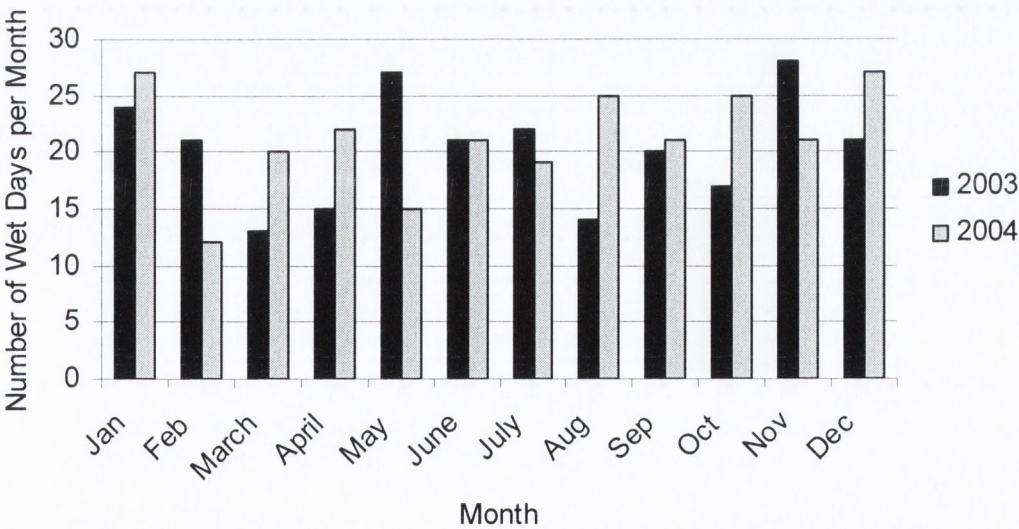


Fig. B.



APPENDIX 3. Individual data for live trapped animals. Trap Acc and 63C0FFB were the two animals that broke their backs while trying to get out of traps. UL003m and LC001 were the two males that would not enter the handling cone and therefore no measurement data was available for them.

N	ID	Lab ID	Date	Site	Trap Point	Sex	Age	Weight (kg)	Total Length (cm)	Head & Body (cm)	Tail (cm)	Hind Foot (cm)	Reproductive Condition	External Parasites
1	63C020B	K27	04/06/03	1	10	M	A	1.8	64	44	20	9.2	Testes visible	None
2	637E593	K13	17/05/04	1	8	M	A	1.6	77.5	46.5	31	10.5	Testes visible	None
3	61D5397	K11	09/07/04	1	4	F	SA	1.1	66.5	37.5	23	8.9	Not Lactating	None
4	63BE083	K12	12/07/04	1	6	F	SA	0.95	56	34	22	8.5	Not Lactating	None
5	61D335F	K24	05/09/04	1	3	F	A	1.2	63	41	22	8.7	Not Lactating	None
6	63C1179	K2	13/04/03	2	1	F	A	1.3	58	40	18	8.4	Not Lactating	None
7	63BEA3F	K7	22/06/03	2	7	F	A	1.5	70	45	25	8.9	Not Lactating	None
			15/08/03	2	8	F	A						Not Lactating	None
			17/05/04	2	8	F	A						Not Lactating	None
8	663BCE6B	K6	22/06/03	2	5	M	SA	1.1	51	35	16	9	No Testes	None
9	6383FFB	K20	05/06/03	3	2	F	SA	1.2	48	33	15	8.1	Not Lactating	None
10	63BF910	K5	09/05/04	3	3	F	A	1.15	66	43	23	9.2	Lactating	None
			12/05/04	3	3	F	A						Lactating	None
			30/06/04	3	3	F	A						Stopped Lact.	None

N	ID	Lab ID	Date	Site	Trap Point	Sex	Age	Weight (kg)	Total Length (cm)	Head & Body (cm)	Tail (cm)	Hind Foot (cm)	Reproductive Condition	External Parasites
11	63C0A53	K18	03/07/04	3	3	F	A							None
			11/05/04	3	5	F	A	1.25	69	44	25	8.2	Lactating	3 ticks on head
12	63BEE9B	K21	11/05/04	3	9	F	A	1.35	73	47	26	9.8	Lactating	None
			14/05/04	3	10	F	A						Lactating	None
			30/06/04	3	10	F	A						Stopped Lact	None
			25/08/04	3	9	F	A						Not Lactating	None
13	Kitten 1	K15	03/07/04	3	2	F	K	0.8	52	33	19	8	Not breeding	None
14	Kitten 2	K16	03/07/04	3	10	M	K	0.85	52	33	19	8.5	Not breeding	None
15	62410B2	K23	25/08/04	3	10	M	SA	1.2	63	40	23	9.1	Not breeding	Tick on left ear
16	6381B02	K17	12/05/03	4	8	F	A	1.3	63	45	18	8.5	Lactating	None
			12/06/03	4	1								Lactating	None
			13/06/03	4	2								Lactating	None
			14/06/03	4	8								Lactating	None
			15/06/03	4	6								Lactating	None
			16/06/03	4	8								Lactating	None
			03/08/03	4	5								Stopped Lact	None
			06/08/03	4	7								Stopped Lact	None
			07/08/03	4	3								Stopped Lact	None
			25/04/04	4	1			1.3					Lactating	None
			26/04/04	4	8								Lactating	None
			27/04/04	4	7								Lactating	None
			29/04/04	4	9								Lactating	None

N	ID	Lab ID	Date	Site	Trap Point	Sex	Age	Weight (kg)	Total Length (cm)	Head & Body (cm)	Tail (cm)	Hind Foot (cm)	Reproductive Condition	External Parasites
			16/06/04	4	10								Stopped Lact	None
			17/06/04	4	2								Stopped Lact	None
			18/06/04	4	6								Stopped Lact	None
17	Trap Acc	K26	13/05/04	4	5	M	A	1.8	77	54	23	9.8	Died in trap	
18	UL003M	none	02/08/03	4	4	M	A						Testes Visible	Tick on ear
					8								Testes Visible	Tick on ear
					5								Testes Visible	Tick on ear
19	63BB818	K25	25/04/04	4	2	M	A	2.1	73	51	22	10	Testes Visible	2 ticks on head
20	63BF258	K19	29/04/04	4	3	F	SA	1.2	64	44	22	9.1	Not Lactating	None
21	642229	K28	14/08/04	4	9	F	A	1.2	63	41	22	8.5	Not Lactating	None
			15/08/04	4	5									
			16/08/04	4	1									
22	63C0FFB	K1	11/04/03	5	2	F	A	1.3	67	48	19	9	Lactating	1 tick on face
			12/04/03	5	4	F	A						Dead at trap	
23	63816BB	K3	14/04/03	5	3	F	A	1.35	68	50	18	9.2	Lactating	None
			25/04/03	5	4	F	A	1.4					Lactating	None
24	61D5855	K9	14/08/04	5	4	F	A	1.2	60	40	20	8.5	Not Breeding	None
25	6243B34	K10	15/08/04	5	9	F	A	1.3	63	40	23	8.3	Not Breeding	None
26	63BEF69	K8	13/05/04	6	9	F	A	1.3	68	46	22	8.9	Not lactating	None
			14/05/04	6	9									
27	63BEDFF	K4	13/05/04	6	10	M	A	2.5	79	49	30	10.2	Testes Visible	None
28	61D280C	K14	30/06/04	6	5	M	A	1.5	74.5	48.5	26	9.9	Testes Visible	None

N	ID		Date	Site	Trap Point	Sex	Age	Weight (kg)	Total Length (cm)	Head & Body (cm)	Tail (cm)	Hind Foot (cm)	Reproductive Condition	External Parasites
29	LC001	none	13/07/03	7	1	M	A						Testes Visible	None
30	62418D6	K22	30/05/04	7	2	M	A	1.7	70	50	20	9.6	Testes Visible	None
			01/08/04	7	6	M	A							
			02/08/04	7	8	M	A							

APPENDIX 4. Description of animals collected from around Ireland. The grid reference is the national grid reference. RTA refers to road traffic accident, while TD refers to trap death. NPWS is National Parks and Wildlife Service. Sex is either male (m) or female (f). Animals were aged as adult (A) or sub-adult (SA).

N	ID	Date	Location	Grid Reference	Sex	Age	Cause of Death	Collector
1	WW1	2002	Glenmalure, Co. Wicklow	T095845	M	A	RTA	John Griffin (NPWS)
2	CE1	01/04/2004	Ballynahinch, Co. Clare	R562811	F	A	RTA	Stefan Jones (NPWS)
3	CE2	26/04/2004	Coolready, Bodyke, Co. Clare	R584817	F	A	RTA	Stefan Jones (NPWS)
4	CE3	05/04/2004	Corbally, Quin, Co. Clare	R420770	M	A	RTA	Stefan Jones (NPWS)
5	CE4	25/03/2004	Kyleglass, Broadford, Co. Clare	R597692	M	A	RTA	Stefan Jones (NPWS)
6	CE5	10/05/2004	Manushare, Ennis, Co. Clare	R372730	F	A	RTA	Stefan Jones (NPWS)
7	CE6	2004	Ballyvaughan, Co. Clare	R230070	M	A	RTA	Stefan Jones (NPWS)
8	CE7	1997	Co. Clare	R562811	M	A	RTA	Rowena Ni Neachtain
9	CE8	2003	Ballyvaughan, Co. Clare	R230070	M	A	RTA	Penny Bartlett (NPWS)
10	G1	25/06/2004	East Galway	M230075	F	A	RTA	Penny Bartlett (NPWS)
11	G2	03/05/2005	Castlefrench, East Bog, Co. Galway	M784465	F	A	RTA	Becky Teesdale (NPWS)
12	G3	2004	Ballynainch, Connemara, Co. Galway	L755477	M	A	RTA	Ger O'Donnell (NPWS)
13	G4	2004	Connemara NP, Co. Galway	L713575	F	A	RTA	Ger O'Donnell (NPWS)
14	G5	17/05/2005	Recess, Co. Galway	L855477	F	A	RTA	Ger O'Donnell (NPWS)
15	G6	11/07/2005	Athenry, Co. Galway	M470260	F	A	RTA	Martino Newcombe
16	KY1	13/05/2003	Ullauns Wood, KNP, Co. Kerry	V910805	M	A	TD	Á. Lynch
17	KY2	03/2003	Tower Lodge, KNP, Co. Kerry	V931828	M	A	RTA	Brendan O'Shea (NPWS)
18	KY3	05/2003	Derrycunihy, KNP, Co. Kerry	V920800	F	A	TD	Á. Lynch
19	KY4	03/04/2005	Pool Wood Road, KNP, Co. Kerry	V931828	M	A	RTA	Brendan O'Shea (NPWS)
20	L1	unknown	Ballyroan Village, Co. Laois	S4489	M	A	RTA	Noreen Corcoran
21	L2	unknown	Co. Laois	S450980	F	A	RTA	Noreen Corcoran

N	ID	Date	Location	Grid Reference	Sex	Age	Cause of Death	Collector
22	L3	29/05/2005	Co. Laois	L519013	M	A	RTA	Ruth Carden
23	OY1	07/04/2004	Eglish Bridge, Co. Offaly	N100972	F	A	RTA	Noel Bugler (NPWS)
24	OY2	12/06/2004	Charleville Wood, Co. Offaly	N313228	M	A	RTA	Noel Bugler (NPWS)
25	KE1	09/05/2005	Rathangan, Co. Kildare	N656026	M	A	RTA	Roy Thompson (NPWS)
26	M1	09/05/2005	Newtown, Partry, Co. Mayo	M144737	M	A	RTA	Eoin McGreal (NPWS)
27	S1	07/08/2004	Turnalayden, Riverston, Co. Sligo	G707229	F	SA	RTA	David Mac Donagh (NPWS)
28	WM1	06/2003	Hill of Uisneach, Co. Westmeath	N292490	M	A	RTA	P O'Donnell (NPWS)

APPENDIX 5. Description of scats analysed: ID, site, date, grid reference, position, season, dry weight (g), dry volume (g) and number of components. W1-W43 was collected and processed by Yvonne McCann. Habitat codes follow Fossit (2000) (Chapter 2). The Total Number of components refers to each scat and is followed by which group those components belonged to. There was no available data for samples W1-W43.

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
TMW1	1	WN1&WD4	08/02/2003	Spring	1.18	0.82	1			1			
TMW2	1	WN1&WD4	08/02/2003	Spring	1.31	0.24	2		1		1		
TMW3	1	WN1&WD4	10/02/2003	Spring	0.88	0.51	1		1	1			
TMW4	1	WN1&WD4	10/02/2003	Spring	4.4	0.37	2	1	1	1			
TMW5	1	WN1&WD4	10/02/2003	Spring	1.06	0.9	1			1			
TMW6	1	WN1&WD4	12/02/2003	Spring	1.95	1.44	2		1	1			
TMW7	1	WN1&WD4	12/02/2003	Spring	4.82	1.74	1		1	1			
TMW8	1	WN1&WD4	12/02/2003	Spring	2.91	1.84	3	1	1			1	
TMW9	1	WN1&WD4	03/04/2003	Spring	2.63	2.23	3		1	1	1		
TMW10	1	WN1&WD4	03/04/2003	Spring	2.02	2.00	3		1	1	1		
TMW11	1	WN1&WD4	05/04/2003	Spring	3.86	0.66	2	1	1				
TMW12	1	WN1&WD4	10/04/2003	Spring	6.46	2.04	3	1	1	1			
TMW13	1	WN1&WD4	10/04/2003	Spring	1.57	1.20	2	1		1			
TMW14	1	WN1&WD4	10/04/2003	Spring	3.12	1.94	2	1	1				
TMW15	1	WN1&WD4	10/04/2003	Spring	3.16	3.05	2	1		1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
TMW16	1	WN1&WD4	10/04/2003	Spring	2.40	0.56	3		1			1	
TMW17	1	WN1&WD4	10/04/2003	Spring	4.41	0.61	2		1	1			
TMW18	1	WN1&WD4	10/04/2003	Spring	3.42	0.43	3		1	1			
TMW19	1	WN1&WD4	10/04/2003	Spring	2.53	2.13	2	1	1				
TMW20	1	WN1&WD4	10/04/2003	Spring	2.01	1.98	3	1	1	1			
TMW21	1	WN1&WD4	12/04/2003	Spring	4.85	3.93	2	1	1				
TMW22	1	WN1&WD4	12/04/2003	Spring	8.02	3.25	3	1	1				1
TMW23	1	WN1&WD4	12/04/2003	Spring	5.16	1.82	2		1	1			
TMW24	1	WN1&WD4	12/04/2003	Spring	6.38	1.01	2	1		1			
TMW25	1	WN1&WD4	12/04/2003	Spring	2.57	0.80	2	1	1				
TMW26	1	WN1&WD4	12/04/2003	Spring	1.98	0.99	5	1	1	1		1	
TMW27	1	WN1&WD4	12/04/2003	Spring	2.53	1.10	2	1		1			
TMW28	1	WN1&WD4	14/04/2003	Spring	2.31	2.34	3	1	1				
TMW29	1	WN1&WD4	14/04/2003	Spring	3.02	2.90	3	1	1		1		
TMW30	1	WN1&WD4	14/04/2003	Spring	3.44	2.18	2	1	1				
TMW31	1	WN1&WD4	14/04/2003	Spring	4.07	2.68	3	1	1				
TMW32	1	WN1&WD4	14/04/2003	Spring	4.68	2.36	2	1	1				1
TMW33	1	WN1&WD4	16/04/2003	Spring	3.51	2.90	3	1	1			1	
TMW34	1	WN1&WD4	27/04/2003	Spring	2.89	0.44	2	1					1
TMW35	1	WN1&WD4	27/05/2003	Summer	2.84	1.08	3	1		1		1	
TMW36	1	WN1&WD4	27/05/2003	Summer	3.8	1.66	3	1		1		1	

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
TMW37	1	WN1&WD4	06/06/2003	Summer	1.98	0.96	3	1		1	1		
TMW38	1	WN1&WD4	06/06/2003	Summer	2.69	1.32	1				1		
TMW39	1	WN1&WD4	01/07/2003	Summer	4.29	2.21	3	1		1	1		
TMW40	1	WN1&WD4	01/07/2003	Summer	1.3	0.14	3	1	1	1			
TMW41	1	WN1&WD4	01/07/2003	Summer	5.86	3.58	2	1			1		
TMW42	1	WN1&WD4	01/07/2003	Summer	2.72	0.94	1				1		
TMW43	1	WN1&WD4	03/07/2003	Summer	2.07	0.88	2	1			1		
TMW44	1	WN1&WD4	03/07/2003	Summer	1.6	1.19	1				1		
TMW45	1	WN1&WD4	05/07/2003	Summer	6.06	3.37	2	1			1		
TMW46	1	WN1&WD4	05/07/2003	Summer	1.9	0.72	5	1	1	1	1	1	
TMW47	1	WN1&WD4	22/08/2003	Autumn	2.75	1.83	1		1				
TMW48	1	WN1&WD4	26/08/2003	Autumn	3.4	2.17	2	1	1				
TMW49	1	WN1&WD4	28/08/2003	Autumn	3.93	2.86	3	1	1	1			
TMW50	1	WN1&WD4	05/04/2004	Spring	4.62	2.87	2	1	1				
TMW51	1	WN1&WD4	07/04/2004	Spring	3.37	2.14	1		1				
TMW52	1	WN1&WD4	09/04/2004	Spring	2.22	1.31	3		1			1	1
TMW53	1	WN1&WD4	04/06/2004	Spring	4.83	1.10	2	1		1			
TMW54	1	WN1&WD4	04/06/2004	Spring	5.04	1.58	2	1			1		
TMW55	1	WN1&WD4	08/06/2004	Summer	3.64	1.05	2	1			1		
TMW56	1	WN1&WD4	13/07/2004	Summer	4.21	1.39	2	1		1			
TMW57	1	WN1&WD4	03/08/2004	Autumn	1.54	0.43	2	1		1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
MF1	2	WD3	15/02/2003	Spring	4.1	1.03	2		1			1	
MF4	2	WD3	22/02/2003	Spring	2.25	0.22	2	1		1			
MF5	2	WD3	11/04/2003	Spring	4.03	2.36	3	1		1	1		
MF6	2	WD3	11/04/2003	Spring	5.5	3.56	4		1	1	1	1	
MF7	2	WD3	22/06/2003	Summer	1.8	1.33	1		1				
MF8	2	WD3	23/06/2003	Summer	1.39	0.87	1			1			
MF9	2	WD3	26/06/2003	Summer	3.02	0.60	2	1			1		
MF10	2	WD3	26/06/2003	Summer	2.3	1.71	3	1	1	1			
MF11	2	WD3	05/08/2003	Autumn	1.85	0.51	2		1	1			
MF12	2	WD3	07/08/2003	Autumn	3.36	2.79	2	1	1				
MF13	2	WD3	07/08/2003	Autumn	3.2	2.95	1		1				
MF14	2	WD3	12/08/2003	Autumn	1.16	0.53	2		1		1		
MF15	2	WD3	14/08/2003	Autumn	2.36	1.74	1		1				
MF16	2	WD3	14/08/2003	Autumn	1.52	1.15	1		1				
MF17	2	WD3	12/04/2004	Spring	2.77	0.81	2		1	1			
MF18	2	WD3	14/04/2004	Spring	2.02	0.55	1		1				
MF19	2	WD3	14/04/2004	Spring	8.79	6.54	4	1	1		1	1	
MF20	2	WD3	09/07/2004	Summer	4.42	1.59	3	1	1			1	
MF21	2	WD3	12/07/2004	Summer	2.83	1.34	6	1	1	1	1	1	1
MF22	2	WD3	12/07/2004	Summer	2.37	0.62	2	1	1				
MP1	3	WN3&WN1	12/02/2003	Spring	3.85	1.67	1	1					

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
MP2	3	WN3&WN1	12/02/2003	Spring	4.52	2.25	2	1		1			
MP4	3	WN3&WN1	15/02/2003	Spring	1.84	1.15	3		1	1	1		
MP5	3	WN3&WN1	17/02/2003	Spring	3.43	2.46	2		1	1			
MP7	3	WN3&WN1	17/02/2003	Spring	2.46	0.35	1	1					
MP8	3	WN3&WN1	17/02/2003	Spring	2.22	0.83	2	1	1				
MP10	3	WN3&WN1	20/03/2003	Spring	2.22	3.09	3	1	1	1			
MP11	3	WN3&WN1	20/03/2003	Spring	3.43	2.54	2	1	1				
MP12	3	WN3&WN1	20/03/2003	Spring	1.17	0.31	2	1					1
MP13	3	WN3&WN1	20/03/2003	Spring	1.33	0.87	2	1	1				
MP14	3	WN3&WN1	20/03/2003	Spring	1.66	0.88	3	1	1	1			
MP15	3	WN3&WN1	20/03/2003	Spring	1.62	0.99	1		1				
MP16	3	WN3&WN1	20/03/2003	Spring	3.86	1.98	3	1	1	1			
MP17	3	WN3&WN1	20/03/2003	Spring	3.02	1.46	2	1		1			
MP19	3	WN3&WN1	25/03/2003	Spring	2.51	1.51	2				1		
MP20	3	WN3&WN1	25/03/2003	Spring	4.19	4.04	4	1	1			1	1
MP21	3	WN3&WN1	25/03/2003	Spring	5.81	1.12	2	1	1				
MP22	3	WN3&WN1	25/03/2003	Spring	2.43	1.60	2	1	1				
MP23	3	WN3&WN1	25/03/2003	Spring	3.75	3.47	4	1	1	1			1
MP24	3	WN3&WN1	25/03/2003	Spring	1.94	1.32	2	1	1				
MP25	3	WN3&WN1	25/03/2003	Spring	3.8	2.25	2	1	1				
MP26	3	WN3&WN1	27/03/2003	Spring	3.19	1.76	3	1	1	1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
MP28	3	WN3&WN1	27/03/2003	Spring	2.8	1.51	3	1	1	1			
MP29	3	WN3&WN1	30/03/2003	Spring	2.6	1.78	3	1	1		1		
MP30	3	WN3&WN1	30/03/2003	Spring	2.46	1.66	3	1	1				1
MP31	3	WN3&WN1	30/03/2003	Spring	2.82	1.55	2	1	1				
MP32	3	WN3&WN1	30/03/2003	Spring	2.05	1.36	2	1	1				
MP34	3	WN3&WN1	30/03/2003	Spring	0.71	0.43	2	1		1			
MP35	3	WN3&WN1	31/03/2003	Spring	1.09	0.60	2	1	1				
MP36	3	WN3&WN1	03/04/2003	Spring	3.72	0.94	2	1		1			
MP37	3	WN3&WN1	04/04/2003	Spring	2.13	1.28	2	1	1				
MP38	3	WN3&WN1	04/04/2003	Spring	1.13	1.09	2	1	1				
MP39	3	WN3&WN1	05/04/2003	Spring	1.85	1.35	4	1	1	1	1		
MP40	3	WN3&WN1	05/04/2003	Spring	2.43	1.51	4	1	1	1	1		
MP41	3	WN3&WN1	17/04/2003	Spring	2.21	0.92	4	1	1	1	1		
MP42	3	WN3&WN1	17/04/2003	Spring	2.75	1.90	1	1					
MP43	3	WN3&WN1	17/04/2003	Spring	1.8	1.85	2	1	1				
MP44	3	WN3&WN1	17/04/2003	Spring	1.94	1.57	2		1	1			
MP45	3	WN3&WN1	17/04/2003	Spring	2.75	0.66	2	1	1				
MP46	3	WN3&WN1	21/04/2003	Spring	4.55	3.51	3	1	1	1			
MP47	3	WN3&WN1	21/04/2003	Spring	3.22	1.96	3	1	1	1			
MP48	3	WN3&WN1	21/04/2003	Spring	4.56	3.38	3	1	1				1
MP49	3	WN3&WN1	21/04/2003	Spring	3.57	3.43	2	1		1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
MP50	3	WN3&WN1	21/04/2003	Spring	9.47	3.41	1		1				
MP51	3	WN3&WN1	21/04/2003	Spring	5.66	1.82	2	1	1				
MP52	3	WN3&WN1	21/04/2003	Spring	7.77	3.01	2	1	1				
MP53	3	WN3&WN1	21/04/2003	Spring	2.31	2.50	2	1	1				
MP54	3	WN3&WN1	21/04/2003	Spring	3.15	2.54	2	1	1				
MP55	3	WN3&WN1	23/04/2003	Spring	3.58	1.45	2	1	1				
MP56	3	WN3&WN1	23/04/2003	Spring	2.51	1.34	4	1	1	1		1	
MP57	3	WN3&WN1	23/04/2003	Spring	2.09	1.28	3		1	1	1		
MP58	3	WN3&WN1	25/04/2003	Spring	3.69	1.70	2	1	1				
MP59	3	WN3&WN1	25/04/2003	Spring	1.87	1.61	4	1	1	1	1		
MP60	3	WN3&WN1	25/04/2003	Spring	2.2	1.81	3		1	1	1		
MP61	3	WN3&WN1	25/04/2003	Spring	2.8	2.53	2	1	1				
MP62	3	WN3&WN1	25/04/2003	Spring	3.48	1.99	2		1			1	
MP63	3	WN3&WN1	27/04/2003	Spring	3.36	1.19	3	1	1		1		
MP64	3	WN3&WN1	27/04/2003	Spring	4.42	1.33	2	1	1				
MP65	3	WN3&WN1	27/04/2003	Spring	2.82	0.78	2						
MP67	3	WN3&WN1	27/04/2003	Spring	1.6	0.20	1	1			1		
MP68	3	WN3&WN1	01/05/2003	Summer	5.33	0.37	1	1					
MP69	3	WN3&WN1	03/05/2003	Summer	9.24	3.57	5	1	1	1	1	1	
MP71	3	WN3&WN1	14/05/2003	Summer	2.05	2.58	4	1	1	1	1		
MP72	3	WN3&WN1	26/05/2003	Summer	8.37	3.36	4	1	1	1		1	

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
MP73	3	WN3&WN1	26/05/2003	Summer	3.55	1.44	2	1				1	
MP74	3	WN3&WN1	26/05/2003	Summer	1.77	5.67	3	1		1			1
MP75	3	WN3&WN1	27/05/2003	Summer	3.51	1.95	2	1		1			
MP76	3	WN3&WN1	27/05/2003	Summer	2.29	0.77	2	1		1			
MP77	3	WN3&WN1	27/05/2003	Summer	2.56	1.13	1			1			
MP78	3	WN3&WN1	02/06/2003	Summer	1.78	0.77	2	1		1			
MP79	3	WN3&WN1	02/06/2003	Summer	3.33	1.78	2	1		1			
MP80	3	WN3&WN1	04/06/2003	Summer	1.82	1.08	3	1	1		1		
MP81	3	WN3&WN1	04/06/2003	Summer	3.47	1.61	4	1	1	1		1	
MP82	3	WN3&WN1	04/06/2003	Summer	2.07	0.92	2	1		1			
MP83	3	WN3&WN1	05/06/2003	Summer	2.7	1.51	2	1		1			
MP84	3	WN3&WN1	05/06/2003	Summer	1.66	1.36	3	1		1	1		
MP85	3	WN3&WN1	06/06/2003	Summer	5.21	2.24	2	1		1			
MP86	3	WN3&WN1	07/06/2003	Summer	2.47	0.75	3	1	1	1			
MP87	3	WN3&WN1	07/06/2003	Summer	1.96	0.93	2	1		1			
MP88	3	WN3&WN1	08/06/2003	Summer	1.5	0.25	1			1			
MP89	3	WN3&WN1	08/06/2003	Summer	1.38	0.70	2	1		1			
MP90	3	WN3&WN1	13/06/2003	Summer	1.02	0.56	2	1		1			
MP91	3	WN3&WN1	13/06/2003	Summer	4.89	2.34	2	1		1			
MP92	3	WN3&WN1	13/06/2003	Summer	5	4.22	1			1			
MP93	3	WN3&WN1	15/06/2003	Summer	4.45	0.53	2	1		1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
MP94	3	WN3&WN1	15/06/2003	Summer	3.27	2.19	2	1		1			
MP95	3	WN3&WN1	15/06/2003	Summer	2.97	1.08	3	1		1	1		
MP96	3	WN3&WN1	15/06/2003	Summer	3	2.43	4	1	1	1		1	
MP97	3	WN3&WN1	17/06/2003	Summer	3.72	1.46	2	1			1		
MP98	3	WN3&WN1	17/06/2003	Summer	0.66	0.30	1			1			
MP99	3	WN3&WN1	17/06/2003	Summer	0.98	0.64	1		1				
MP100	3	WN3&WN1	22/08/2003	Autumn	6.56	3.94	3	1	1	1			
MP101	3	WN3&WN1	22/08/2003	Autumn	3.96	3.11	3	1	1	1			
MP102	3	WN3&WN1	22/08/2003	Autumn	7.31	5.05	3	1	1	1			
MP103	3	WN3&WN1	24/08/2003	Autumn	3.98	2.26	3	1	1	1			
MP104	3	WN3&WN1	24/08/2003	Autumn	6.48	4.28	2	1	1				
MP105	3	WN3&WN1	01/04/2004	Spring	4.41	1.06	5	1	1	1	1		1
MP106	3	WN3&WN1	01/04/2004	Spring	1.79	2.88	1		1				
MP107	3	WN3&WN1	01/04/2004	Spring	1.64	1.39	3	1	1	1			
MP108	3	WN3&WN1	01/04/2004	Spring	3.85	1.26	2	1	1				
MP109	3	WN3&WN1	01/04/2004	Spring	2.15	0.39	2	1				1	
MP110	3	WN3&WN1	04/04/2004	Spring	4.45	2.65	2	1		1			
MP111	3	WN3&WN1	06/04/2004	Spring	2.24	1.68	3	1	1	1			
MP112	3	WN3&WN1	06/04/2004	Spring	2.3	1.72	3	1	1	1			
MP113	3	WN3&WN1	06/04/2004	Spring	1.6	2.26	3	1	1		1		
MP114	3	WN3&WN1	08/04/2004	Spring	3.59	2.87	2		1		1		

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
MP115	3	WN3&WN1	08/04/2004	Spring	1.7	2.14	3	1	1	1			
MP116	3	WN3&WN1	08/04/2004	Spring	2.73	1.11	2	1			1		
MP117	3	WN3&WN1	08/04/2004	Spring	4.46	0.043	3	1	1	1			
MP118	3	WN3&WN1	14/05/2004	Summer	3.98	3.41	2	1	1				
MP119	3	WN3&WN1	04/06/2004	Summer	1.53	0.45	2	1			1		
MP120	3	WN3&WN1	06/06/2004	Summer	2.98	1.19	2	1		1			
MP121	3	WN3&WN1	06/06/2004	Summer	4.35	1.73	1				1		
MP122	3	WN3&WN1	06/06/2004	Summer	4.7	0.82	2	1			1		
MP123	3	WN3&WN1	06/06/2004	Summer	1.09	1.21	2	1	1				
MP124	3	WN3&WN1	06/06/2004	Summer	2.63	1.52	2	1		1			
MP125	3	WN3&WN1	08/06/2004	Summer	1.51	0.87	2	1		1			
MP126	3	WN3&WN1	30/06/2004	Summer	2.18	1.25	3	1		1			
MP127	3	WN3&WN1	04/08/2004	Autumn	6.11	5.20	1		1				
MP128	3	WN3&WN1	06/08/2004	Autumn	2.43	2.26	2	1	1				
MP129	3	WN3&WN1	06/08/2004	Autumn	2.62	2.07	3	1	1	1			
MP130	3	WN3&WN1	06/08/2004	Autumn	4.88	3.71	1		1				
MP131	3	WN3&WN1	06/08/2004	Autumn	6.11	2.21	2	1	1				
MP132	3	WN3&WN1	10/08/2004	Autumn	2.86	2.51	2		1			1	
UL1	4	WN1	25/02/2003	Spring	1.55	0.50	1	1					
UL2	4	WN1	01/03/2003	Spring	1.36	0.76	1			1			
UL3	4	WN1	05/03/2003	Spring	0.57	0.26	1			1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
UL4	4	WN1	26/03/2003	Spring	0.76	0.43	1			1			
UL5	4	WN1	26/03/2003	Spring	6.99	5.40	1		1				
UL6	4	WN1	26/03/2003	Spring	4.43	2.36	4	1	1	1		1	
UL7	4	WN1	26/03/2003	Spring	1.76	0.52	1			1			
UL8	4	WN1	26/03/2003	Spring	4.62	2.55	2			1		1	
UL9	4	WN1	26/03/2003	Spring	1.32	0.11	2	1	1				
UL10	4	WN1	30/03/2003	Spring	1.51	0.60	1		1				
UL11	4	WN1	02/04/2003	Spring	0.61	1.42	1		1				
UL12	4	WN1	02/04/2003	Spring	0.78	0.44	1		1				
UL13	4	WN1	02/04/2003	Spring	2.54	1.10	1		1				
UL14	4	WN1	02/04/2003	Spring	1.47	3.52	3	1	1	1			
UL15	4	WN1	02/04/2003	Spring	1.6	1.61	2		1	1			
UL16	4	WN1	11/05/2004	Spring	4.82	2.05	1		1				
UL17	4	WN1	12/06/2003	Summer	3	1.26	2		1	1			
UL18	4	WN1	13/06/2003	Summer	2.92	1.54	1		1				
UL19	4	WN1	14/06/2003	Summer	3.96	1.66	2	1	1				
UL20	4	WN1	15/06/2003	Summer	0.86	0.32	1		1				
UL21	4	WN1	16/06/2003	Summer	2.11	0.76	3	1	1	1			
UL22	4	WN1	02/07/2003	Summer	2.34	0.94	2	1		1			
UL23	4	WN1	04/07/2003	Summer	3.91	1.33	2	1			1		
UL24	4	WN1	04/07/2003	Summer	1.98	1.09	2		1	1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
UL25	4	WN1	04/07/2003	Summer	2.17	2.43	2	1				1	
UL26	4	WN1	04/07/2003	Summer	2.32	1.59	1			1			
UL27	4	WN1	06/07/2003	Summer	4.46	0.95	4	1	1	1		1	
UL28	4	WN1	03/08/2003	Autumn	2.93	2.11	1	1					
UL29	4	WN1	07/08/2003	Autumn	0.74	0.09	1	1					
UL30	4	WN1	03/09/2003	Autumn	1.97	1.12	4	1	1	1	1		
UL31	4	WN1	03/09/2003	Autumn	3.92	1.02	2	1	1				
UL32	4	WN1	05/09/2003	Autumn	1.48	1.37	1		1				
UL33	4	WN1	07/09/2003	Autumn	2.85	1.13	1		1				
UL34	4	WN1	19/04/2004	Spring	7.12	0.55	1		1				
UL35	4	WN1	24/04/2004	Spring	3.15	2.89	2	1	1				
UL36	4	WN1	24/04/2004	Spring	2.59	1.24	2	1	1				
UL37	4	WN1	28/04/2004	Spring	2.23	1.63	3	1	1	1			
UL38	4	WN1	16/05/2004	Summer	5.58	2.65	2	1	1				
UL39	4	WN1	16/05/2004	Summer	4.32	1.55	4	1	1	1		1	
UL40	4	WN1	16/05/2004	Summer	5.2	2.90	1		1				
UL41	4	WN1	20/05/2004	Summer	4.2	2.76	2	1	1				
UL42	4	WN1	20/05/2004	Summer	1.93	1.28	2	1	1				
UL43	4	WN1	10/07/2004	Summer	7.14	4.02	5	1	1	1	1	1	
UL44	4	WN1	10/07/2004	Summer	2.32	1.44	1	1					
UL45	4	WN1	13/07/2004	Summer	2.67	1.55	1			1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
DC1	5	WN1	27/02/2003	Spring	4.45	1.60	2	1			1		
DC2	5	WN1	01/03/2003	Spring	1.24	0.41	2			1	1		
DC3	5	WN1	03/03/2003	Spring	0.86	0.46	3		1	1	1		
DC4	5	WN1	03/03/2003	Spring	1.04	0.95	1			1			
DC5	5	WN1	03/03/2003	Spring	1.9	1.35	3	1	1	1			
DC6	5	WN1	05/03/2003	Spring	1.06	0.81	2		1	1			
DC7	5	WN1	31/03/2003	Spring	3.17	2.36	2	1	1				
DC9	5	WN1	31/03/2003	Spring	3.1	2.02	1		1				
DC10	5	WN1	31/03/2003	Spring	2	0.75	3	1	1	1			
DC11	5	WN1	31/03/2003	Spring	3.22	1.93	1		1				
DC12	5	WN1	02/04/2003	Spring	3.34	1.96	2	1	1				
DC13	5	WN1	04/04/2003	Spring	3.1	1.58	3	1	1		1		
DC14	5	WN1	04/04/2003	Spring	2.66	1.65	1		1				
DC15	5	WN1	04/04/2003	Spring	1.91	1.22	2	1			1		
DC16	5	WN1	06/04/2003	Spring	3.31	1.07	1		1				
DC17	5	WN1	06/04/2003	Spring	3.31	1.08	1		1				
DC18	5	WN1	10/04/2003	Spring	3.1	1.97	1		1				
DC19	5	WN1	10/04/2003	Spring	2.69	2.16	2		1	1			
DC20	5	WN1	12/04/2003	Spring	3.97	2.16	2		1	1			
DC21	5	WN1	12/04/2003	Spring	2.72	2.55	1		1				
DC22	5	WN1	11/04/2003	Spring	2.97	2.80	2		1	1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
DC23	5	WN1	11/04/2003	Spring	1.17	1.71	2	1		1			
DC24	5	WN1	02/06/2003	Summer	1.69	1.77	1		1				
DC25	5	WN1	02/06/2003	Summer	3.46	1.72	2		1			1	
DC26	5	WN1	02/06/2003	Summer	2.19	2.61	4	1	1			1	1
DC27	5	WN1	02/06/2003	Summer	6.42	0.50	2		1	1			
DC28	5	WN1	26/06/2003	Summer	2.2	0.94	2	1		1			
DC29	5	WN1	26/06/2003	Summer	2.03	0.65	3	1	1	1			
DC30	5	WN1	12/08/2003	Autumn	4.91	2.60	4	1	1	1		1	
DC31	5	WN1	16/08/2003	Autumn	3.01	0.37	1		1				
DC33	5	WN1	08/09/2003	Autumn	3.83	2.19	2	1	1				
DC34	5	WN1	10/09/2003	Autumn	7.2	2.94	4	1	1	1		1	
DC35	5	WN1	24/04/2004	Spring	8.54	3.22	4	1	1	1		1	
DC36	5	WN1	25/04/2004	Spring	9.4	4.54	2		1		1		
DC37	5	WN1	26/04/2004	Spring	2.73	1.74	2		1		1		
DC38	5	WN1	26/04/2004	Spring	2.98	2.37	2	1	1				
DC39	5	WN1	26/04/2004	Spring	2.51	2.37	2	1			1		
DC40	5	WN1	27/04/2003	Spring	5.91	4.47	3	1	1		1		
DC41	5	WN1	29/04/2004	Spring	5.36	2.98	2		1	1			
DC42	5	WN1	09/05/2004	Summer	3.49	1.22	2		1	1			
DC43	5	WN1	11/05/2004	Summer	3.54	2.70	1		1				
DC44	5	WN1	11/05/2004	Summer	3.33	2.18	2	1	1				

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
DC45	5	WN1	13/05/2004	Summer	4.45	2.64	1			1			
DC46	5	WN1	13/05/2004	Summer	2.85	2.09	2		1		1		
DC47	5	WN1	13/05/2004	Summer	3.39	2.92	2		1			1	
DC48	5	WN1	28/06/2004	Summer	4.91	1.20	2	1		1			
DC49	5	WN1	02/07/2004	Summer	1.83	1.28	3	1		1	1		
TW2	6	WN1	16/07/2003	Summer	1.57	1.20	3	1	1			1	
TW3	6	WN1	22/07/2003	Summer	2.78	1.73	3	1	1			1	
TW5	6	WN1	12/06/2004	Summer	4.05	2.75	3	1	1			1	
LC1	7	WN1	11/07/2003	Summer	3.72	1.57	2		1	1			
LC2	7	WN1	14/07/2003	Summer	1.74	3.56	2	1				1	
LC3	7	WN1	15/07/2003	Summer	2.25	1.41	1			1			
LC4	7	WN1	07/09/2003	Summer	2.9	0.41	1	1					
LC5	7	WN1	26/05/2004	Summer	1.6	1.75	2		1	1			
TOF1	8	WD3	21/04/2003	Spring	4.91	0.62	3	1	1	1			
TOF2	8	WD3	21/04/2003	Spring	1.87	0.60	3	1	1		1		
TOF3	8	WD3	23/04/2003	Spring	6.6	2.44	3	1	1			1	
TOF4	8	WD3	23/04/2003	Spring	4.7	0.44	3	1	1	1			
TOF5	8	WD3	23/04/2003	Spring	2.21	2.02	1		1				
TOF6	8	WD3	23/04/2003	Spring	2.7	1.66	3		1	1	1		
TOF7	8	WD3	23/04/2003	Spring	1.25	0.19	2	1		1			
TOF8	8	WD3	23/04/2003	Spring	1.25	0.19	3	1	1	1			

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
TOF9	8	WD3	22/06/2003	Summer	5.15	1.42	2	1			1		
TOF10	8	WD3	22/06/2003	Summer	0.87	2.38	1	1					
TOF11	8	WD3	24/06/2003	Summer	3.13	0.30	3	1		1		1	
TOF12	8	WD3	24/06/2003	Summer	1.41	1.83	3	1	1		1		
TOF13	8	WD3	24/06/2003	Summer	1.54	1.25	2	1			1		
TOF14	8	WD3	24/06/2003	Summer	0.9	1.23	2					1	1
TOF15	8	WD3	14/08/2003	Autumn	4.21	0.47	2	1				1	
TOF16	8	WD3	16/08/2003	Autumn	2.89	2.07	3		1	1		1	
TOF17	8	WD3	12/04/2004	Spring	1.49	1.18	2	1	1				
TOF18	8	WD3	14/04/2004	Spring	1.45	0.87	3	1	1	1			
TOF19	8	WD3	14/04/2004	Spring	1.38	0.68	2	1				1	
TOF20	8	WD3	31/07/2004	Summer	5.56	4.06	2	1	1				
TOF21	8	WD3	31/07/2004	Summer	2.02	0.99	2	1		1			
TOF22	8	WD3	02/08/2004	Autumn	6.14	0.92	3	1	1			1	
TOF23	8	WD3	02/08/2004	Autumn	1.7	5.61	2	1	1				
TOF24	8	WD3	02/08/2004	Autumn	3.86	1.32	2	1	1				
TOF25	8	WD3	04/08/2004	Autumn	3.09	3.36	2	1	1				
BPC1	9	WN6&WD4	24/04/2003	Spring	5.24	4.26	2			1	1		
BPC2	9	WN6&WD4	24/04/2003	Spring	2.72	1.99	2		1	1			
GA1	10	WD1&WN3	26/04/2003	Spring	4.54	4.26	2	1	1				
GA2	10	WD1&WD3	13/07/2003	Summer	1.31	0.91	2	1			1		

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
GA3	10	WD1&WD3	14/04/2004	Spring	3.83	2.19	2	1		1			
GA4	10	WD1&WD3	31/07/2004	Summer	2.34	2.21	2	1		1			
GA5	10	WD1&WD3	04/08/2003	Autumn	4.85	2.06	3	1		1			
GnaM1	11	WN1	21/03/2003	Spring	1.32	1.29	1			1			
GnaM2	11	WN1	21/03/2003	Spring	2.15	0.70	2		1	1			
GnaM4	11	WN1	27/03/2003	Spring	6.55	3.09	3		1	1		1	
GnaM5	11	WN1	27/03/2003	Spring	1.89	1.21	2		1	1			
GnaM6	11	WN1	27/03/2003	Spring	1.4	0.49	1		1			1	
GnaM7	11	WN1	05/03/2004	Spring	2.28	1.27	1	1					
GanM8	11	WN1	17/04/2003	Spring	3.2	2.17	3	1	1	1			
GnaM9	11	WN1	14/05/2004	Summer	1.56	2.18	2		1	1			
C1	12	WN1	22/03/2003	Spring	4.38	1.31	2		1	1			
C2	12	WN1	22/03/2003	Spring	2.41	2.29	1			1			
C3	12	WN1	31/03/2003	Spring	0.92	1.18	1			1			
C4	12	WN1	16/06/2003	Spring	3	0.68	1		1	1			
W1	3	WN3&WN1	20/11/2004	Winter	8.49	4.874	2						
W2	3	WN3&WN1	20/11/2004	Winter	2.62	9.429	3						
W3	3	WN3&WN1	20/11/2004	Winter	3.58	1.04	4						
W4	3	WN3&WN1	20/11/2004	Winter	4.29	2.017	3						
W5	3	WN3&WN1	20/11/2004	Winter	6.39	12.21	4						
W6	3	WN3&WN1	20/11/2004	Winter	5.56	13.516	5						

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
W7	3	W/N3&W/N1	06/01/2005	Winter	4.96	4.041	2						
W8	3	W/N3&W/N1	06/01/2005	Winter	4.45	3.878	4						
W9	3	W/N3&W/N1	06/01/2005	Winter	4.31	2.905	4						
W10	3	W/N3&W/N1	06/01/2005	Winter	3.67	10.593	4						
W11	3	W/N3&W/N1	06/01/2005	Winter	4.61	0.485	2						
W12	3	W/N3&W/N1	06/01/2005	Winter	2.76	0.198	3						
W13	3	W/N3&W/N1	06/01/2005	Winter	2.46	15.631	2						
W14	3	W/N3&W/N1	24/01/2005	Winter	2.26	8.18	4						
W15	3	W/N3&W/N1	24/01/2005	Winter	2.81	1.553	2						
W16	3	W/N3&W/N1	24/01/2005	Winter	1.54	4.682	3						
W17	3	W/N3&W/N1	24/01/2005	Winter	1.12	0.352	1						
W18	3	W/N3&W/N1	24/01/2005	Winter	1.15	6.851	2						
W19	3	W/N3&W/N1	24/01/2005	Winter	3.46	10.764	4						
W20	3	W/N3&W/N1	24/01/2005	Winter	2.98	21.639	2						
W21	3	W/N3&W/N1	23/11/2004	Winter	4.62	9.073	4						
W22	3	W/N3&W/N1	23/11/2004	Winter	5.44	3.86	2						
W23	3	W/N3&W/N1	23/11/2004	Winter	8.39	17.637	3						
W24	3	W/N3&W/N1	23/11/2004	Winter	6.97	4.601	3						
W25	3	W/N3&W/N1	23/11/2004	Winter	5.12	3.74	3						
W26	3	W/N3&W/N1	23/11/2004	Winter	5.34	8.124	4						
W27	3	W/N3&W/N1	23/11/2004	Winter	3.38	2.044	2						

ID	Site	Habitat Type	Date	Season	Dry weight (g)	Dry Weight after washing (g)	Total Number of Components	Arthropod	Fruit	Mammal	Bird	Other	Earthworm
W28	3	WN3&WN1	23/11/2004	Winter	7.17	3.696	3						
W29	3	WN3&WN1	23/11/2004	Winter	4.53	3.813	3						
W30	3	WN3&WN1	23/11/2004	Winter	7.31	2.73	1						
W31	3	WN3&WN1	24/11/2004	Winter	5.74	3.289	3						
W32	3	WN3&WN1	24/11/2004	Winter	5.76	21.437	3						
W33	3	WN3&WN1	24/11/2004	Winter	5.66	2.322	6						
W34	3	WN3&WN1	24/11/2004	Winter	5.14	2.973	3						
W35	3	WN3&WN1	24/11/2004	Winter	5.13	11.835	2						
W36	3	WN3&WN1	24/11/2004	Winter	6.62	5.381	3						
W37	3	WN3&WN1	24/11/2004	Winter	6.05	3.777	2						
W38	3	WN3&WN1	24/11/2004	Winter	2.58	1.059	3						
W39	3	WN3&WN1	24/11/2004	Winter	3.11	1.319	5						
W40	3	WN3&WN1	24/11/2004	Winter	0.52	17.78	5						
W41	3	WN3&WN1	24/11/2004	Winter	3.29	1.948	3						
W42	3	WN3&WN1	24/11/2004	Winter	4.21	1.06	4						
W43	3	WN3&WN1	24/11/2004	Winter	2.41	10.79	4						

APPENDIX 5. % Frequency Occurrence (FO) for each component for each season (1: spring; 2: summer; 3: autumn; 4: winter). N_{scats} = 387.

Figure in parentheses is the actual number of components.

Season	Coleoptera	Hymenoptera	Lepidoptera	Isopoda	Larvae	Insecta Unknown	Blackberry	Bilberry	Ivy	Rowan	Yew	Apple	Hawthorn	Pine	Fruit Unknown
1	22.22	0.24	0.24	0.24	0.24	3.86	0.24	0.96	28.74	0.24	0.48	0.48	0.24	0	0.48
(413)	(92)	(2)	(2)	(2)	(2)	(16)	(2)	(4)	(117)	(2)	(3)	(3)	(2)	(0)	(3)
2	27.08	1.17	0.39	0	0	5.49	0.39	2.7	10.98	0	0.39	1.17	0	0	2.35
(255)	(69)	(3)	(1)	(0)	(0)	(14)	(1)	(7)	(28)	(0)	(1)	(3)	(0)	(0)	(6)
3	22.5	1.25	3.75	0	0	3.75	17.5	2.5	0	3.75	18.75	0	0	0	3.75
(80)	(18)	(1)	(3)	(0)	(0)	(3)	(14)	(2)	(0)	(3)	(15)	(0)	(0)	(0)	(3)
4	18.04	1.49	0	5.26	0	1.49	2.98	0	0	2.98	25.56	0	0	1.49	1.49
(133)	(24)	(2)	(0)	(7)	(0)	(2)	(4)	(0)	(0)	(4)	(34)	(0)	(0)	(2)	(2)

Season	Woodmouse	Bank Vole	Pygmy Shrew	Lagomorph	Rat	Squirrel	Deer	Mammal Unknown	Passerine	Falciforme	Egg Shell	Bird Unknown	Frog	Mollusc	Debris	Earthworm
1	7.0	3.86	0.96	0.72	0.24	0.96	0.24	8.69	3.14	0	0.24	5.55	3.14	0.48	3.86	1.69
	(29)	(16)	(4)	(3)	(2)	(4)	(2)	(36)	(13)	(0)	(0)	(23)	(15)	(2)	(12)	(7)
2	6.66	6.66	0.39	0	0	0	0	9.41	1.69	0.39	0.39	9.02	7.84	1.96	1.96	0.39
	(17)	(17)	(1)	(0)	(0)	(0)	(0)	(24)	(7)	(1)	(1)	(23)	(20)	(5)	(5)	(1)
3	3.75	0	0	0	0	0	0	8.75	0	0	0	3.75	5	1.25	1.25	0
	(3)	(0)	(0)	(0)	(0)	(0)	(0)	(6)	(0)	(0)	(0)	(3)	(4)	(1)	(1)	(0)
4	1.49	4.47	4.47	0.74	0	0	0	0.74	5.97	0	0	0	5.97	0	0.74	14.18
	(2)	(5)	(5)	(1)	(0)	(0)	(0)	(1)	(8)	(0)	(0)	(0)	(8)	(0)	(1)	(19)

APPENDIX 5. % Mass (M) for each component for each season (1: spring; 2: summer; 3: autumn; 4: winter).

Season	Coleoptera	Hymenoptera	Lepidoptera	Isopoda	Larvae	Insecta Unknown	Blackberry	Bilberry	Ivy	Rowan	Yew	Apple	Hawthorn	Pine	Fruit Unknown
1	4.84	0.04	0.03	0.03	0.09	0.28	0.62	1.65	55.56	0.34	0.1	0.09	0.24	0	0.21
2	8.10	0.41	0.06	0	0	1.98	0.07	4.84	27.64	0	0.02	0.49	0	0	1.57
3	2.83	0.49	0.36	0.46	0	0.49	28.17	4.63	0	1.66	53.4	0	0	0	1.56
4	1.36	0.02	0.003	0.009	0	0.003	0.04	0	0	0.045	31.94	0	0	0.09	0.02

Season	Woodmouse	Bank Vole	Pygmy Shrew	Lagomorph	Rat	Squirrel	Deer	Mammal Unknown	Passerine	Falciforme	Egg Shell	Bird Unknown	Frog	Mollusc	Debris	Earthworm
1	5.33	5.44	1.20	1.12	0.2	0.49	0.06	8.36	1.26	0	0.23	10.13	5.69	10.76	0	2.23
2	11.53	7.53	0.96	0	0	0	0	9.74	2.6	1.4	0	10.92	7.61	0.07	0.23	2.39
3	0.51	0	0	0	0	0	0.71	1.41	0	0	0	0.05	1.39	0.06	0	1.7
4	0.06	0.82	1.04	0.03	0	0	0	0	0.04	0	0	0	0.23	0	64.22	64.22

APPENDIX 5. % Fresh Weight Ingested (FWI) for each component for each season (1: spring; 2: summer; 3: autumn; 4: winter).

Season	Coleoptera	Hymenoptera	Lepidoptera	Isopoda	Larvae	Insecta Unknown	Blackberry	Bilberry	Ivy	Rowan	Yew	Apple	Hawthorn	Pine	Fruit Unknown
1	1.63	0.01	0.02	0.01	0.03	0.09	0.59	1.56	52.3	0.32	0.09	0.09	0.23	0	0.19
2	2.67	0.14	0.047	0	0	0.65	0.06	4.47	25.52	0	0.015	0.46	0	0	1.45
3	1.03	0.18	0.32	0.17	0	0.18	28.8	4.7	0	1.74	54.6	0	0	0	1.6
4	0.46	0.006	0.002	0.003	0	0.001	0.03	0	0	0.04	30.49	0	0	0.09	0.01

Season	Woodmouse	Bank Vole	Pygmy Shrew	Lagomorph	Rat	Squirrel	Deer	Mammal Unknown	Passerine	Falciforme	Egg Shell	Bird Unknown	Frog	Mollusc	Debris	Earthworm
1	7.88	8.06	1.79	3.26	0.29	0.73	0.27	12.36	3.13	0	0.02	5.8	0.03	6.89	0.38	0.13
2	16.73	10.92	1.39	0	0	0	0	14.13	6.31	3.42	0.006	26.66	0.07	9.03	0.50	0.001
3	0.82	0	0	0	0	0	0	2.27	0	0	0	0.14	0.07	1.83	0.10	0
4	0.08	1.24	1.56	0.208	0	0	0	0	0.12	0	0	0	0	0.28	0	4.38

APPENDIX 5. Table of reported values for the dietary components of the pine marten. Values are reported as fully as possible but many authors only chose to report percentage frequency of occurrence (%FO) but where the percentage fresh weight ingested (%FW) was also reported, this was included. Where it was not possible to record values within the table, these studies have been summarised below. All references quoted in the table appear in Chapter 5 and some, though not all, in Chapter 1. The country where the study was carried out and the number of scats analysed (N = X, if stomach contents then this will appear as the second number quoted) are listed under the authors. BS is the Dietary Niche Breadth. The seasons are 1 (spring), 2 (summer), 3 (autumn) and 4 (winter).

	Arthropod		Fruit		Mammal		Bird		Other		Earthworm		BS	Study
	FO	FW	FO	FW	FO	FW	FO	FW	FO	FW	FO	FW		
Annual	29.33	1.87	24.6	23.2	10.7	29.1	10.2	21.2	10.73	3.28	13.77	21.55	0.798	NíNeachtain (1998)
(scrub) (Conifer)	37.99	2.93	10.66	9.84	10.9	14.2	12.8	26.2	14.48	21.96	13.17	24.87		
Seasonal 1	20		14		14		12		16		23		0.67	Ireland N = 80 per site
2	25		12		12		23		14		15		0.54	
3	29		23		11		5		15		18		0.59	
4	26		18		18		5		15		20		0.62	
Annual	21.8		16.14		12.74		23.36		2.44		23.57		0.803	Warner & O'Sullivan (1982) Ireland N = 609
Seasonal 1	10		10		20		15		2		4.7			
2	25		10		20		6		2		7.5			
3	25		20		5		6		7		5.9			
4	10		20		5		15		7		3.2			
Annual	33.54		20.23		24.19		20.37		1.7		0			Clevenger (1993c) Minorca N = 723
Seasonal 1	33.57		7.9		40.98		17.23		0.2		0			
2	28.11		9.5		21.88		33.57		7.3		0			
3	36.2		35.88		11.23		16.47		0.2		0			
4														
Annual	13.5		28.57		35.6		11.2		4.6		6.3		0.6579	Marchesi & Mermod (1989) Switzerland N = 823, 31
Seasonal 1	8		16		58		9		7				0.4053	
2	25		21		32		18		4				0.7994	
3	22		45		21		5		4				0.5649	
4	1		45		39		15		2				0.4236	

	Arthropod		Fruit		Mammal		Bird		Other		Earthworm		BS	Study
	FO	FW	FO	FW	FO	FW	FO	FW	FO	FW	FO	FW		
Annual	22.55		11.39		47.4		12.5		6.15					Balharri (1993b)
Seasonal 1	1		0		30		18		4					Scotland
2	7		2		35		22		5					N = 1304
3	32		6		35		6		2					
4	0		2		30		4		1					
Annual	27		11		41		20		1					Halliwell (1997)
Seasonal 1														Scotland
2														N = 1938
3														
4														
Annual	20.73		11.95		40.82		12.9		13.56					Jedrzejewski
Seasonal 1														<i>et al.</i> (1993)
2														Poland
3														N = 1735
4														
Annual		1.7		42.7		39.9		15.7						Goszczyński
Seasonal 1		5.2		1.2		52.6		41						(1976)
2		0.8		70.6		26.1		2.5						Poland
3		0.8		70.6		26.1		2.5						N = 835
4		0.1		28.2		55		16.7						
Annual		2.95		37.15		40.33		19.08		0.49				Goszczyński
Seasonal 1														(1986)
2														Poland
3														N = 813
4														

Studies quoted in table

Halliwell (1997) Author analysed a total of 1938 scats containing 4006 components but only 0.77% contained red squirrel remains [5 = winter, 4 = spring, 4 = summer, 3 = autumn]. Figures taken from Birks (2002).

Other Studies

Clevenger (1993a) Author investigated the feeding ecology of pine martens on an island (Minorca) and on the mainland (Cantabrian Mountains, Spain) by analysis of food habits, food niche breadth, prey size index and prey weight distribution. Mammals, birds, fruits and insects were the four main dietary components of both populations. In Minorca all four were important during the year, whereas small mammals and fleshy fruits were dominant in the Cantabrian diet. The insular pine martens were characterised as having a wide food niche breadth and exploited all foods equally compared to the Cantabrian martens and from those from two other mainland populations [$BS_{\text{Minorca}} = 0.754$ & $BS_{\text{CantabrianMountains}} = 0.380$].

Gurnell et al. (1994) (Scotland; N = 240). Found that mammals were the most important food item, in particular voles. Coleoptera were the most important of the insects and were actively taken throughout the year except January. Birds and fruit were seasonally important. Reptiles and frogs were preyed upon but were less important.

Jedrzejewski & Jedrzejewska (1993) (Poland) The pine marten exploited alternative prey [other small mammals] when rodents weren't available and were responsible for between 10-15% of all total rodent mortality during autumn and winter.

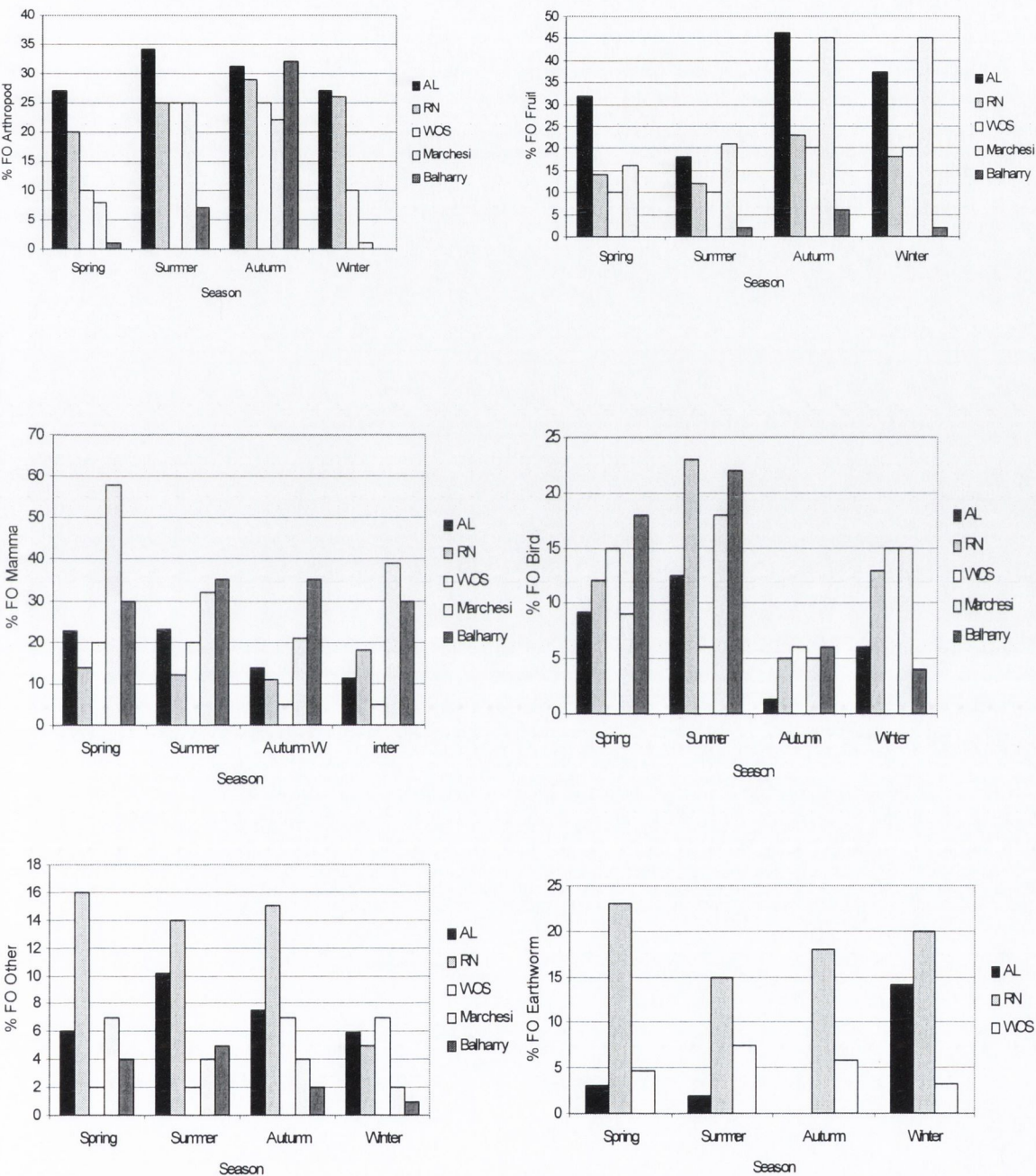
Pulliainen (1981) (Finland; N = 2700 and observing animals eating by locating them with radio telemetry) Berries are an important early winter food but are eaten throughout this season when available (take the flesh but the skin and seed are left untouched). Also reports that

pine martens will frequently eat the spore cases of mushrooms. Microtids and shrews are the most important of all the mammals, but carrion and squirrels (momentary importance) are eaten too. Pine martens will eat bird carcasses whenever found but will also take the eggs. The author found no significant difference between the diets of male and female animals. Author reported that the pine marten killed more individuals than it ate immediately. These excess prey items were buried and dug out following winter when microtids were in low abundance and eaten.

Putman (2000) (Scotland; N = 174). Mammals were the backbone of the diet, particularly voles. Fruit was important in autumn followed by winter then spring. Birds were most often taken in summer and winter. Beetles were taken frequently. Carrion was likely to be important if encountered, while frogs were only taken in July. Earthworms were important in winter and less so in summer but were rarely consumed at any other time during the year.

Zalewski (1996) (Poland; N = 1996). Findings show that in spring pine marten preyed upon bank voles generally over 8 months in age. By comparison in the summer they took animals between 2-4 months and in winter between 4-8 months old. In all seasons pine martens consumed lighter voles than tawny owls who took animals heavier than the average for the population.

APPENDIX 5. Graphs showing seasonal peaks in the % FO of the six main food groups in the diet of the pine marten. AL = this study; RN = NíNeachtain (1998); WOS = Warner & O’Sullivan (1982); Marchesi (1989); Balharry (1993).



APPENDIX 6. Microsatellite data for Killarney samples, showing the number of alleles found for each loci. --/-- indicates no PCR product, therefore the sample could not be scored at that locus. The figure in parentheses is the number of alleles found at each locus in Kyle *et al.* (2003).

n	Animal	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
		MA1	MA2	MA18	MA19	GG7	GG14	Lut604	Ggu454
1	K1	200/200	--/--	150/150	208/208	--/--	128/128	110/110	126/126
2	K2	200/200	--/--	150/150	208/208	160/160	128/128	110/110	126/126
3	K3	200/200	--/--	150/150	208/208	160/160	128/128	110/110	126/126
4	K4	200/200	168/170	150/150	208/208	160/160	128/128	110/110	126/128
5	K5	200/200	170/172	150/150	208/208	160/160	128/128	110/110	126/128
6	K6	200/200	170/170	15/150	208/208	168/168	128/128	110/110	126/128
7	K7	200/200	168/170	150/150	208/208	168/168	128/128	110/110	126/128
8	K8	200/200	--/--	150/150	208/208	160/164	128/128	110/110	126/128
9	K9	200/200	--/--	150/150	208/208	162/164	128/128	110/110	126/128
10	K10	200/200	--/--	150/150	208/208	168/168	128/128	110/110	126/128
11	K11	200/200	170/170	150/150	208/208	160/168	128/128	110/110	126/128
12	K12	200/200	--/--	150/150	208/208	160/168	128/128	110/110	126/128
13	K13	200/200	172/172	150/150	208/208	160/168	128/128	110/110	126/128
14	K14	200/200	172/172	150/150	208/208	160/168	128/128	110/110	126/126
15	K15	200/200	168/172	150/150	208/208	160/160	128/128	110/110	126/128
16	K16	200/200	168/172	150/150	208/208	160/168	128/128	110/110	126/128
17	K17	200/200	170/170	150/150	208/208	168/168	128/128	110/110	126/128
18	K18	200/200	168/168	150/150	208/208	160/168	128/128	110/110	126/128
19	K19	200/200	170/170	150/150	208/208	168/168	128/128	110/110	126/126
20	K20	200/200	170/170	150/150	208/208	160/160	128/128	110/110	126/126
21	K21	200/200	--/--	150/150	208/208	160/168	128/128	110/110	126/126
22	K22	200/200	168/170	150/150	208/208	160/168	128/128	110/110	126/126
23	K23	200/200	170/170	150/150	208/208	160/168	128/128	110/110	126/126
24	K24	200/200	170/170	150/150	208/208	164/168	128/128	110/110	126/128
25	K25	200/200	170/170	150/150	208/208	160/168	128/128	110/110	126/126
26	K26	200/200	170/172	150/150	208/208	160/168	128/128	110/110	126/128
27	K27	200/200	170/172	150/150	208/208	164/168	128/128	110/110	126/126
28	K28	200/200	170/170	150/150	208/208	160/168	128/128	110/110	126/128
No.									
Alleles/									
Locus		1(1)	3(4)	1(2)	1(1)	(4)	1(2)	1(1)	(2)

APPENDIX 7. Microsatellite data for Ireland samples, showing the number of alleles found for each loci. --/-- indicates no PCR product, therefore the sample could not be scored at that locus. The figure in parentheses is the number of alleles found at each locus in Kyle *et al.* (2003).

n	ID	Loc1 MA1	Loc2 MA2	Loc3 MA18	Loc4 MA19	Loc5 GG7	Loc6 GG14	Loc7 Lut604	Loc8 Ggu454
1	Ww1	200/200	172/172	148/148	208/208	160/160	128/128	110/110	126/126
2	Ce1	200/200	172/174	150/150	208/208	160/160	128/128	110/110	126/128
3	Ce2	--/--	--/--	--/--	208/208	160/160	--/--	110/110	126/128
4	Ce3	200/200	170/170	148/148	--/--	158/160	128/128	110/110	126/128
5	Ce4	200/200	168/172	150/150	208/208	160/164	128/128	110/110	126/128
6	Ce5	200/200	--/--	148/148	208/208	160/160	128/128	--/--	126/128
7	Ce6	200/200	168/172	150/150	208/208	160/164	128/128	110/110	126/128
8	Ce7	200/200	174/174	150/150	208/208	160/164	128/128	110/110	126/128
9	Ce8	200/200	168/174	150/150	208/208	168/170	128/128	110/110	126/128
10	Ky1	200/200	168/172	150/150	208/208	160/166	128/128	110/110	126/126
11	ky2	200/200	168/172	150/150	208/208	158/160	128/128	110/110	126/128
12	Ky3	200/200	170/172	150/150	208/208	160/166	128/128	110/110	126/128
13	Ky4	200/200	164/168	150/150	208/208	164/164	128/128	110/110	126/126
14	Oy1	200/200	170/172	150/150	208/208	160/164	128/128	110/110	126/126
15	Oy2	200/200	168/172	150/150	208/208	158/160	128/128	110/110	126/126
16	G1	200/200	170/170	150/150	208/208	158/160	128/128	110/110	126/128
17	G2	200/200	168/168	150/150	208/208	158/160	128/128	110/110	126/126
18	G3	200/200	170/170	150/150	208/208	158/160	128/128	110/110	126/126
19	G4	200/200	168/170	--/--	208/208	164/164	128/128	110/110	126/126
20	G5	200/200	170/170	150/150	208/208	158/160	128/128	110/110	126/126
21	G6	--/--	168/170	148/148	202/202	162/164	128/128	--/--	--/--
22	S1	200/200	170/174	150/150	208/208	162/164	128/128	110/110	126/128
23	L1	200/200	--/--	--/--	208/208	160/160	--/--	--/--	126/128
24	L2	200/200	168/172	150/150	208/208	--/--	128/128	110/110	126/126
25	L3	200/200	168/172	150/150	208/208	164/164	128/128	110/110	126/126
26	Ke1	200/200	164/168	150/150	208/208	164/164	--/--	110/110	126/126
27	M1	200/200	170/170	150/150	202/202	158/160	128/128	110/110	126/126
28	Wm1	200/200	168/168	150/150	208/208	160/162	128/128	110/110	126/126
29	+VE	200/200	170/170	150/150	208/208	158/164	128/128	110/110	126/128
No. Alleles/ Locus		1(1)	5(4)	2(2)	2(1)	7(4)	1(2)	1(1)	2(2)

APPENDIX 8. Mitochondrial DNA sequences for the control region and cytochrome *b* gene.

CONTROL REGION

KY1

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCCCTCTCCCTATGTC
TTAATTCATATATTTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCTTGCCCCATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCAATTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAGA
CTGAAACTATACCTGG

KY2

CGATGAGTACCCAAAGGCTGACATTCTAACTAAACTATTCCCTGATTTCCCTCTCCCTATGTC
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TATGTACATCGTGCAGTTAGTGGCTTGCCCCATGCATATAAGCATGTACATATTATGCTTG
ATCTTGCAATTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCAT
CAACCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAG
ACTGAAACTATACCTGG

KY3

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TCTTGCAATTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCCTTGCCCGATGTGTACCTCTACTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAGA
CTGACACTATACCTGG

KY4

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCCCTCTCCCTATGTC
TTAATTCATATATTTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCGTGCCCGATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCAATTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAGA
CTGACACTATACCTGG

CE6

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCCCTCTCCCTATGTC
TTAATTCATATATTTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCTTGCCCCATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCAATTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAGA
CTGAAACTATACCTGG

CE7

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCCCTCTCCCTATGTC
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TCTTGCAATTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAGA
CTGAAACTATACCTGG

CE8

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
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TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAGA
CTGAAACTATACCTGG

CE1

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
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TATGTACATCGTGCATTAGTGGCTTGCCCCATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAGA
CTGAAACTATACCTGG

CE2

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
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TCTTGCATTTCGGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCAT
CAACCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGCCCATAACATGTGGGGGTTTCTAG
ACTGACACTATACCTGG

G1

CCGTCAGCTCCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
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TCTTGCATTTCGGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCAT
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ACTGACACTATACCTGG

G2

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ATCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCAT
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G3

CCGTCAGCTCCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
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CTGACACTATACCTGG

G4

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TATGTACATCGTGCAGTTAGTGGCTTGCCCGATGCATATAAGCATGTACATATTATGCTTG
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ACTGACACTATACCTGG

G5

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TATGTACATCGTGCATTAGTGGCTTGCCCCATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCTTGCCCGATGTGTACCTCTACTCGCTCCGGGGCCATAACATGTGGGGGTTTCTAGA
CTGACACTATACCTGG

G6

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TCTTGCATTTCGTGCACCTCACTTAGACCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
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CTGAAACTATACCTGG

M1

CCATCAGCACCCCAAAGCTGACATTCTAACTAAACTATTCCCTGAATTCCTCTCCCTATGTC
TTAATTCATATATTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCGTGCCCGATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCTTGCCCGATGTGTACCTCTACTCGCTCCGGGGCCATAACATGTGGGGGTTTCTAGAC
TGACACTATACCTGG

S1

CCATCAGCGCCCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTTCGTCTCCCTATGTC
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TATGTACATCGTGCATTAGTGGCGTGCCCGCTGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGACCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCTTGCCCGATGTGTACCCCTACTCGCTCCGGGGCCATAACATGTGGGGGTTTCTAGA
CTGACACTATACCTGG

OY1

CCATCAGCACCCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCCCTCTCCCTATGTC
TTAATTCATATATTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCTTGCCCCATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGGCCATAACATGTGGGGGTTTCTAGA
CTGAAACTATACCTGG

OY2

CCGTGAGCACCCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCCCTCTCCCTATGTC
TTAATTCATATATTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCTTGCCCGATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGGCCATAACATGTGGGGGTTTCTAGA
CTGAAACTATACCTGG

L2

CCATCAGCGCCCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTTCGTCTCCCTATGTC
TTAATTCATATATTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCGTGCCCGCTGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGGCCATAACATGTGGGGGGTTTCTAGA
CTGACACTATACCTGG

L3

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
TTAATTCATATATTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCTTGCCCCATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
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CTGAAACTATACCTGG

WM1

CCATCAGCACCCAAAGCTGACATACTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
TTAATTCATATATTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCGTGCCCGCTGCATATAAGCATGTACATATTATGCTTG
ATCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCAT
CAACCCTTGCCCGATGTGTACCTCTACTCGCTCCGGGGCCATAACATGTGGGGGTTTCTAG
ACTGACACTATACCTGG

KE1

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
TTAATTCATATATTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
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TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
AACCCCTTGCCCGATGTGTACCTCTTCTCGCTCCGGGGCCATAACATGTGGGGGTTTCTAGA
CTGAAACTATACCTGG

WW1

CCATCAGCACCCAAAGCTGACATTCTAACTAAACTATTCCCTGATTTCTCTCCCTATGTC
TTAATTCATATATTTAATAACATTTACTGTGCCTCCCCAGTATGTACTTTTTCCCCACCCC
TATGTACATCGTGCATTAGTGGCTTGCCCCATGCATATAAGCATGTACATATTATGCTTGA
TCTTGCATTTCGTGCACCTCACTTAGATCACGAGCTTAATCACCAGGCCTCGAGAAACCATC
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CTGAAACTATACCTGG

CYTOCHROME B**KY1**

GGCAGAACGTAACCTATGAATGCTGTTGCTATAACTGCGAATAATAGGATGATGCCAATGT
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ACGTCTCGGCAAATGTGGGTGACTGATGAGAAGGCTGTGGCTGTGTCTGATGTGTAGTGTA
TGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATCCCAAGGAGGGAGCCGAA
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KY2

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GTGTATGGCTAGAAATAAACCTGTAAGATTCTGTAGGATTAGGCAGATTCCAAGGAGGGAG
CCGAAATTTTCATCATGCGGAAATGTTTGATGGCAGCAGCTAAGTCGATGAATGAATTGTTG
ATGA

KY3

GGCAGAACGTAACCTATGTAATGCTGTTGCTATAACCTGCGAATAATAGGATGATGCCAAT
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GTGTATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAG
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ATGA

KY4

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TGTATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGC
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GA

CE1

GGCAGAACGTAACCTATGTAATGCTGTTGCTATAACTGCGAATAATAGGATGATGCCAATG
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GGCAGATGAAGAATATGGAAGCCCCGTTGGCATGTATATATCGGATAATCCAGCCGTAGTT
GACGTCTCGGCAAATGTGGGTGACATGATGAGAAGGCTGTGGCTGTGTCTGATGTGGTAGT
GTATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCC
GAAATTTTCATCATGCGGAAATGTTTGATGGCAGCAGGTAAGTCGATGAATGAATTGTTGAT
GA

CE2

GGCAGAACGTAACCTCTATGTAATGCTGTTGCTATAACCTGCGAATAATAGGATGATGCCAA
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ACAGGCACGATGAAGAATATGGAAGCCCCGTTGGCATGTATATATCGGATAATCCAGCCGT
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GTGTATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATATAGGCAGATTCCAAGGAGGGA
GCCGAAATTTTCATCATGCGGAAATGTTTGATGGCAGCAGGTAAGTCGATTAATGAATTGTT
GATGA

CE6

GGCAGAACGTAACCTATGAATGCTGTTGCTATAACTGCGAATAATAGGATGATGCCAATGT
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GCAGATGAAGAATATGGAAGCCCCGTTGGCATGTATATATCGGATAATCCAGCCGTAGTTG
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TGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCCGAA
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CE7

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TGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCCGAA
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CE8

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G1

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GATGA

G2

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TGACGTCTCGGCAAATGTGGGTGACTGATGAGAAGGCTGTGTCTTTGTCTGCATGTGTAGT
GTATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCC
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A

G3

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TGATGA

G4

GGGAGAACGTAACCTATGAATGCTGTTCTTTAACTGCGAACAATAGGATGATGCCAATGT
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GACGTCTCGGCAAATGTGGGTGACATGATGAGAAGGCTGTGGCTGTGTCTCGATGTGTAGT
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A

G5

GACACAGCGTAACATTTCTTCCAAGTCCGTGTAACCTTCAAATATTAGTATGATGCCGAGGT
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GACGTCTCGGCAAATGTGGGTGACTGATGAGAAGGCTGTGGCTGTGTCTCATGTGTAGTGT
ATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCCGA
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G6

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TGACGTCTCGGCAAATGTGGATTACATGCTGAGAAGGCTGTGCGCTGTGTCTGATGTGTAG
TGTACTGGCTAGAAATAAACCTGTAAGAATCTGTAGGATATAGGCAGATTCCAAGGAGGGA
GCCGAAATTTTCATCATGCGGAAATGTTTGATGGCAGCAGGTAAGTCGATGAATGAATTGTT
GATGA

M1

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TTGACGTCTCGGCAAATGTGGGTGACTGATGAGAAGGCTGTGGCTGTGTCTCGATGTGTAG
TGCATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGC
CGAAATTTTCATCATGCGGAAATGTTTGATGGCAGCAGGTAAGTCGATGAATGAATTGTTGA
TGA

OY1

GGCAGAACGTAACCTATGAATGCTGTTGCTATAACTGCGAATAATAGGATGATGCCAATGT
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GCAGATGAAGAATATGGAAGCCCCGTTGGCATGTATATATCGGATAATCCAGCCGTAGTTG
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TGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCCGAA
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OY2

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TGACGTCTCGGCATATGTGGGTGACTGATGAGAAGGCTGTGGCTGTGCATCTGATGTGTAGT
GCATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCC
GAAATTTTCATCATGCGGAAATGTTTGATGGAGCAGGTAAGTCGATGAATGAATTGTTGATG
A

WM1

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TGACGTCTCGGCAAATGTGGGTGACATGATGAGAAGGCTGTGGCTGTGCCTGATGTGTAGT
GTATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCC
GAAATTTTCATCATGCGGAAATGTTTGATGGAGCAGGTAAGTCGATGAATGAATTGTTGATG
A

KE1

GGCAGAACGTAACCTATGAATGCTGTTGCTATAACTGCGAATAATAGGATGATGCCAATGT
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GCAGATGAAGAATATGGAAGCCCCGTTGGCATGTATATATCGGATAATCCAGCCGTAGTTG
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TGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGCCGAA
ATTTTCATCATGCGGAAATGTTTGATGGAGCAGGTAAGTCGATGAATGAATTGTTGATGA

WW1

GGCAGAACGTAACCTATGTAATGCTGTTGCTATAACCTGCGAATAATAGGATGATGCCAAT
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CAGGCAGATGAAGAATATGGAAGCCCCGTTGGCATGTATATATCGGATAATCCAGCCGTAG
TTGACGTCTCGGCAAATGTGGGTGACTGATGAGAAGGCTGTGGCTGTTGTCTGATGTGTAG
TGTATGGCTAGAAATAAACCTGTAAGAATCTGTAGGATTAGGCAGATTCCAAGGAGGGAGC
CGAAATTTTCATCATGCGGAAATGTTTGATGGCAGCAGGTAAGTCGATGAATGAATTGTTGA
TGA

APPENDIX 9. The number of times each primer set had to be typed for samples K1-K28. After 5 rounds of PCR with primers MA2, GG7 and GG454 new primers were purchased and resuspended. Reagents were also checked for viability. A MgCl₂ curve (1-6mM) and annealing temperature gradient run. Eventually a hotstart PCR and new PCR machine was used for PCR 8 and PCR 9. NP indicates no product was observed.

	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8
Sample	MA1	MA2	MA18	MA19	GG7	GG14	Lut604	Ggu454
-VE	NP	NP	NP	NP	NP	NP	NP	NP
K1	2	NP	2	3	NP	6	1	9
K2	2	NP	1	3	8	6	1	9
K3	4	NP	1	3	8	1	1	9
K4	1	9	1	2	8	6	1	9
K5	1	9	1	3	8	1	1	9
K6	4	9	1	3	8	1	1	9
K7	4	9	1	3	8	1	1	9
K8	4	NP	1	3	8	1	1	9
K9	4	NP	1	6	8	1	1	9
K10	4	NP	1	2	8	1	1	9
K11	2	8	2	1	8	1	3	9
K12	2	NP	2	1	8	1	3	9
K13	2	8	2	1	8	1	3	9
K14	2	8	2	1	8	1	3	9
K15	2	8	2	1	8	1	3	9
K16	2	8	2	1	8	1	3	9
K17	2	8	2	1	8	1	3	9
K18	2	8	2	1	8	1	3	9
K19	2	8	2	1	8	1	3	9
K20	2	8	2	1	8	1	3	9
K21	1	NP	1	1	8	1	1	9
K22	1	8	1	1	8	6	1	9
K23	1	8	1	1	8	1	1	9
K24	1	8	1	1	8	1	1	9
K25	1	8	1	1	8	1	1	9
K26	1	8	1	1	8	1	1	9
K27	1	8	1	1	8	1	1	9
K28	1	8	1	1	8	1	1	9
+VE	1	6	1	1	6	1	1	6

APPENDIX 9. The number of times each primer set had to be typed for samples WW1 – WM1. Samples that had not amplified after five replications of PCR were excluded from analysis. Only two replications were carried out for Mitochondrial primers (Mt Primers).

Sample	<u>Microsatellite Primers</u>								<u>Mt Primers</u>	
	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8	CR	Cyt <i>b</i>
	MA1	MA2	MA18	MA19	GG7	GG14	Lut604	Ggu454		
-VE	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Ww1	1	1	2	1	1	1	5	1	1	1
Ce1	3	1	1	1	1	NP	2	1	1	1
Ce2	NP	NP	NP	1	1	1	2	1	NP	NP
Ce3	1	1	2	NP	1	1	2	2	NP	NP
Ce4	1	1	5	1	1	5	1	1	NP	NP
Ce5	1	1	1	1	1	1	NP	1	1	1
Ce6	1	1	1	1	1	1	1	1	1	1
Ce7	1	1	1	1	1	1	1	1	1	1
Ce8	1	2	1	1	1	1	2	1	1	1
Ky1	3	1	2	1	1	1	5	2	1	1
ky2	3	1	2	1	1	1	5	2	1	1
Ky3	3	1	3	1	1	1	5	2	2	2
Ky4	3	1	2	1	1	1	5	2	2	1
Nb1	1	1	1	1	1	1	1	1	1	NP
Nb2	1	1	2	1	1	1	2	1	1	1
G1	1	1	2	1	1	1	2	1	1	2
G2	1	1	2	1	1	1	2	1	2	1
G3	3	1	2	1	1	1	5	2	2	1
G4	4	1	NP	1	1	3	5	3	NP	NP
G5	1	1	2	1	1	1	2	1	1	NP
G6	NP	1	3	1	1	1	NP	NP	1	NP
S1	1	1	2	1	1	1	2	1	1	1
L1	1	NP	NP	1	2	NP	NP	1	1	2
L2	3	1	2	1	NP	1	5	2	1	1
L3	3	1	2	1	1	1	5	2	2	2
Ke1	1	1	2	1	1	NP	2	1	1	1
M1	3	1	3	1	2	2	1	2	1	2
Wm1	1	1	1	1	1	1	1	1	1	1

NATIONAL PARKS & WILDLIFE

Wildlife Act, 1976 (as amended) - Sections 22, 23, 32 and 34

LICENCE TO CAPTURE ALIVE WILD BIRDS OR WILD MAMMALS FOR
EDUCATIONAL SCIENTIFIC OR OTHER PURPOSES

The Minister for the Environment and Local Government in exercise of the powers conferred on him by Sections 9, 22, 23, 32 and 34 of the Wildlife Act, 1976, (as amended) authorises:

Áine Lynch, Wildlife Ecology Group, Department of Zoology, University of Dublin, Trinity College, Dublin 2.

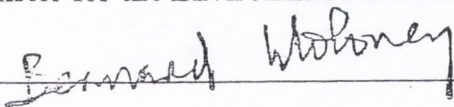
to capture specimens of the species of wild bird or wild mammal specified in Column 1 of the Schedule hereunder in the area specified in Column 2 by the means specified in Column 3 for the purposes of **research** for the period beginning on date of this licence and ending on the 30th of September, 2003, subject to the conditions listed below:

SCHEDULE

1	2	3
Species	Area(s)	Means of capture
Pine Marten (<i>martes martes</i>)	Killarney National Park	Specifically designed traps for live capture

Dated this 25th day of February, 2003.

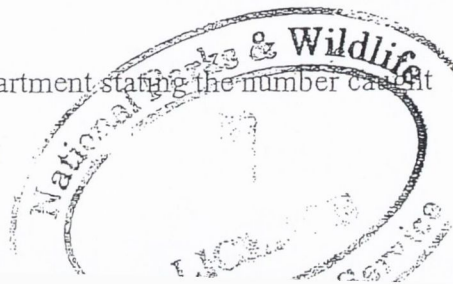
For the Minister for the Environment and Local Government



CONDITIONS

1. This licence shall be produced for inspection on a request being made on that behalf by a member of the Garda Síochána or any person appointed by the Minister for the Environment and Local Government under Section 72 of the Wildlife Act, 1976, to be an authorised person for the purposes of that Act.
2. Any traps, snares or nets used shall not be placed or operated in any place or in such a manner so as to endanger farm or domestic animals or other species for which it is not intended and shall be visited at least once every twenty four hours and be removed at the end of the period specified in the licence.
3. On expiry of this licence, a return should be sent to this Department stating the number caught and/or killed.

NOTE: This licence does not confer right of entry on any land



NATIONAL PARKS & WILDLIFE

Wildlife Act, 1976 (as amended) - Sections 23, 32 and 34

LICENCE TO CAPTURE ALIVE WILD BIRDS OR WILD MAMMALS FOR EDUCATIONAL SCIENTIFIC OR OTHER PURPOSES

The Minister for the Environment, Heritage and Local Government in exercise of the powers conferred on him by Sections 9, 23, 32 and 34 of the Wildlife Act, 1976 (as amended), authorises:

Aine Lynch, Wildlife Ecology Group, Zoology Department, Trinity College, Dublin 2.

to disturb specimens of the species of wild bird or wild mammal specified in Column 1 of the Schedule hereunder in the area specified in Column 2 by the means specified in Column 3 for the purposes of **conservation** during the period beginning on the date of this licence and ending on the **31st December 2006**, subject to the conditions listed below:

SCHEDULE

1	2	3
Species	Area(s)	Means of capture
Pine Marten (<i>Martes martes</i>)	Killarney National Park	Specifically designed traps for live capture

Dated this 19th day of December, 2003.

For the Minister for the Environment, Heritage and Local Government

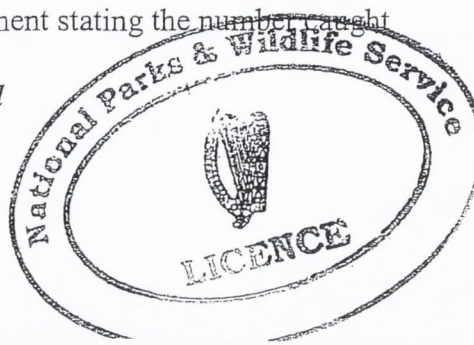
CONDITIONS

This licence shall be produced for inspection on a request being made on that behalf by a member of the Garda Síochána or any person appointed by the Minister for the Environment, Heritage and Local Government under Section 72 of the Wildlife Act, 1976 (as amended), to be an authorised person for the purposes of that Act.

Any traps, snares or nets used shall not be placed or operated in any place or in such a manner so as to endanger farm or domestic animals or other species for which it is not intended and shall be visited at least once every twenty four hours and be removed at the end of the period specified in the licence.

On expiry of this licence, a return should be sent to this Department stating the number caught and/or killed.

NOTE: This licence does not confer right of entry on any land



APPENDIX 11. Reagents and consumables used in this study.

Reagent/Consumable	Concentration/Details	Company	Catalogue #	Storage
DNeasy Tissue Kit	Proteinase K, Buffers AL, ATL, AW1, AW2 & AE	Qiagen	69504	Room temp
Chelex-100 Resin	100g	Bio-Rad	143-2832	Room temp
Proteinase K	20 mg/ml	Invitrogen	25539-049	-20°C
dNTPS	25 µmol	Invitrogen	10297-018	-20°C
Taq Polymerase	5U/µl	Invitrogen	10342-020	-20°C
PCR Buffer	10 X	Invitrogen	10342-020	-20°C
MgCl ₂	50mM	Invitrogen	10342-202	-20°C
HotStar Taq PCR Mix	1000U	Qiagen	203445	-20°C
Spreadex Gels S-2 x 25	50 wells, optimum separation range 65-250 bp	Elchrom Scientific	P/N 34 43	+ 4°C
Spreadex Gels S-2x 13	26 wells, optimum separation range 65-250 bp	Elchrom Scientific	P/N 34 27	+ 4°C
Agrose (Routine Use)	100g	Sigma	A9539	Room temp
M3 Marker	50 µl	Elchrom Scientific	P/N 32 03	-20°C
10bp Ladder	1 µg/µl	Invitrogen	10821-015	-20°C
Loading Buffer	5 X concentrate	Elchrom Scientific	P/N 30 33	-20°C
Sybr Gold Stain	10, 0000 X	Invitrogen	S11494	-20°C
Ethidium Bromide	0.625 mg/ml	Mercury	5450	Room temp
Tris Borate EDTA (TBE)	5 X concentrate	Sigma	T-6400	Room temp
TAE Running Buffer	1.2 M (50ml)	Elchrom Scientific	P/N 30 31	Room temp

APPENDIX 12. List of websites on which or from which programs for estimating genetic statistics were used in this study

FROM CHAPTER 6.

GENEPOP: <http://wbiomed.curtin.edu.au/genepop>

(P_n) Chakraborty *et al.* (1992) $P_n = H_{exp} - H_{obs} / H_{exp} + H_{obs}$

[where H_{exp} is the expected heterozygosity and H_{obs} is the observed heterozygosity]

ML-NULLE ALLELE ESTIMATOR: www.montana.edu/kalinowski

MSA (Microsatellite Analyzer): <http://il22server.vu-wien.ac.at/MSA>

WHICHRUN: www-bml.ucdavis.edu/whichrun.htm