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The vagility of an invasive species, the grey squirrel (*Sciurus carolinensis*) - studies of population genetic structure, control and recolonisation, range expansion and pox virus.

By

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Department of Zoology
2011

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Micheal McGoldrick

Micheal S. McGoldrick

For: Jane, Ciara, Sinéad

James and Ella.

With love,

Michael

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Summary

About a dozen grey squirrels were introduced to Castleforbes, Co. Longford in 1911 and have since expanded their range, almost exclusively to counties east of the Shannon, at a mean rate of 1.94km/year.

The molecular ecology of the grey squirrel in Ireland was studied by analysing 5 microsatellites in 7 populations throughout its range. Overall mean observed heterozygosities and mean expected heterozygosities were 0.63 and 0.61 respectively. No evidence of inbreeding was detected in any population. Differences in allele frequencies over all seven populations were significant ($P<0.001$). Overall differentiation between the seven study sites was moderate ($F_{ST} = 0.10$). F_{ST} values were low between the adjacent sites of Clongowes Wood, Mountarmstrong and the K Club (pairwise F_{ST} 0.02-0.03). Isolation by distance was significant over all 7 populations (Mantel test, $r = 0.79$, $P<0.001$). There was a sharp discontinuity in allele frequencies across the River Boyne between Castle Leslie and Beau Parc, which may indicate that the River Boyne is a barrier to dispersal in grey squirrels. The Irish grey squirrel population is not panmixic, but is significantly differentiated into subpopulations at both a local and a national scale. Significant evidence for recent population bottlenecks was found in Castle Leslie and Mount Juilet. Evidence for sex biased breeding dispersal was significant. No evidence for sex biased natal dispersal was detected.

Grey squirrels in 3 study sites surrounding Clongowes Wood were trapped and tagged in spring from 2003-2005. Three 14 day culls were conducted in Clongowes Wood in April, June and July 2003. Population recovery was monitored in Clongowes Wood in February and June of 2004-2005 by trapping. Only 1 tagged immigrant grey squirrel was detected on the study site from 2003-2005. Beech mast was generally moderate-good in 2003 and poor in 2004. 5 microsatellites were analysed for all individuals as before. A parentage analysis to detect direct dispersal was unsuccessful.

On an unrooted UPGMA tree of Nei's genetic distance for all populations in all years, the Clongowes Wood populations of 2003-2005 partitioned together 86 percent of the time, following 1000 bootstrap replicates of the allele frequencies data. Population recovery over a two year period was predicted by an individual based model, incorporating high fecundity and low mortality, and could be achieved with or without immigration. Overall, the data suggest that the Clongowes Wood populations of 2004 and 2005 are derived largely from survivors of the cull in 2003 and not from immigrants from surrounding study sites.

A distribution survey of grey squirrels was conducted by contacting interested parties - conservation rangers, foresters, gun club members etc.- mainly by phone, and a questionnaire completed to determine range expansion in the Republic of Ireland since 1995. 233 responses were obtained. Only first hand or second hand information was used. Physical evidence of grey squirrels presence (trapped, shot, roadkill or in hairtubes) was sought. Since 1995, grey squirrels have expanded into Wicklow, Wexford, south Tipperary, north Waterford, east Donegal with unconfirmed reports in north Cork, east Limerick and north Roscommon.

Grey squirrel serum was tested for squirrel poxvirus (SQPV) for 205 individuals from 5 populations throughout its range. SQPV was absent from one population in the south. Seroprevalence ranged from 27-60% in the other 4 populations. The rate of decline in red squirrel distribution was calculated, by different methods, from 1950-1995 at 62km^2/year and from 1911-1995 at 81km^2/year. These rates of declines are comparable with Cumbria (76km^2/year) and Norfolk (92 km^2/year) where SQPV is also present. In Scotland and Italy, where SQPV is absent, rates of decline were 4.4 km^2/year and 3.6 km^2/year.

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1. Introduction

1.1 Non-native species

Non-native species are those that have been moved outside their normal geographic range via human activities. Those non-native species that have a clear ecological or economic impact are also described as invasive species, although the terms are often used interchangeably (Cassey *et al.*, 2005).

Ecologists describe biological invasions as a series of stages: (1) transport – the uptake of a species from its native range and transport to the new environment; (2) introduction – the release or escape from captivity; (3) establishment - the transition from released or escaped individuals to a self-sustaining wild population; (4) spread - the expansion of geographical range beyond the point of establishment; (5) impact - these may be ecological and/or economic impacts which vary from low to high (Williamson, 1996; Lockwood, 1999; Blackburn and Duncan, 2001b, Kolar and Lodge, 2001; Forsyth *et al.*, 2004; Lockwood *et al.*, 2005; Jeschke and Strayer, 2005).

The introduction of non-native species can be divided into three broad categories (1) deliberate introductions for aesthetic reasons, to control pests, for commerce, food, hunting or sport (2) accidental introductions, often transported with cargo and goods and (3) introductions which had a limited purpose but escaped from inadequate biological or physical containment (Levin, 1989). Many introduced species fit into several categories. Some introductions of the house sparrow (*Passer domesticus)* may have been deliberate, notably to control the canker worm in the United States, but many were for purely aesthetic reasons (Roots, 1976). The accidental introduction of the brown treesnake (*Boiga irregularis*), from New Guinea to Guam which has led to the extinction of the majority of the islands endemic birds and mammals species (Fritts and Rodda, 1998). The escape of North American mink (*Mustela vison*) from

confinement on mink farms has resulted in populations establishing in Sweden, Norway, Denmark, Iceland, Ireland, Finland and Great Britain (Long, 2003). They are now found in most counties in the U.K. and are threatening the long-term survival of the water vole (*Arvicola terrestris*) (Bonesi and Palazon, 2007).

Williamsom (1996) noted that most non-native species that have an opportunity to move to the next stage of invasion do not do so. He suggested that an average of only 10% of non-native species successfully move from one invasion stage to the next and called this pattern the Tens Rule. Data has been gathered which roughly fits the Tens Rule, for plants (Lonsdale, 1999; Williamson and Fitter, 1996) and invertebrates (Boag and Yeates, 2001). However, Jeschke and Strayer (2005) examined data for all fish, bird and mammal introductions between North American and Europe categorised by taxon. Mean establishment success and mean spread success both exceed 50% indicating that non-native vertebrates are more likely to establish and spread than predicted by the Tens Rule.

Cohen and Carlton (1998) found that non-native species have been arriving and establishing at an accelerating rate, particularly in the last 35 years, in a study of aquatic organisms (plants, protists, invertebrates, vertebrates) introduced to San Franciso Bay estuary between 1850-1995. Lockwood *et al.*, (2005) attribute this acceleration to free trade and globalisation. Moulton *et al.*, (2001) also found an accelerating rate of introduction of non-native birds introduced to the six Hawaiian islands, particularly from 1900-1975, but with a dramatic drop in recent decades. Many of the recent introductions in the Cohen and Carlton (1998) study were accidental introductions of smaller organisms while, in Hawaii, most of the bird introductions were deliberate. This suggests that a decrease in the number of deliberate introductions of larger vertebrate species has occurred in recent decades due to increased awareness of the problems of invasive species.

One of the central questions in invasion biology involves why some introductions succeed and others fail. Several geographic patterns in the numbers of non-native species have been documented in an attempt to answer this question. For example, islands were thought to be more invasible than mainlands, as they tend to be more isolated environments with a lower diversity of native species and it has been documented that there are comparatively more non-native species found on islands than mainlands (Elton, 1958). Similarly, the New World (Americas) was thought to be more invasible than the Old (Europe) (Elton, 1958). For Hawaii, New Zealand, Australia and North America there is a strong negative relationship between percent of fauna that is comprised of established exotics and the number of native species (Brown, 1989). In contrast to this finding, Blackburn and Duncan (2001b) found no relationship between the number of established non-native birds globally and island/mainland status when the number of attempted introductions was controlled for. Introductions tended to be concentrated in relatively few, predominantly temperate and island, locations. Similarly, Jeschke and Strayer (2005) found no difference in invasion success between North America and Europe (New World and Old) and noted that the characteristics of the species and locations chosen for introductions were not representative of species and locations in general. This fact limits the ability to draw general conclusions from historical records, and generates problems of confounding and non-independence in statistical analyses of introduction success (Lockwood *et al.*, 2007).

There is an increasing emphasis on the importance of introduction effort (propagule pressure) i.e. the number of individuals released (propagule size) and the number of release events (propagule number) on establishment success (Kolar and Lodge, 2001; Lockwood *et al.*, 2005). The probability of successful establishment of exotic birds in New Zealand was strongly influenced by the number of individuals released during the main period of introductions. 83% of species that had more than 100 individuals released within a 10-year period became

established, compared with 21% of species that had less than 100 birds released (Green, 1997). Similar findings have been noted for birds in Australia (Duncan *et al.*, 2001), and globally (Blackburn & Duncan 2001a; Cassey *et al.*, 2004a), for ungulates introduced into New Zealand (Forsyth & Duncan, 2001) and for mammals in Australia (Forsyth *et al.*, 2004). Relatively few individuals were required to establish wild populations of ungulates in Victoria, Australia. All but one release of seven or more individuals succeeded, whereas releases of four or fewer individuals always failed. A similar threshold (six individuals) was found for New Zealand ungulate introductions implying that escape or release of just a few of these animals has a high risk of establishing a population (Forsyth *et al.*, 2004). Threshold sizes for successful establishment differ among taxa. Birds, for example, have a higher threshold than ungulates, requiring the release or escape of considerably more individuals to achieve a comparable establishment rate (Green, 1997; Forsyth & Duncan 2001; Forsyth *et al.*, 2004).

In Australia, several of the factors identified as explaining variation in the establishment of mammal species also explained variation in the establishment of bird species introduced to the same region (Duncan *et al.*, 2001; Forsyth *et al.*, 2004). For both mammals and birds in Australia, species were more likely to become established if they had a greater extent of climatically matched habitat in Australia, a larger overseas range size and if they had been introduced successfully elsewhere. Migration also significantly explained establishment outcomes for mammals in Australia when phylogenetic relatedness was controlled for. Species that were non-migratory in their native range were more likely to become established than were migratory species (Forsyth *et al.*, 2004). Migratory status failed to significantly explain establishment outcomes for exotic birds introduced into Australia (Duncan *et al.*, 2001) and exotic ungulates introduced into New Zealand (Forsyth and Duncan 2001), however it was significant for exotic birds introduced into New Zealand (Veltman *et al.*, 1996); and worldwide (Cassey, *et al.*, 2004a).

Several further correlates of establishment success have been identified, although these alone cannot identify the mechanisms underlying the establishment process (Martin and Fitzgerald, 2005). Disturbance was positively correlated with the likelihood of non-native fish establishment in California, particularly urban development, aqueducts, water diversions and agriculture. However, 8 of the 45 non-native fish species in the state were negatively associated with disturbance (Marchetti *et al.*, 2004). This supports the argument of Higgins and Richardson (1998) that the effects of disturbance on non-native species are context specific. Cassey *et al.*, (2004b) found that for parrots a sedentary lifestyle and broad diet were correlated with establishment success. Other correlations with establishment success include being associated with *Homo sapiens*, for example, the Black rat (*Rattus rattus*) and the Norway rat (*Rattus norvegicus*), having a broad diet and being vagile, for example, the coyote (*Canis latrans*) and being able to tolerate a wide range of physical conditions. The mosquito fish (*Gambusia affinis*) can survive in water from 6-35^{0}C, with extreme low oxygen concentrations and high salinities and because of its use as a biological control agent may be the most widely distributed species of freshwater fish (Ehrlich, 1989).

Cassey *et al.*, (2004a) found that the number of individuals released (propagule size) was highly correlated with almost all the other explanatory variables for establishment success considered for non-native birds worldwide. This included a positive relationship between number of individuals released and (i) large body mass, (ii) high annual fecundity (iii) large geographic range and (iv) being released on islands. This suggests that studies that have shown a correlation between these variables and establishment success may simply be reflecting the hidden effect of propagule size and not the proposed effect of the variable itself Cassey *et al.*, (2004a). Human bias in choice of release organism and location seems to drive these correlations. For example, government agencies in New Zealand and Australia have introduced several large

bodied game birds in high numbers. Species with high annual fecundity and large geographic range may be captured more easily and in greater numbers for introduction attempts. Acclimatization societies formed on island states seem to have been particularly deliberate in their efforts and thus may have released comparatively more individuals than their mainland counterparts (Lockwood *et al.*, 2007).

Biotic interactions such as competition, predation and mutualism may resist or facilitate establishment and spread. Predation by the native red rock crab (*Cancer productus*) has prevented the European green crab (*Carcinus maemas*) from establishing along the pacific coast of Oregon, USA (Hunt and Yamada, 2003). In contrast, the non-native bluegill sunfish increases the survival of the tadpoles of the non-native bullfrog (*Rana catesbeiana*), by reducing the population size of the bullfrog's predators, dragonfly nymphs, again in Oregon, USA (Adams *et al.*, 2003). There is no clear pattern showing a negative relationship between species richness and non-native species establishment, although it is suggested that this pattern may operate at small spatial scales. Hooper *et al.*, (2005) conclude that susceptibility to invasion by non-native species is strongly influenced by species composition and, under similar environmental conditions, generally decreases with increasing species richness. However, several other factors, such as propagule pressure, disturbance regime, and resource availability also strongly influence invasion success and often override effects of species richness in comparisons across different sites or ecosystems.

Once established, a population must grow and disperse in order to spread. Caswell *et al.*, (2003) used sensitivity analysis to examine the relative importance of population growth and dispersal in the spread of three bird species, pied flycatcher, *Ficedula hypoleuca*, starling *Sturnus vulgaris* and sparrowhawk *Accipiter nisus*. Although dispersal was more important for the pied flycatcher and the starling, population growth was critical for all three. With dispersal, the most important factor is long distance dispersal (Shigesada and Kawasaki, 1997). Caswell *et al.*,

(2003) also found that the 10% of individuals who moved the furthest had the largest single effect on the spread of the three bird species, regardless of the relative importance of population growth or dispersal.

Factors that explain the variation in the establishment of non-native species may also explain their spread. For both mammal and bird species introduced to Australia, established species that have spread tend to have a greater extent of closely climatically matched habitat in Australia, to have a larger overseas range size, and to be species of small body mass with fast development times that produce more broods per season (for birds) or more offspring per year (for mammals) (Duncan et *al.*, 2001; Forsyth *et al.*, 2004).

Many mathematical models have been developed to describe the geographical spread of non-native species and predict future spread (for example, grey squirrels in Europe; Bertolino *et al.*, 2008). In developing models, the life history or ecological conditions that are most important in allowing rapid range expansion may be identified and the likely impact of control efforts assessed. Rushton *et al.*, (2002) modelled the impacts and cost of two types of grey squirrel control, removal by trapping and immunocontraception, on the viability of red squirrel populations. Removal of by trapping was predicted to lead to red squirrel population persistence whereas immunocontraception was unlikely to be effective. Models that contrast the effect of removing either the expanding edge of large populations, or new satellite populations arising from dispersal, generally find that control of satellites is a better way to manage the spread of non-native species (Shigesada and Kawasaki, 1997; Taylor and Hastings, 2004; Bertolino *et al.*, 2008).

With the spread of a non-native species come potential large-scale ecological and economic impacts. Invasive species are responsible for most extinction where the direct cause of species extinction is identifiable. The single biggest vertebrate extinction event in recent history is the probable loss of at least 200 of the 300 endemic cichlid

species of fish in Lake Victoria as a result of the deliberate introduction for food of the Nile perch, (*Lates niloticus*) in the mid 1950's (Lowe-McConnell, 1993). Invasive mammals are responsible for all but one of the nine known extinctions of endemic vertebrate species or sub-species from the islands of northwest Mexico, and virtually all of the avian extinctions on Pacific islands have been due to invasive mammals (including *Homo sapiens*). Globally, almost 20% of vertebrates thought to be in danger of extinction are threatened in some way by invasive species (McNeely, 2001).

Ecological impacts extend beyond species extinction. In New Zealand streams, the presence of non-native brown trout has resulted in altered nutrients budgets and a six-fold increase in primary production (Huryn, 1998). Hybridisation between non-native mallard duck and native congeneric ducks, for example New Zealand's grey duck (*Anas supercilosa supercilosa*) and the Hawaiian duck (*Anas wyvillana*), may threaten the persistence of these endemic ducks as distinct species (Allendorf *et al.*, 2001). Invasive species may also spread disease and parasites. The Australian brush-tail possum has acted as a reservoir for the maintenance and transmission of bovine tuberculosis to domestic cattle in New Zealand and also damages native forest trees and plants (Arthur *et al.,* 2004).

Pimentel *et al.*, (2000) estimate that the annual cost of non-native species to the USA economy is $136 billion per year; of this non-native mammals account for $13 billion, non-native birds $2 billion and non-native fish $1 billion. However, economically, some people also benefit from non-native species. The brush tailed possum above was introduced to New Zealand to establish a fur trade, and with fewer competitors, predators and parasites, has reached densities 10 times that in Australia. This has proved to be a benefit to the fur industry, albeit at a much greater cost to the New Zealand economy (McNeely, 2001).

1.2 The grey squirrel as an invasive species

Squirrel species have been introduced worldwide several times and have often proven to be invasive (Long, 2003). In Europe, apart from the grey squirrel, three other non-native species are already established with many populations: Pallas's squirrel (*Callosciurus erythraeus*), Finlayson's squirrel *(Callosciurus finlaysonii)* and the Siberian chipmunk *(Eutamias sibiricus)*, all from Asia. In Italy, for example, there are a total of eight populations of the above three non-native species, while in France there are ten populations of *E. sibiricus* together with a population of *C. erythraeus*. These and other populations come from deliberate release of animals or from escapes from pet shops or private owners (Bertolino, 2008). In Argentina, the Asiatic red-bellied beautiful squirrel, *Callosciurus erythraeus*, is the first known introduction of a squirrel into South America (Guichon and Doncaster, 2008).

Elton (1958) expected competitive interactions to act as a strong defence against invasion by non-native species. However, in Ireland, Britain and Italy, the grey squirrel has successfully established and spread despite the presence of a congeneric native species, the red squirrel, *Sciurus vulgaris*, occupying a similar ecological niche. Both grey and red squirrels inhabit a similar range of broadleaved, mixed and conifer woodland, with grey squirrels avoiding spruce dominated monoculture. Both are granivore-herbivores, tree seeds are the primary food for both species, and both scatterhoard trees seeds in autumn and recover them in late winter/sping when food is scarcer (Moller, 1983). Both have similar home range/core area sizes and are diurnal with similar activity patterns (Gurnell, 1987; 1990). Females of both species breed once or twice per season and on average have about 3 young per litter (Gurnell, 1990). Adult survival (~70% (Gurnell, 1983; 1987)) and juvenile survival (~15-25% (Gurnell, 1983; 1987)) are similar in both species and are positively correlated with food availability (Gurnell, 1983; Gurnell, 1996). There are two main differences between the species besides coat colour. Adult greys are somewhat heavier than adult reds (adult grey: 450-710g

(Koprowski, 1994); adult red 250-400g (Gurnell, 1990). Also, typical population densities are considerably higher in grey squirrels (6 per hectare in broadleaved woodland (Gurnell, 1987; 1996)) than red squirrels (<1 per hectare in broadleaved/conifer woodland (Gurnell, 1987), with red squirrels maintaining greater inter-individual distances than grey squirrels (Wauters and Dhondt, 1992). The grey squirrel has slowly replaced the red squirrel in Scotland and Italy, in the absence of squirrel pox virus (SQPV), through some form of feeding competition, indicating that the two species share a similar niche and competitive exclusion (Gurnell *et al.,* 2004a) is occurring. Therefore, in this example, the presence of a congeneric native species in a very similar ecological niche to that required by the invasive species, did not provide the biotic resistance necessary to prevent establishment and spread.

Grey squirrels became established in Ireland following a single introduction of just 12 individuals to one location, Castleforbes, Co. Longford (Watt, 1923). As noted above, introduction success is positively related to introduction effort. In Britain, grey squirrels were released on 31 occasions and in 24 cases the population increased in size; in 11 of these cases, less than ten individuals were released (Laidler, 1980). In Italy, three out of four introductions with 4–6 animals were successful (Bertolino and Genovesi, 2003). Therefore, as with other examples of invasive mammals, the release of a relatively small number of individuals can have high probability of successfully establishing a population.

Grey spread population growth and spread in Ireland has been similarly successful. Watt (1923) noted that in Castleforbes estate in 1919, 8 years after their introduction, 'over three hundred were killed in the course of the year'. The rate of early dispersal given by Watt (10 miles in 12 years or 1.33 km/year) is consistent with later data given by O'Teangana *et al.*, (2000) who calculated a mean dispersal rate for grey squirrels in Ireland from 1911-1997 of 1.94 km/year and a two dimensional diffusion coefficient of 1.15 km^2/yr. In England, the pattern

of grey squirrel spread in England has been described as random dispersal, with occasional major advances. The recorded mean rate of spread was 7.7 km/yr, while the two-dimensional diffusion coefficient was 17.9 km^2/yr (Williamson and Brown, 1986). As grey squirrels were successfully introduced to many locations in Britain their mean rate of spread is greater than in Ireland. Comparable data are not available for Italy, but estimations from range size and number of years present gives a range increase of 17.2 km^2/yr (Bertolino and Genovesi, 2003).

The characteristics that predicted successful bird and mammal establishment and spread in Australia (Duncan et *al.*, 2001; Forsyth *et al.*, 2004) are found in the successful establishment and spread of the grey squirrel in Ireland. Grey squirrels occupy a large native range (eastern and midwestern United States and to the southerly portions of the eastern provinces of Canada) (Gurnell, 1987). Closely climatically matched habitat i.e broadleaved and mixed woodland in a temperate climate is found in Ireland (NPWS/EHS 2008). Grey squirrels have small body mass (less than 710g), with fast development times (sexual maturity at about 12 months), can produce many offspring per year (may breed twice in one season producing on average 3 young per litter) and are non-migratory (Gurnell, 1990; Koprowski, 1994). In addition, grey squirrels have a broad diet within woodlands (Moller, 1983; Gurnell, 1983), which Cassey *et al.*, (2004b) found to predict establishment success in parrots worldwide.

Similar to other invasive species, grey squirrels have significant ecological and economic impacts. The grey squirrel is replacing the red squirrel in Ireland (O'Teangana *et al.*, 2000), Britain (Gurnell and Pepper, 1993) and Italy (Bertolino and Genovesi, 2003). This replacement appears to occur in two ways. Firstly, through a slow process of competitive exclusion (Gurnell *et al.*, 2004a), arising from some type of feeding competition. Secondly, grey squirrels carry a pathogen, squirrel poxvirus, which they are immune to but which is lethal to red squirrels (Sainsbury *et al.*, 2000) and which leads to a much

more rapid replacement of reds by greys (Rushton *et al.*, 2006). They cause economic damage to forestry by stripping the bark of broadleaved trees and chewing the underlying vascular tissue. Although generally fewer than 5% of damaged trees die, many more have degraded timber value through rot and broken tops (Pepper and Currie, 1998). Typical of other invasive species, predation has little impact on population dynamics (Gurnell, 1990). Control is practised by warfarin poisoning, trapping and shooting to reduce damage to trees locally but little long-term impact on population sizes (Pepper and Currie, 1998).

1.3 Report outline

Once established, the vagility of a non-native species is critical to the rate and extend of its spread, and consequently to its impact. Vagility can be defined as 'the inherent power of movement possessed by individuals' (Allaby, 1994). Movement takes different forms (Dingle, 1996), for example dispersal, migration, exploration etc., and has both distance and frequency components (Carr and Fahrig, 2001). This report will focus on the vagility of the grey squirrel, with an emphasis on dispersal movements and gene flow, through studies of population genetic structure (chapter 2), control (chapter 3) and range expansion (chapter 4). Finally, it deals with the effects of this vagility and the presence of a lethal pathogen, squirrel pox virus, on the rate of decline in red squirrel distribution in Ireland (Chapter 5).

There has been virtually no data published describing the molecular ecology, population genetic structure, genetic diversity etc. of non-native populations of grey squirrels. Most studies have concentrated on the genetic diversity of declining red squirrel populations and how this might affect their long-term persistence (Wauters *et al.*, 1994; Barratt *et al.*, 1999). An analysis of the molecular ecology of the Irish grey squirrel population (chapter 2) will enable questions concerning non-native population establishment, population structure, gene flow and sex-biased dispersal to be explored.

Control of invasive species is difficult and rarely successful. A bounty scheme to control grey squirrels in Britain in the 1950's, made no significant impact on grey squirrel populations and was considered ineffective (Sheail, 1999). In individual woodlands grey squirrel population recovery occurred in 6-10 weeks following control by trapping (Lawton, 1999). The raises a key question for the control of invasive species. Does control fail because the control effort is insufficient or because dispersal of individuals from adjacent populations into the control area (the vacuum effect) negates the effect of the control? Molecular genetic data will be used to resolve this question by identifying individuals found in the study area following control as being either residents of the study area or immigrants from surrounding populations.

Reilly (1997) mapped the grey squirrel invasion of the Republic of Ireland by means of a questionnaire based distribution survey. This distribution map requires updating to determine the extent of recent grey squirrel range expansion. This is for two reasons. First, to determine if grey squirrels have encroached on important red squirrel populations, particularly west of the River Shannon, and second to alert foresters to those areas where grey squirrels pose a new threat to broadleaved forestry (chapter 4).

Neither grey nor red squirrels in the Republic of Ireland have been tested for squirrel pox virus (SPV), nor has SPV disease been reported clinically for either species. Yet the transmission of SPV from grey squirrels to red squirrels is now believed to be the primary cause of the rapid decline in red squirrel distribution in England and Wales, compared to the more gradual decline in red squirrel distribution in Scotland and Italy, where SPV is absent (Rushton *et al.,* 2006). SPV is present in grey squirrels in Northern Ireland. The presence or absence of SPV in the Republic of Ireland, the rate of spread of grey squirrels and the associated the rate of decline in red squirrel distribution must be determined to demonstrate the urgency of the threat to red squirrels in Ireland (chapter 5).

2. Molecular Ecology of the Irish Grey Squirrel (*Sciurus carolinensis*) Population

2.1.1 Molecular genetic analysis of non-native species

Molecular genetic analysis of non-native species can provide valuable information about the source and genetic diversity of non-native populations, the size and frequency of introductions (Kolbe *et al.*, 2004), the pattern of spread (Stone and Sunnucks, 1993), even the identification of the non-native species itself (Collins *et al.*, 2002). This information, which is important to prevent further introductions (Kolbe *et al.*, 2004) and develop effective methods of control (Robertson and Gemmell, 2004), is often difficult to observe directly in the field. It is also sometimes essential to distinguish between native species and native-invasive hybrids (Spruell *et al.*, 2001).

The size and frequency of introductions, and the source and genetic variability of a non-native species, may influence invasion success. Genetic analysis of mitochondrial DNA of brown anole (*Anolis sagrei*) in Florida reveals higher levels of genetic variation than found in native populations. It suggests that the brown anole was introduced to Florida on at least eight independent occasions from across the lizard's native range in the Caribbean Islands (Kolbe *et al.*, 2004). Recently introduced brown anole populations worldwide originate, not from the Caribbean Islands, but from Florida and some have maintained these high levels of genetic variation. In contrast, other non-native populations have established and spread despite having much lower genetic diversity than populations in their native range and despite some degree of inbreeding, for example, the common myna (*Acriotheres tristis*) introduced into New Zealand, Australia, Fiji, Hawaii and South Africa from southeast Asia (Baker and Moeed, 1987).

A broad survey of the literature on the genetic diversity of non-native

populations suggest that in many cases non-native populations loose very little genetic diversity (Wares *et al.*, 2005). Where source populations show a high level of genetic structure, and introductions are made of individuals from across this source population, then the resulting population admixture will have greater genetic diversity than the native source populations (Lockwood *et al.*, 2005). Merila *et al.*, (1996) found higher genetic diversity in introduced populations of greenfinch (*Carduelis chloris*) on New Zealand than in native populations. Baker (1992) reported that chaffinches from 8 populations in New Zealand have an average heterozygosity significantly greater than 10 native European populations. Only if a population bottleneck is very severe will significant loss of average heterozygosity occur, although rare alleles will be lost. For example, a population bottleneck of 10 individuals, with an intrinsic rate of growth near 1, is predicted to experience very little reduction in average heterozygosity (Nei *et al.*, 1975).

The pattern of genetic differentiation found across an invasive species range can provide information on its spread. Stone and Sunnucks (1993) have described the invasion of northern and western Europe by the gallwasp *Andricus quercusalicis*. Populations further from the native range show reduced allelic diversity and heterozygosity. This suggests that this species has experienced a series of bottlenecks over the last 300-400 years as it spread throughout Europe following a stepping-stone process.

Genetic analysis may be necessary to identify the non-native species with a genus. Collins *et al.*, (2002) sampled four locations in Florida and Georgia and found that these non-native populations of Asian swamp eels (genus *Monopterus*) were genetically distinct, representing two and possibly three different species, from three distinct source populations. Genetics may also allow the detection of a non-native morph that is conspecific with a native species. Genner *et al.*, (2004) detected a non-

native morph from southeast Asia of the gastropod *Melanoides tuberulata* in Lake Malawi, Africa, which is sympatric with indigenous forms of the same species. This non-native morph was not present in historical collections and appears to be spreading rapidly and replacing the indigenous form.

The loss of native cutthroat trout by hybridisation with introduced rainbow trout has been recognised for over 75 years as a major threat in western USA (Allendorf and Leary, 1998). Likewise hybridisation of bull trout and introduced brook trout is a major threat to the persistence of bull trout (Spruell *et al.*, 2001). Genetic analysis of hybrids and hybridisation is based upon diagnostic loci that are fixed or nearly fixed for different alleles in two hybridising populations (Cornuet *et al.*, 1999). Genetic analysis has been used to identify pure bull trout found in a single stream in Crater Lake National Park Oregon. These were removed, held in hatcheries and, following chemical treatment of their stream to kill all brook trout and hybrids, returned to this steam and have increased in abundance and distribution (Spruell *et al.*, 2001).

Molecular genetic techniques may be applied to questions relating to the general ecology of native and invasive species. Questions relating to population genetic structure, dispersal/gene flow, reproductive strategies, relatedness and kin selection etc. may best be answered using genetic techniques (Grapputo *et al.*, 2005; Lizarralde *et al.*, 2008; Reitzel *et al.*, 2008). For example, Fleischer *et al.*, (2008) studied the increase in common raven (*Corvus corax*) populations in Mojave Desert regions of southern California and Nevada. Patterns of movement inferred from genetic data suggested that immigration into the West Mojave Desert was from southern California and the Central Valley while immigration into the East Mojave Desert was from northern Nevada.

Understanding the molecular ecology of introduced species may not be necessary for their management and control. A policy of 'Shoot first, ask

questions later' is in agreement with basic population biology. The best way to reduce the probability that a non-native species becomes invasive is to eliminate it before it becomes abundant and widespread (Simberloff, 2001). However, once established, genetics can be used to identify isolated reproductive units that are appropriate units for eradication on the basis of patterns of genetic divergence (Calmet *et al.*, 2001; Robertson and Gemmell, 2004). Eradication of invasive species, where possible, is a valuable tactic in restoration (Myers *et al.*, 2000). Successful eradication requires a low risk of recolonisation as found in isolated island populations or populations limited to isolated 'habitat islands'. However, other islands or regions that have no barriers between populations are more problematic. The eradication of a portion of a population, or a sink population from within an unidentified source-sink dynamic, results in rapid recolonisation and a waste of resources (Myers *et al.*, 2000). Robertson and Gemmell (2004) examined two populations of brown rats separated by a glacier on South Georgia and showed there was limited or negligible gene flow between the populations. The eradication of rats from the entire island is a daunting task because of its size. However discrete populations could be considered as eradication units and eradication proceed sequentially with low risk of natural recolonisation (Robertson and Gemmell, 2004).

2.1.2 Hypotheses

12 grey squirrels were introduced into Ireland in one introduction event from Woburn Abbey, UK (Watt, 1923). The population at Woburn Abbey, UK was derived from 10 individuals introduced from the USA in 1890 (Shorten, 1954). It was hypothesised that the genetic diversity of the Irish grey squirrel population would be less than that of grey squirrels in the U.K and less also than native squirrel populations worldwide. It was also hypothesised that inbreeding would be evident in the Irish grey squirrel population given the small number of initial founders. The importance of genetic diversity and inbreeding to the successful

establishment and spread of invasive species will be discussed in relation to the findings.

Grey squirrels are highly dependent on woodland habitat and populations may be partially isolated by physical barriers (rivers or urban areas) or deforested areas with few corridors for dispersal. While the grey squirrel is a vagile animal, data in the literature on dispersal distance and frequency is somewhat contradictory. For example, Taylor *et al.*, (1971) suggest that dispersal is usually limited to about 1.5km or less, with occasional longer distances (5km) moved, while Lawton and Rochford (2007) suggest that movements over longer distances (>2km) can counteract the effects of control within 6-10 weeks. It was hypothesised that population genetic structure would be significant at a national scale (at distances greater than 70km) but at a local scale (at distances less than 3.5km) populations would be effectively panmixic.

Isolation by distance (IBD), a correlation between genetic differentiation and geographical distance (Wright 1969), has been invoked to suggest that geographical distance alone could explain genetic structure. Slatkin (1993) theoretically showed that, in a species expanding its range to a new habitat, IBD should be low at first and attained at short geographical distances, then increase progressively over larger geographical distances when sufficient time has elapsed for patterns to be established and stabilized. However, IBD may also depend on the scale of analysis and may not account for the effect of physical barriers, for example rivers. So, depending on the timescale covered by the genetic marker, the impact of recent and historical events, the scale of analysis and the presence of potential barriers, different outcomes can be expected (Trizio *et al.*, 2005).

Following introduction, the Irish grey squirrel population expanded rapidly (Watt, 1923) and dispersing individuals established populations throughout the north, east and south in the subsequent 100 years

(Stelfox, 1927; Moffat, 1938; Henderson, 1947; Shorten, 1953, Jackson, 1961; National Parks and Wildlife Service, 1973, Crichton, 1974; Ni Lamhna, 1979; Reilly, 1997; O'Teangana *et al.*, 2000; O'Neill and Montgomery, 2003). Only the River Shannon and the Shannon callows have acted as a barrier to the spread of the grey squirrel westwards (O'Teangana *et al.*, 2000). Given this generally successful spread, it was hypothesised that the pattern of genetic diversity would be mainly determined by equilibrium between gene flow and genetic drift and that IBD would be expected to be strong and significant over local and national geographical scales. However, if recent founder effects/bottlenecks and disruption to gene flow have had an additional impact on the present genetic structure of squirrel populations, a complex distribution of genetic variability is expected and IBD will not fully explain the population structure (Trizio *et al.*, 2005).

Clobert *et al.*, (2001) broadly define natal dispersal as the movement of an individual between the natal area or social group and the area or social group where breeding first takes place and breeding dispersal as the movement of an individual between two successive breeding areas or social groups. This definition is useful as it requires a statement of what type of dispersal we wish to measure and what sampling strategy and statistical tools we need to measure it. Other authors describe these types of dispersal more generally as short distance dispersal and long distance dispersal, for example, Gull (1977, in Gurnell, 1987).

Depending on a species life history strategy the costs and benefits of dispersal are expected to affect the sexes differently. This may lead to a sex biased dispersal pattern. Male–biased dispersal is more common in mammals and female biased dispersal more common in birds (Greenwood, 1980) but many exceptions to this general rule exist (Lawson Handley and Perrin, 2007). Following breeding, female grey squirrels tend to occupy small core areas well separated from other females (Gurnell, 1987). Familiarity with the natal area would be

beneficial in selecting a high quality home range/core areas and would favour philopatry in females. The benefits of kin cooperation, described by Koprowski (1996), may provide further competitive advantages to residents and thereby promote philopatry. Inbreeding avoidance is also speculated to be a primary cause of sex-biased dispersal in birds and mammals (Greenwood, 1980). Dispersal lowers the risk of mating with relatives and hence inbreeding depression. Kin recognition could avoid the potential for close inbreeding and indirectly select for dispersal in the sex (males) that less crucially depends on maintaining high quality core areas and social bonds (Lehmann and Perrin, 2003). Observational data indicate that both natal and breeding dispersal are male biased in grey squirrels. It was hypothesised that genetic data would indicate likewise.

2.1.3 Objectives

Determine the level of genetic diversity within and between seven Irish populations of grey squirrels.

Test for inbreeding in Irish grey squirrel populations.

Describe the population genetic structure of the Irish grey squirrel population

Test for isolation by distance in the Irish grey squirrel population

Test for population bottlenecks in Irish grey squirrel populations.

Determine if natal dispersal and/or breeding dispersal is sex biased in grey squirrels

2.2 Methods

2.2.1 Study areas

Grey squirrel tissue samples were obtained from 7 locations in Ireland described below listed from north to south (Fig. 2.0). A vegetation survey was conducted in four woodlands: Clongowes Wood, Mountarmstrong, the K Club and Loughbollard. Line transects were marked in each woodland along straight line paths or tracks. All tree species of seed bearing age visible from the transects were identified. This data was used to estimate the number and percentage of each tree species of seed bearing age present in the woodland and is presented in Section 3.3.3 (Table 3.5a,b).

Portglenone (PG)

Portglenone is a 45 hectare (ha) woodland outside Portglenone, Co. Antrim (C 98 02). It is divided by the River Bann and consists mainly of beech (*Fagus sylvatica*), sycamore (*Acer pseudoplatanus*), ash (*Fraxinus excelsior*), and stands of Sitka spruce (*Picea sitchensis*). Ecological evidence that Portglenone has been continuously wooded with mainly native broadleaved trees comes from the species composition of the ground flora, such as wood anemone (*Anemone nemorosa*), an indicator of an ancient woodland site (The Woodland Trust, 2006).

Castle Leslie (CL)

Castle Leslie estate is located in the village of Glaslough, Co. Monaghan (H 72 41) and contains a 200 hectare woodland composed mainly of mature oak (*Quercus* sp.) and beech (*Fagus sylvatica*), interspersed with ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*). A 5 hectare stand of Norway Spruce (*Picea abies*) was present within the study site. There is little or no ground cover under the mature oak, beech or Norway

Spruce. Elsewhere there was a ground layer of nettle (*Urtica dioica*), ferns (*Dryopteris* sp.), bramble (*Rubus fruticosus*) and grass.

Beau Parc (BP)

Beau Parc estate is midway between Navan and Slane in Co. Meath. The woodland on the estate (N 94 72) forms part of a larger block of about 500 hectares of deciduous woodland, approximately 200 years old, running along the south bank of the River Boyne. The woodland is mainly mature beech (*Fagus sylvatica*) and oak (*Quercus* sp.) with some sycamore (*Acer pseudoplatanus*) and horse chestnut (*Aesculus hippocastanum*). There was a dense layer of bramble (*Rubus fruticosus*) and fern (*Dryopteris* sp.) throughout much of the woodland. Much of the woodland was present on a steep bank sweeping down to the River Boyne and was difficult to access. Traps were placed on a path running through the woodland on the top of this bank.

Clongowes Wood (CW)

Clongowes Wood is a 12 hectare woodland (N 87 29), approximately 150 years in age, surrounding Clongowes Wood College in Clane, Co. Kildare. It is a mixed woodland composed mainly of beech (*Fagus sylvatica*) but also containing oak (*Quercus* sp.), yew (*Taxus baccata*), horse chestnut (*Aesculus hippocastanum*), ash (*Fraxinus excelsior*), hazel (*Corylus avellana*) and sycamore (*Acer pseudoplatanus*). Two 1km rows of limes (*Tilia x europaea*) line the avenue to the college. In roughly half the woodland, where the canopy was open, there was a dense layer of ferns (*Dryopteris* sp.), bramble (*Rubus fruticosus*) and grass.

Mountarmstrong (MA)

Mountarmstrong is a 6 hectare woodland of mature beech (*Fagus sylvatica*), located 1.5km northwest of Clongowes Wood at their nearest

points (N 86 31). There was some Sitka spruce (*Picea sitchensis*) in the centre of the woodland and an isolated stand of 0.5 hectare of Sitka spruce at the western edge of the woodland. There were few breaks in the canopy and no ground cover. Throughout the woodland the ground was covered with a layer of old beech mast.

K Club (KC)

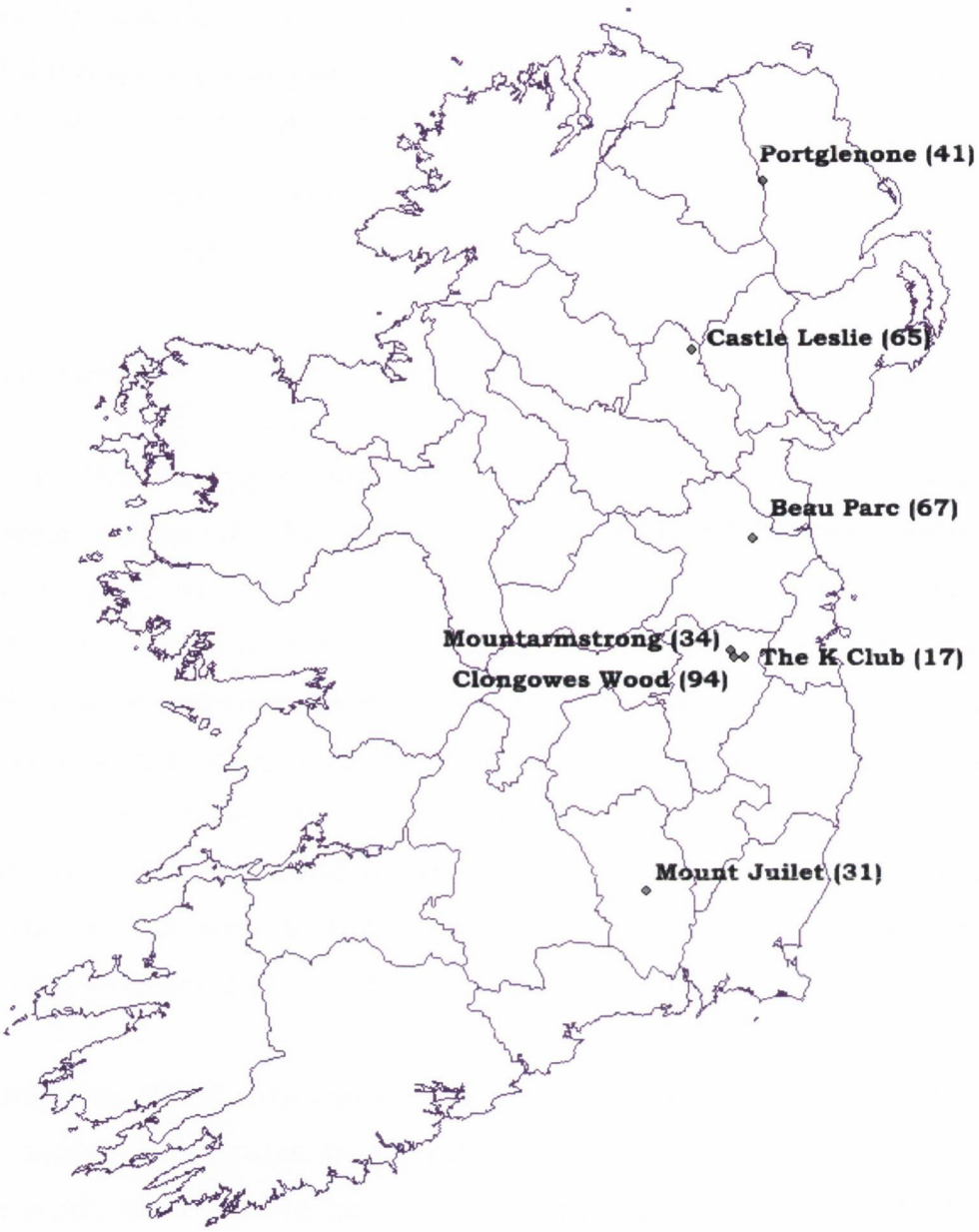
The K Club is approximately 2.5 km east of Clongowes Wood at their nearest points (N 91 29) and 4.5km east of Mountarmstrong. The woodland in the K Club extends around the perimeter of the golf courses and along the driveways. This was very mixed woodland consisting of both native species: beech (*Fagus sylvatica*), oak (*Quercus* sp.), ash (*Fraxinus excelsior*) and hazel (*Corylus avellana*); many ornamental species such as willows (*Salix* sp.) Japanese larch (*Larix kaempferi*) etc. and areas of rhododendron (*Rhododendron ponticum*). The study area, located to the west of the estate, was chosen as it consisted mainly of natural woodland of beech, oak, ash and hazel.

Clongowes Wood, Mountarmstrong and the K Club are located in north Co. Kildare, separated by farmland given over to pasture. Fields between the study sites tend to be relatively small and are bounded by hedgerows consisting mainly of hawthorn (*Crataegus* spp.) with some ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*). There were no continuous tree lines along the hedge rows or between the study sites. One secondary road separated Clongowes Wood from each of Mountarmstrong and the K Club and both secondary roads separated Mountarmstrong and the K Club.

Mount Juliet (MJ)

Mount Juliet estate is on the edge of Thomastown, Co. Kilkenny (S 55 43). It is a 700 hectare estate with approximately 100 hectares of mature

ash (*Fraxinus excelsior*), oak (*Quercus* sp.), beech (*Fagus sylvatica*) and sycamore (*Acer pseudoplatanus*). There was a dense ground layer in most of the woodland consisting of nettle (*Urtica dioica*), ferns (*Dryopteris* sp.), bramble (*Rubus fruticosus*) and grass.



2.0 Sites where grey squirrel tissue samples were obtained for the molecular genetic analysis. Number of samples obtained in brackets.

2.2.2 Trapping and Sampling Techniques

In Portglenone, during the spring of 2006, grey squirrels were shot by the local wildlife ranger, placed in sample bags and stored at -24^0 C in a freezer. They were collected from this freezer by the author and transferred within two hours to a freezer in Trinity College Dublin to prevent thawing and degradation of DNA.

In all other locations, grey squirrels were trapped in 'Albi-Trap humane live trap animal cage traps - Mink/Squirrel Trap type 007' (Albion Manufacturing, The Granary, Silfield Road, Wymondham, Norfolk, NR18 9AU, Phone:-01953 605983, Fax:-01953 606764, Email:-sales@albionmanufacturing.com, www.albionmanufacturing.com). These are live catch traps made from 2mm X 2mm galvanised weld mesh with a spring operated door activated by a foot lever. The traps are 60cm X 20cm X 20cm. A baseboard of 9mm plywood measuring 80cm X 20cm was added to the bottom of each trap and a nest box, also made from 9mm plywood, measuring 20cm X 20cm X 20cm, with a hinge door at the back, was placed on the baseboard at the end of each trap. A hole measuring 10cm X 10cm was cut in the mesh at the end of the trap and a circular hole of 10cm diameter was cut in the front of the nest box to allow the squirrel access to the nest box. After cutting the mesh, the sharpened edges were filed to prevent injury to the squirrels. The mesh cage was wrapped in black, 'bin-liner', plastic in the field to prevent the squirrels from being startled by the presence of people in their vicinity. The 'bin liner' was secured by placing branches and stones on top of the trap.

Traps were placed in a woodland following a trapping grid or line at intervals of between 25-50 meters depending on the area and shape of the woodland, the number of traps available and the ease of access into the woodland from woodland paths.

Traps were prebaited every 1-2 days for 5 days using a mixture of peanuts and yellow, whole maize (Power Seeds and Hardware, Portglorian, Kilcock, Co. Kildare. Phone: 01 6287541). This involved spreading bait in approximately a 20m arc around each trap and placing bait within the trap, with the trap door locked in the open position. On subsequent prebaiting days bait was spread in an ever decreasing arc and again within the trap. Following prebaiting, traps were then set at dawn (or as close to dawn as possible) and bait was placed on the bait tray at the back of the trap and a few grains on top of the nest box and at the entrance to the trap.

Traps were checked once or twice daily. If a squirrel was present in a trap it was approached quietly from behind so as not to startle the squirrel. A hessian sack was placed over the nestbox and the hinged door opened to allow the squirrel into the sack. The top of the sack was secured to a cylindrical mesh squirrel handling 'cone' using expandable bicycle cable tie. The squirrels was then allowed to enter the cone, the base of the cone was blocked by hand, the sack removed and the squirrel secured in the cone by inserting two pens into the mesh at base of the case and clipping the pens onto the mesh. There were no squirrel trap deaths during the course of the study.

In May 2004 in Beau Parc and in May 2005 in Castle Leslie, grey squirrels were trapped and euthanized using 2.0mls of Dolethal (sodium pentobarbitone) injected under the sternum towards the heart.

In 2003 in Clongowes Wood the first 54 grey squirrels trapped were euthanized. Thereafter, due to disturbance of traps by animal rights activists, the remaining 49 grey squirrels trapped during the 'culls' were tagged with one tag in each ear (National Tag and Band Company, tag number 1005) and released in a 30 hectare broadleaved woodland, 10km away. In Clongowes Wood in 2004 and 2005 grey squirrels were trapped, tagged and released in Clongowes Wood.

In 2003-2005 in Mountarmstrong and the K Club, and in June 2004 in Mount Juliet grey squirrels were trapped, tagged and then released.

In all cases a piece of tissue was taken from the tip of one ear using a square ear punch. This piece of tissue was then cut into smaller pieces with the ear punch and stored in a sample container containing 1.5ms of 100% ethanol. These containers were stored at 4-8^{0}C.

Prior to taking each sample, the ear punch was cleaned with a pre-injection swab containing 70% isopropyl alcohol. The swab was then held lightly in the punch and lit to flame the punch, thus destroying any trace DNA and preventing cross contamination.

Where squirrels were dead prior to taking the ear tissue sample a piece of ear tissue measuring approximately 10mm x 3mm was taken. Where grey squirrels were alive and to be released, a smaller piece of ear tissue measuring about 5mm x 2mm was taken.

Each grey squirrel was weighed to the nearest 10g using a Pesola 1000g spring balance, sex and breeding status were determined and each squirrel was given a unique identification number.

2.2.3 Microsatellite analysis

The benchtop was wiped with Industrial Methylated Spirited and covered with benchcote. A jeweller's forceps was flamed in alcohol and allowed to cool. A small piece of ear tissue (about 3-5mm x 1-2 mm) was removed from the sample container using the forceps and placed in a sterilised screw cap Eppendorf tube containing 1.5mls of 100% ethanol. These samples were stored at 4-8^{0}C until transfer to Eurofins Medigenomix Gmbh, Fraunhoferstraße 22, D-82152 Martinsried, Germany (Phone: ++49 (0)89-899892-0, Fax: -90, info@medigenomix.de) in a cool box by overnight courier (DHL), where they were again stored at 4-8^{0}C.

Slivers of frozen muscle tissue were taken to act as positive controls for the pieces of ear tissue preserved in alcohol. First, the scalpel blade was wiped clean with an alcohol swab and flamed in alcohol. Then, the hair from the lower leg muscle of a frozen squirrel was scraped away. The scalpel was used to remove the top layers of skin and muscle tissue. When 'red' muscle tissue was clearly exposed a sliver (10mm x 10mm x 1mm) was taken, placed in a sterile Eppendorf tube and restored the freezer. Before shipment, these samples were placed in a small steel thermos flask with ice cubes and stored in the freezer overnight. The thermos flask was removed from the freezer at approximately 5pm on the day of shipment, shipped in a cold box as above and refrozen on arrival in Germany at approximately 1pm the following day.

Medigenomix performed the extraction of the DNA, amplification of the target microsatellite sequences using Polymerase Chain Reaction (PCR) and identification of the size of the microsatellite allele using an Applied Biosystems DNA sequencer.

DNA was extracted using NucleoSpin® Food DNA Purification Kit (MACHEREY-NAGEL GmbH & Co. KG, Neumann Neander Str. 6-8, D-52355 Düren, Germany. Tel. +49-(0)2421-969-0, Fax. +49-(0)2421-969-199, e-mail: sales@mn-net.com) which guarantees good recovery rates even for small genomic DNA fragments (< 1 kb) from samples which may have very low DNA contents and/or well as poor quality, degraded DNA.

Microsatellite loci were amplified in 15µl reactions following Hale *et al.,* (2001a) [1µl template DNA, 1 x Taq buffer, 2.5mM $MgCl_2$, 0.2mM each dNTP, 0.33µM each primer, 0.6 U Taq as follows: 95^{0}C for 12min, 10 cycles of 94^{0}C for 15sec, annealing temperature (T_a) for 15 sec, 72 ^{0}C for 15sec, followed by 30 cycles of 89^{0}C for 15sec, annealing temperature (T_a) for 15 sec, 72 ^{0}C for 15 sec, then 72^{0}C for 10mins.

Polymerase chain reaction products were separated on an ABI Prism

3100 Genetic Analyzer (Applied Biosystems, Lingley House, 120 Birchwood Boulevard, Warrington WA3 7QH, UK. Tel 44 1925 825650. Email uk.orders@eur.appliedbiosystems.com) and visualised using Genescan size standards and Genetyper software. The ABI Prism 3100 Genetic Analyzer is a multi-color fluorescence-based DNA analysis system with 16 capillaries operating in parallel which enables efficient sample processing and offers high-quality data.

Hale *et al.,* (2001a) isolated 21 microsatellites for red squirrels and found these microsatellites amplified and were polymorphic for grey squirrels also. The 10 most polymorphic of these microsatellites described below were chosen for an initial screening.

2.2.4 Data analysis

Data were collected in successive years (2003-2005) from Clongowes Wood, Mountarmstrong and the K Club. In the analysis of the molecular ecology of the Irish grey squirrel population, only data from 2003 was used, for each of the above populations, together with data from Portglenone, Castle Leslie, Beau Parc and Mount Juliet. This gave population genetic data from seven grey squirrel populations throughout the grey squirrel's range in Ireland.

2.2.4.1 Molecular markers

10 ear tissue samples were chosen from each of the Clongowes Wood, Mount Juliet, Castle Leslie and Beau Parc populations (i.e. 40 samples in total) and the following 10 microsatellites from Hale *et al.,* (2001a) were analysed for each tissue sample: Scv 3, Scv 4, Scv 6, Scv 9, Scv 10, Scv 14, Scv 15, Scv 18, Scv 19, Scv 31.

Then, the following 312 samples were analysed using only the 6 most polymorphic microsatellites obtained, (Scv 4, Scv 6, Scv 15, Scv 18, Scv 19, Scv 31) based on the above results.

As Scv 31 was not in Hardy Weinberg equilibrium the remaining 133 samples were analysed at 5 microsatellites (Scv 4, Scv 6, Scv 15, Scv 18, Scv 19).

When testing the data for HWE and linkage disequilibrium only, data from all populations and all years was used. This additional data will be explored further in Chapter 3.

Exact tests for deviations from Hardy Weinberg equilibrium (HWE) were performed using the GENEPOP package 3.3 (Raymond and Rousset, 1995). The program performs a probability test using a Markov chain Monte Carlo simulation (Guo and Thompson, 1992) (dememorisation 1000, batches 100, iterations per batch 1000). The probability of the observed sample is used to define the rejection zone, and the *P*-value of the test corresponds to the sum of the probabilities of all tables (with the same allelic counts) with the same or lower probability (Raymond and Rousset, 1995). Significance levels were calculated per locus and per population. Sequential Bonferroni corrections (Rice, 1989) were applied.

A randomisation test in FSTAT was used to test for linkage disequilibrium (LD) across all pairs of loci in all study sites. This option allows testing the significance of association between genotypes at pairs of loci in each population. The statistic used to test the tables when testing for Linkage Disequilibrium is the log-likelihood ratio G-statistic. Genotypes at the 2 loci are associated at random a number of times (2400 for this data set) and the statistic is recalculated on the randomised data sets. The P-value is estimated as the proportion of statistics from randomised data sets that are larger or equal to the observed. Sequential Bonferroni corrections were applied (Goudet, 2001).

2.2.4.2 Genetic diversity

Several indices of genetic diversity were measured namely the number of

alleles sampled, the number of private alleles, allelic richness, observed heterozygosity (H_O), gene diversity (Nei, 1978) and F_{IS}. The number of private alleles and the observed heterozygosity were measured with GENALEX (Peakall and Smouse, 2006), the remaining indices were measured with FSTAT version 2.9.3 (Goudet, 2001).

2.2.4.3 Genetic differentiation and genetic distance

A randomisation test was used to test for population differentiation between each pair of study sites and overall in FSTAT (Goudet, 2001) assuming HW equilibrium within populations. For the tests of differentiation, FSTAT uses as an overall test statistic, the sum of individual loci G-values. Pairwise significance is reported at the 5% nominal level after standard Bonferroni corrections (Goudet, 2001).

Overall pairwise Wrights F_{ST} (Weir and Cockerham, 1984) were estimated using FSTAT (Goudet, 2001). Pairwise Nei's standard genetic distance, Ds (Nei, 1978) were estimated using GENALEX (Peakall and Smouse, 2006).

A graphical representation of the distance matrix was constructed using the UPGMA algorithm of PHYLIP version 3.5c. The UPGMA option constructs a tree by successive (agglomerative) clustering using an average-linkage method of clustering (Felsenstein, 2004). The data input to PHYLIP are the allele frequencies of each population. The following programs in PHYLIP were used to generate the tree. GENDIST generated a pairwise table of Nei's genetic distance D_S. NEIGHBOUR constructed the tree using the UPGMA algorithm. DRAWTREE plotted the tree as a postscript (.ps) file. The scale on the tree was set at 75cm per unit branch length. To provide support for the nodes in the tree 1000 bootstrap replicates of the original gene frequency data set were generated. The sequence of programs used was SEQBOOT, GENDIST (set to analyse 1000 data sets), NEIGHBOUR (set to analyse 1000 data

sets), CONSENSE (to generate the consensus tree using the Majority-Rule Extended rule) and DRAWTREE (Felsenstein 2004).

A hierarchical Analysis of Molecular Variance (AMOVA) (Excoffier *et al*, 1992) was performed using ARLEQUIN (version 2.000) (Schneider *et al.*, 2000). This analysis calculated the partitioning of the molecular variance among groups, among populations within groups and among individuals (within populations), using allele frequency data (F_{ST}). Study sites were grouped into two regions for the purpose of the hierarchial analysis (Group 1 (hereafter called North): Portglenone and Castle Leslie, Group 2 (hereafter called South): Beau Parc, Clongowes Wood, K Club, Mountarmstrong and Mount Juliet). This grouping was based on the results obtained from the above UPGMA tree of the genetic distance between population.

2.2.4.4 Isolation by Distance

Isolation by Distance (IBD) was investigated using IBD Web Server v 3.03 (Jensen *et al.*, 2005). Data were first used from five populations - Portglenone, Castle Leslie, Beau Parc, Clongowes Wood and Mount Juliet. Pairwise F_{ST}, $F_{ST}/1- F_{ST}$ (Rousetts distance) and Nei's genetic distance were each plotted against pairwise minimum geographic distance (km). The above data were also plotted as semi-natural logarithm (ln) and ln-ln plots. Significance in each of the isolation by distance relationships was tested statistically using a Mantel test.

IBD was further investigated by plotting ln minimum geographic distance v pairwise F_{ST} for all 7 populations and for the 5 most southern populations Beau Parc, Clongowes Wood, the K Club, Mountarmstrong and Mount Juliet (excluding Portglenone and Castle Leslie) and testing for significance with a Mantel test.

This Mantel test assesses whether the pairwise (or ln pairwise) genetic

distance matrix is correlated with the pairwise (or ln pairwise) geographic distance matrix. A null distribution is generated by randomizing rows and columns of one matrix while holding the other constant. Because entire rows (populations) are treated as a single unit, the Mantel test is more appropriate than alternatives that assume that each population pair is independent (Bohonak, 2002).

Regression techniques must be used to estimate the slope and intercept of the IBD relationship, since the Mantel test provides only an assessment of whether the association is significant. Reduced Major Axis (RMA) regression was used as recommended in Bohonak (2002).

A linear regression analysis of ln minimum geographic distance (km) V pairwise F_{ST} was performed for all pairwise comparisons between Clongowes Wood and the other six populations.

2.2.4.5 2MOD

The demographic history of grey squirrels in Ireland was investigated using 2MOD (Ciofi *et al.,* 1999; Beaumont, 2001). This program can be used to estimate the relative likelihoods of a model of immigration-drift equilibrium versus drift since a certain time. The model of immigration-drift is assumed to be either an infinite island or continent-island model of gene flow, which both give rise to the same likelihood function. The calculation of likelihoods for the pure drift case is implemented by the program dlik1.1. 2MOD estimates the relative likelihoods of the two models using an MCMC procedure as described in Ciofi *et al.,* (1999).

The key assumptions are: under a gene-flow model, mutation is much rarer than immigration so that the descendents of each immigrant are identical-by-descent, and also that the gene-frequency distribution from which immigrants are drawn does not change substantially since the time of the oldest immigrant that leaves descendents in the sample; under a founder/drift model the time between founding and taking the

samples is sufficiently short that mutations will not have strongly affected the gene frequencies (i.e. there will be no allelic variants that have arisen from mutations since the population was founded - another way of putting it is that the descendents of each founder that are present in the sample are identical-by-descent).

The models were used to detect if the genealogical history of alleles in the given populations could be explained by one of the two alternative demographic scenarios: the gene flow model (an equilibrium between gene flow and drift) or the (pure) drift model. Likelihoods of the gene flow and pure drift models were computed in three independent runs choosing 100,000 iterations. As suggested by the author, the first 10% of output calculations were discarded to avoid dependence on starting values.

2.2.4.6 Bottleneck detection

All populations were tested for a recent reduction of their effective population size using the Wilcoxon sign rank test implemented in BOTTLENECK (Piry *et al.,* 1999). In a population at mutation-drift equilibrium (i.e., the effective size of which has remained constant in the recent past), there is approximately an equal probability that a locus shows a gene diversity excess or a gene diversity deficit. To determine whether a population exhibits a significant number of loci with gene diversity excess, three tests are implemented in BOTTLENECK, namely a sign test, a standardized differences test, and a Wilcoxon sign-rank test (Piry *et al.,* 1999). The first test suffers from low statistical power. The second test in not very useful since it requires at least 20 polymorphic loci. The Wilcoxon test provides relatively high power and it can be used with as few as four polymorphic loci and any number of individuals (15-40 individuals and 10-15 polymorphic loci is recommended to achieve high power). A qualitative descriptor of the allele frequency distribution ("mode-shift" indicator), which discriminates bottlenecked populations from stable populations, is also given. However this qualitative method

is not a proper statistical test in that the type I error rate varies with sample size. It cannot be used (with confidence) with samples of fewer than 30 individuals and less than 10-20 polymorphic loci (Piry *et al.,* 1999). For these reasons the Wilcoxon sign rank test was chosen to test each of the seven grey squirrel populations.

2.2.4.7 Sex biased dispersal

2.2.4.7.1 Sex biased breeding dispersal

For the purposes of this study, breeding dispersal is assumed to be inter-population dispersal occurring between different woodlands. Goudet *et al.,* (2002) introduced a number of statistical tests for sex bias in dispersal between different populations, implemented in FSTAT. These are mean corrected Assignment Index (mAI_C), variance of corrected Assignment Index (vAI_C), F_{IS} and F_{ST}.

Mean corrected Assignment index (mAI_C)

An assignment test is a statistical test of the hypothesis that the multilocus genotype of an individual in question arose from a particular population (Manel *et al.*, 2005). This statistic was first introduced in Favre *et al.,* (1997). For each individual in each population, the program calculates the probability that its genotype should appear in the population. Because populations can contain very different levels of gene diversity, the multi-locus probabilities of individuals in different populations are not directly comparable. To remove this problem, the average probability of the sample is subtracted from the individual multi-locus probability, after log-transformation to avoid rounding errors with very small numbers. This gives the AI_C, the corrected assignment index of the individual in the population. The distribution of AI_C will therefore be centered around 0. A positive value indicates a genotype more likely than average to occur in its sample (likely a resident individual), while a negative value indicates a genotype less likely than

average (potentially a disperser) (Goudet *et al.,* 2002).

Because immigrants tend to have lower AI_C values than residents, under sex biased dispersal, the average index for the sex that disperses most is expected to be lower than that for the more philopatric sex. A t-statistic is used for the test, where n_p and n_d are the number of individuals in the more philopatric and the more dispersing group respectively (Goudet *et al.,* 2002).

Variance of AI_C (vAI_C)

As members of the dispersing sex will include both residents (with common genotypes) and immigrants (with rare genotypes), vAI_C for the sex dispersing most should be largest (Goudet *et al.,* 2002).

F_{IS}

F_{IS} is a statistic describing how well the genotype frequencies within populations fit with Hardy Weinberg expectation (Hartl & Clark, 1997). If only males disperse, the males sampled from a single patch will be a mixture of two populations, residents and immigrants; due to the Wahlund effect, the sample should show a heterozygote deficit and a positive F_{IS}. In general, members of the dispersing sex should therefore display a higher F_{IS} than the more philopatric sex. Weir & Cockerham's (1984) estimator of F_{IS} is used for the test (Goudet *et al.,* 2002).

F_{ST}

F_{ST} is a statistic expressing the proportion of the total genetic variance that resides among populations (Hartl & Clark, 1997). Allelic frequencies for individuals of the sex dispersing most should be more homogeneous than those for individuals of the more philopatric sex. We therefore expect F_{ST} for the more philopatric sex to be higher than that of the more

dispersing sex. Weir & Cockerham's (1984), estimator of F_{ST} is chosen because it is unbiased and is the most commonly used (Goudet *et al.,* 2002).

To test whether these statistics differ significantly between the two sexes, a randomisation approach is used. Under the null hypothesis that males and females disperse equally, the four statistics do not depend on the variable 'sex'. Letting X_d and X_p be the statistic of interest for the dispersing and the philopatric sex respectively, the program proceeds as follows for one sided tests. It first calculates the statistic for each sex over all populations and either takes the difference for F_{IS} and F_{ST}, the t-statistic for $m\text{AI}_C$; or the ratio for $v\text{AI}_C$. It randomly assigns a sex to each of the multi-locus genotypes (keeping the genotypes in their original sample, and the sex ratio in each sample constant). It recalculates the appropriate difference or ratio for the randomised data set. The probability that dispersal is unbiased by sex is then estimated as the proportion of times where the relevant statistic is larger or equal to the observed one (Goudet *et al.,* 2002).

The relative power of these indices to detect a sex bias in dispersal depends on the magnitude of the bias, dispersal rates, sampling scheme and markers examined. They also assume individuals are sampled post dispersal, therefore juveniles must be excluded from the populations tested (Goudet *et al.,* 2002).

These tests have been implemented for a variety of species, for example, the greater white toothed shrew (*Crocidura russula*) (Favre *et al.,* 1997), red-billed quelea (*Quelea quelea*) (Dallimer *et al.,* 2002), the bullfrog (*Rana catesbeiana*) (Austin *et al.,* 2003), the common frog (*Rana temporaria*) (Palo *et al.,* 2004), brook charr (Salvelinus fontinalis) (Fraser *et al.,* 2004) and White's skink (*Egernia whitii*) (Chapple and Keogh, 2005).

Palo *et al.,* (2004) note that if the geographic distance between pairs of populations studied exceeds the dispersal ability of the species in question, among population statistics such as F_{ST} will bear little information on dispersal rates. Therefore mAI_C, vAI_C and F_{IS} were calculated for all seven populations (Portglenone, Castle Leslie, Beau Parc, Mount Juliet, Clongowes Wood, The K Club and Mountarmstrong). F_{ST} was also calculated for Clongowes Wood, The K Club and Mountarmstrong. Each of these populations is located within grey squirrel dispersal range of the others (<5km apart).

2.2.4.7.2 Sex biased natal dispersal

For the purposes of this study, natal or short distance dispersal in grey squirrels is considered to be intra-population dispersal, within the same woodland. Assuming (i) natal dispersal occurs over a regular and fairly narrow range of dispersal distances (roughly 0-400m, Taylor *et al.,* 1971) and (ii) dispersing individuals, once settled, remain in that area for life (Taylor *et al.,* 1971, Gurnell, 1987) and (iii) individuals are sampled (trapped) in that area, then the sampled population should display some genetic structure. In general, pairwise comparison of individuals sampled within the regular range of dispersal distances should reveal greater autocorrelation than pairwise comparison of individuals sampled at distances greater than the regular dispersal distances.

If dispersal is sex biased then individuals of the philopatric sex should display greater pairwise autocorrelation within the range of dispersal distances than the dispersing sex. The analysis was conducted for males and females separately, to test for a sex bias in natal dispersal within each sex, and for the complete population.

The spatial autocorrelation method of Smouse and Peakall (1999) employs a multivariate approach to simultaneously assess the spatial signal generated by multiple genetic loci. Unlike classical spatial autocorrelation analysis, usually executed one allele at a time, the

procedure is intrinsically multivariate, avoiding the need for allele-by-allele, locus-by-locus analysis. By combining alleles and loci, the spatial signal is strengthened by reducing stochastic (allele-to-allele and locus-to-locus) noise. Both pairwise geographic and pairwise squared genetic distance matrices are required as inputs. The autocorrelation coefficient generated (r) is a proper correlation coefficient, bounded by [–1, +1]. The autocorrelation coefficient (r) provides a measure of the genetic similarity between pairs of individuals whose geographic separation falls within the specified distance class. The results are often summarized by a correlogram. Statistical significance is tested by obtaining random permutation and bootstrap estimated of r, called r_p and r_{bs} respectively.

A spatial autocorrelation analysis using GENALEX (Peakall and Smouse, 2006) was conducted to study the genetic structure of individuals along a linear transect of 26 trap points, each trap approximately 50 meters apart, in a strip of riverine woodland 1.45km in length and approximately 100m in width in Beau Parc (Figure 2.1). An additional 5 trap points were located at the midpoint of the linear transect and at right angles to it. Trap points were mapped on an x-y scale, with coordinates of 10-300, in units of 10, on the x-axis and from 10-50, in units of 10 on the y-axis. Each unit of 10 was approximately equal to 50 meters. Traps were not placed in the woodland directly behind Beau Parc house leading to a gap of 4 trap points from 110-140 inclusive on the x axis.

To implement this procedure, first, a pairwise, individual-by-individual ($N \times N$) genetic distance matrix was calculated. For a single-locus analysis, with i-th, j-th, k-th and l-th different alleles, a set of squared distances is defined as $d2(ii, ii) = 0$, $d2(ij, ij) = 0$, $d2(ii, ij) = 1$, $d2(ij, ik) = 1$, $d2(ij, kl) = 2$, $d2(ii, jk) = 3$, and $d2(ii, jj) = 4$ (Smouse and Peakall, 1999). A corresponding pairwise linear geographic distance matrix was then calculated. A single population spatial autocorrelation analysis was conducted using the genetic and matching geographic distance matrices

as input. The number of permutation and the number of bootstraps were both set at 999. An analysis was conducted for each of 10 different distance size classes from 10-100 ie. Distance size class 10, No. of Distance Classes 30, Distance Size Class 20, No. of Distance Classes 15,....Distance Size Class 100, No. of Distance Classes 3) for males and females separately and for the entire population. This analysis was repeated excluding the individuals trapped in the 5 trap points at right angles to the main linear transect.



Figure 2.1 Map of Beau Parc study site. Traps were placed in a line in woodland on the south side of the River Boyne. Source: Ordnance survey map of County Meath. Sheet 14. Revised 1958. Published 1968. Scale 1:10,560

2.3 Results

2.3.1 Microsatellite markers

The initial screen of 40 individuals for 10 microsatellites yielded 6 polymorphic microsatellites (Scv15, Scv19, Scv4, Scv18, Scv6, Scv31), 3 microsatellites with 2 alleles (Scv 3, Scv14, Scv9) and 1 microsatellite (Scv10) which failed to amplify reliably. Following a further screen of 312 individuals for the 6 polymorphic microsatellites above, locus Scv 31 had the highest number of alleles per locus at 7, but was not in HWE. Therefore, further analysis of the remaining 133 individuals used only 5 microsatellite loci Scv15, Scv19, Scv4, Scv18, Scv6 and Scv31 was excluded from all further data analysis.

Two loci failed to amplify in two tissue samples and three loci failed to amplify in one tissue sample, possibly due to poor quality DNA obtained from the tissue. One tissue sample gave three alleles at each of two loci, presumably due to contamination. Data from these samples were not used.

2.3.2 Genetic diversity

The five microsatellites used in this study were moderately polymorphic. Total number of alleles in 465 individuals for 5 loci was 26. Numbers of alleles per locus ranged from 4-6 and only 1 population had a private allele (Beau Parc: Scv 18, Allele 242). The number of alleles obtained per locus is dependent on sample size. Allelic richness is a measure of the number of alleles per locus independent of sample size, allowing a comparison between different sample sizes. Allelic richness per locus ranged from 3.9-5.7 (Table 2.1)

The number of alleles per population ranged from 19 (Mount Juliet) – 23 (Beau Parc) and allelic richness per population ranged from 16.6 (Mount Juliet) – 19.9 (Beau Parc) (Table 2.1).

Mean observed heterozygosities (H_O) and mean gene diversities (expected heterozygosities, H_E) across all loci were 0.63 and 0.61 respectively. Gene diversity (expected heterozygosity) fell within a narrow range from 0.46-0.76 across all loci/populations (Table 2.1). However expected heterozygosities were calculated only from the most polymorphic loci known in the literature (Hale *et al.,* 2001a) and following a further screen described above, and do not represent a mean expected heterozygosity for grey squirrels in any way. A random screen of microsatellite loci in grey squirrels would yield a combination of monomorphic and polymorphic loci and a much lower expected heterozygosity.

Following the exclusion of locus Scv 31, no locus in any population showed a significant deviation from HWE (Adjusted P value for 5% nominal level is 0.0004; All P>0.03).

No linkage disequilibrium was evident across the 120 locus pair\study site comparisons (P value for genotypic disequilibrium based on 2400 permutations; Adjusted P value for 5% nominal level is 0.0004. All P >0.005).

For all loci the frequency of null alleles was below the critical value 0.02, as measured and defined in CERVUS.

There was no evidence of inbreeding in any of the populations. A positive F_{IS} is an indication of heterozygote deficit and inbreeding. F_{IS} was marginally negative in 6 out of 7 populations and was not significant in any population (randomisation test in FSTAT, 1000 permutations, P>0.5 in all cases).

Table 2.1 Measures of genetic diversity among the seven populations at five microsatellite loci. *All values for F_{IS} per population were nonsignificant.

Population		PG	CL	BP	MJ	CG	KC	MA	Overall
No of individuals sampled		41	65	67	31	94	17	34	349
Number of alleles sampled	Scv15	3	3	4	3	3	3	3	4
	Scv19	4	5	4	5	5	4	5	5
	Scv4	4	4	5	3	5	4	5	6
	Scv18	5	5	5	5	4	4	4	6
	Scv6	4	4	5	3	4	4	3	5
No.of private alleles per population		0	0	1	0	0	0	0	1
Allelic Richness	Scv15	3.0	3.0	3.8	2.6	3.0	3.0	3.0	3.9
	Scv19	3.9	3.7	3.3	4.6	4.5	3.6	4.1	4.9
	Scv4	3.7	4.0	4.5	2.6	4.0	3.9	4.5	5.7
	Scv18	3.8	4.5	4.0	4.4	3.9	4.0	3.9	4.6
	Scv6	4.0	3.9	4.3	2.4	3.2	3.4	3.0	4.4
Observed heterozygosity	Scv15	0.44	0.71	0.73	0.52	0.46	0.59	0.68	0.59
	Scv19	0.59	0.58	0.66	0.81	0.77	0.47	0.53	0.63
	Scv4	0.66	0.75	0.61	0.56	0.57	0.41	0.65	0.60
	Scv18	0.63	0.72	0.61	0.81	0.64	0.65	0.56	0.66
	Scv6	0.90	0.72	0.48	0.44	0.65	0.82	0.68	0.67
Mean across loci		0.64	0.70	0.62	0.63	0.62	0.59	0.62	0.63
Gene diversity	Scv15	0.49	0.61	0.69	0.54	0.46	0.57	0.63	0.57
	Scv19	0.57	0.57	0.59	0.76	0.72	0.60	0.65	0.64
	Scv4	0.67	0.73	0.58	0.50	0.55	0.45	0.59	0.58
	Scv18	0.65	0.74	0.65	0.74	0.60	0.55	0.57	0.64
	Scv6	0.75	0.70	0.52	0.42	0.64	0.55	0.67	0.61
Mean across loci		0.62	0.67	0.61	0.59	0.60	0.54	0.62	0.61
Fis	Scv15	0.10	-0.16	-0.06	0.04	0.00	-0.03	-0.08	-0.03
	Scv19	-0.03	-0.02	-0.11	-0.06	-0.06	0.22	0.18	0.02
	Scv4	0.01	-0.03	-0.05	-0.13	-0.04	0.09	-0.09	-0.03
	Scv18	0.03	0.02	0.07	-0.10	-0.06	-0.17	0.02	-0.03
	Scv6	-0.21	-0.03	0.06	-0.05	-0.01	-0.50	-0.02	-0.11
Fis per population		-0.03*	-0.04*	-0.02*	-0.06*	-0.04*	-0.08*	0.00*	

2.3.2 Genetic Differentiation and Genetic Distance

Differences in allele frequencies over all seven populations were significant ($P<0.001$). All pairwise differences in allele frequencies between the seven populations were also significant ($P<0.002$). Overall pairwise Wrights F_{ST}, pairwise Nei's genetic distance Ds and pairwise distances in km between the study sites are shown in Table 2.2.

Overall differentiation between the seven study sites was moderate (F_{ST} = 0.10; 95% confidence limits 0.07-0.13).

As expected F_{ST} values are low between the three adjacent study sites in Co. Kildare: Clongowes Wood, Mountarmstrong and the K Club (pairwise F_{ST} 0.02-0.03). Pairwise F_{ST} between Clongowes Wood/Mountarmstrong and other study sites are similar, while equivalent values between the K Club and other sites are more variable. The sample size for the K Club was low at 17, ideally a minimum of 30 samples per population are needed to obtain a reliable estimate of F_{ST} (Beebee and Rowe, 2004).

If 5 study sites are considered (by omitting the adjacent study sites of the K Club and Mountarmstrong), overall differentiation was a little higher (F_{ST} = 0.12; 95% confidence limits 0.08-0.15) and again statistically significant ($P=0.001$). Each of these 5 study sites was separated from the nearest neighbouring site by distances of 43-91km.

The most striking feature of the data is the low pairwise F_{ST} between Portglenone and Castle Leslie (pairwise F_{ST} 0.03, distance 67km - little genetic differentiation, Hartl and Clarke, 1997) and the high pairwise F_{ST} between Castle Leslie and Beau Parc (pairwise F_{ST} 0.15, distance 73 km - great genetic differentiation, Hartl and Clarke, 1997).

Similar low pairwise F_{ST} is found elsewhere only at short distances of 1.5-4.5km between the three populations of Clongowes Wood, Mountarmstrong and the K Club (pairwise F_{ST} 0.02-0.03). Similar high

pairwise F_{ST}, is found only at greater distances, for example, at distances of 134km between Beau Parc and Mount Juliet (pairwise F_{ST} of 0.14), 263km between Portglenone and Mount Juliet (pairwise F_{ST} 0.15) and 132km between Portglenone and Castle Leslie (pairwise F_{ST} 0.17).

The data suggest that the populations of Portglenone and Castle Leslie are genetically similar but strongly differentiated from all other populations. This pattern is further evident in the unrooted UPGMA tree of Nei's genetic distance obtained using PHYLIP v 3.5 (Figure 2.2). The two most northerly populations, Portglenone and Castle Leslie, group closely together and are clearly separated from the more southerly populations, Beau Parc, the Clongowes Wood/Mountarmstrong/K Club cluster and Mount Juliet. The numbers on the branches indicate (in percentage) the number of times the partition of the species into the two sets which are separated by that branch occurred among the trees, out of 1000 bootstrapped trees. This north/south partition occurs in 100% of bootstrap samples. The remaining three southerly populations/groupings are also clearly separated from each other with high bootstrap support for the branches.

The results of the hierarchial AMOVA indicate that the highest percentage of variation is found within populations (85.98%, $P<0.001$), variance among populations within groups is low but significant (7.90%, $P<0.001$), as is variance between groups (6.12%, $P=0.048$) (Table 2.3).

Overall the tests for genetic differentiation among populations show that the Irish grey squirrel population is not panmixic, but is significantly differentiated into subpopulations at both a local (Clongowes Wood, Mountarmstrong and the K Club in Co. Kildare) and a national scale. The extent of this population substructure, as measured by pairwise F_{ST}, however varies depending on the scale of the comparison (i.e. the minimum geographic distance between populations) and on what region the populations are located in (North or South).

Pairwise Population Matrix of F_{ST}

	PG	CL	BP	CG	MA	KC	MJ
PG	0	67	132	174	173	174	263
CL	0.03	0	73	113	112	113	198
BP	0.17	0.15	0	43	42	43	134
CG	0.11	0.12	0.08	0	1.5	2.5	91
MA	0.10	0.11	0.08	0.02	0	4.5	92
KC	0.15	0.16	0.05	0.02	0.03	0	94
MJ	0.14	0.14	0.15	0.11	0.09	0.14	0

Pairwise Population Matrix of Nei's Genetic Distance Ds

	PG	CL	BP	CG	MA	KC	MJ
PG	0	67	132	174	173	174	263
CL	0.07	0	73	113	112	113	198
BP	0.41	0.39	0	43	42	43	134
CG	0.23	0.28	0.16	0	1.5	2.5	91
MA	0.23	0.29	0.16	0.05	0	4.5	92
KC	0.34	0.4	0.07	0.06	0.08	0	94
MJ	0.3	0.35	0.32	0.2	0.18	0.27	0

Table 2.2 Pairwise Wrights F_{ST} (upper table, lower diagonal) and pairwise Nei's genetic distance D_S (lower table, lower diagonal) for all populations. Pairwise minimum geographic distance in km (both tables, upper diagonal) for all populations.

PG: Portglenone, CL: Castle Leslie, BP: Beau Parc, MT: Mount Juliet, CG: Clongowes Wood, KC: The K Club, MA: Mountarmstrong

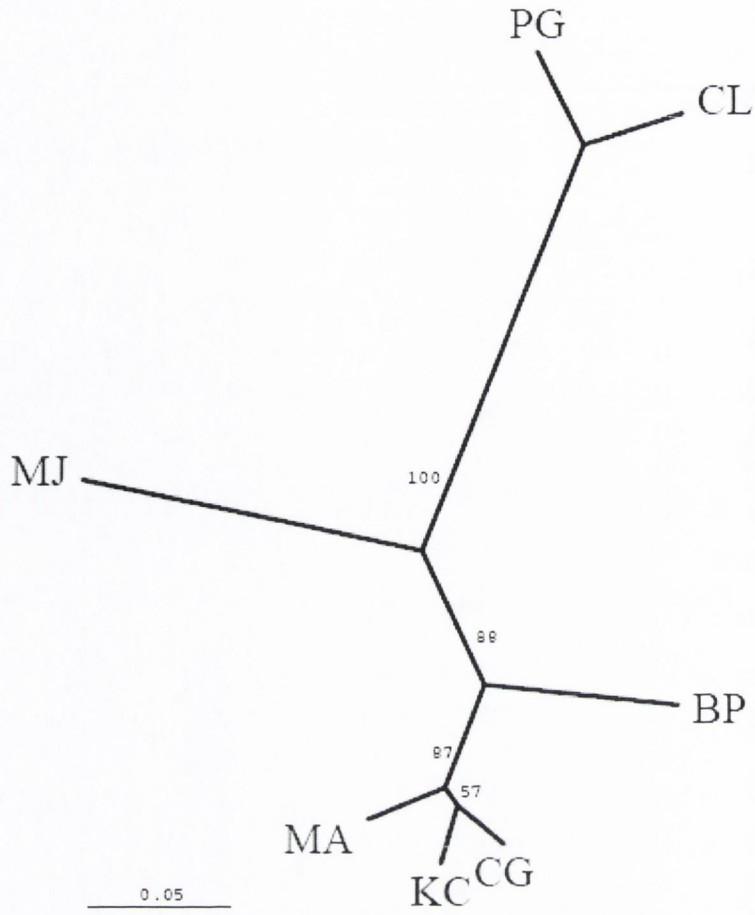


Figure 2.2 Unrooted UPGMA tree of Nei (1978) genetic distance obtained using PHYLIP v 3.5. The numbers on the branches indicate the number of times (in percentage) the partition of the species into the two sets which are separated by that branch occurred among the trees, out of 1000 bootstrapped trees.

Table 2.3 AMOVA results for grey squirrel data using Arlequin (Schneider *et al.,* 2000). Populations were assigned into two groups (Group 1: Portglenone and Castle Leslie; Group 2: Clongowes Wood, Mountarmstrong, K Club and Mount Juliet as identified by the UPGMA tree (Figure 2.2).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*P*-values
Among groups	1	55.70	0.14	7.90	0.048
Among populations within groups	5	57.55	0.10	6.12	<0.001
Within populations	693	1058.87	1.53	85.99	<0.001

2.3.4 Isolation by Distance

No isolation by distance relationship was observed among the five populations tested (Portglenone, Castle Leslie, Beau Parc, Clongowes Wood and Mount Juliet). Correlation between geographical distance and pairwise F_{ST} values was not significant (Mantel test, r=0.45, P=0.11) (Figure 2.3). Correlation between ln geographic distance and genetic distance, geographic distance and ln genetic distance, ln geographic distance and ln genetic distance, for all three genetic distance measurements, pairwise FST, Fst/1-Fst (Rousetts distance) and Neis genetic distance was not significant (Mantel test, all $r<0.53$, all $P>0.08$).

When all seven population were tested for isolation by distance, by correlating ln geographic distance with pairwise F_{ST} a significant relationship was observed (Mantel test, r =0.79, $P<0.001$) (Figure 2.4). Similiarly, when the two most northern populations, Portglenone and Castle Leslie, were excluded a strong isolation by distance relationship was observed in the five remaining southern populations (Mantel test, r=0.94, $P<0.001$). This effect can be attributed to the effect of incorporating the cluster of three populations in Co. Kildare, Clongowes Wood, the K Club and Mountarmstrong as independent samples in the larger scale analysis. The cluster of three populations in Co. Kildare, Clongowes Wood, the K Club and Mountarmstrong, are separated by 1.5-4.5km and have pairwise F_{ST} values ranging from 0.02-0.03 and similar pairwise F_{ST} values with each of the other populations (Table 2.2). Consequently, when included in an analysis the isolation by distance relationship is significant.

A linear regression analysis of ln (minimum geographic distance in km) V pairwise F_{ST} for all pairwise comparisons between Clongowes Wood and the other six populations was highly significant (d.f =5, $R^2=0.96$, P=0.001) (Fig. 2.5)

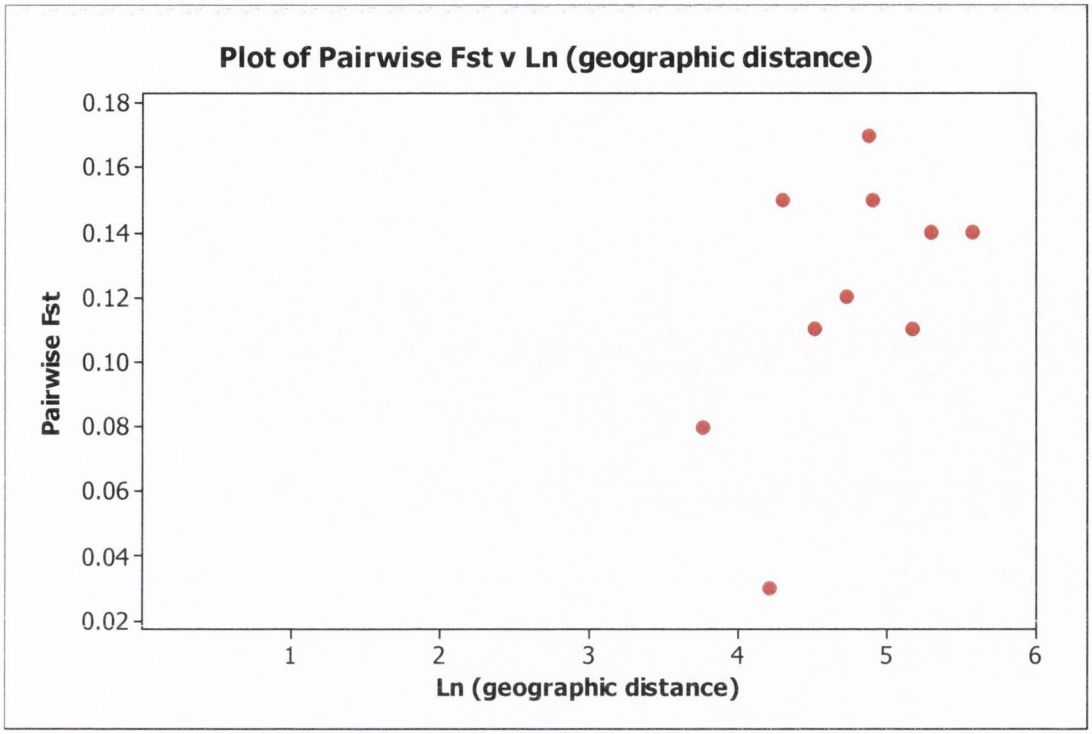


Figure 2.3 Natural logarithm of geographical distance (in km) is plotted against pairwise F_{ST} for five population pairs - Portglenone, Castle Leslie, Beau Parc, Clongowes Wood and Mount Juliet, to test for isolation by distance. No significant correlation between geographic distance and genetic distance was observed (Mantel test, r = 0.45, P = 0.11).

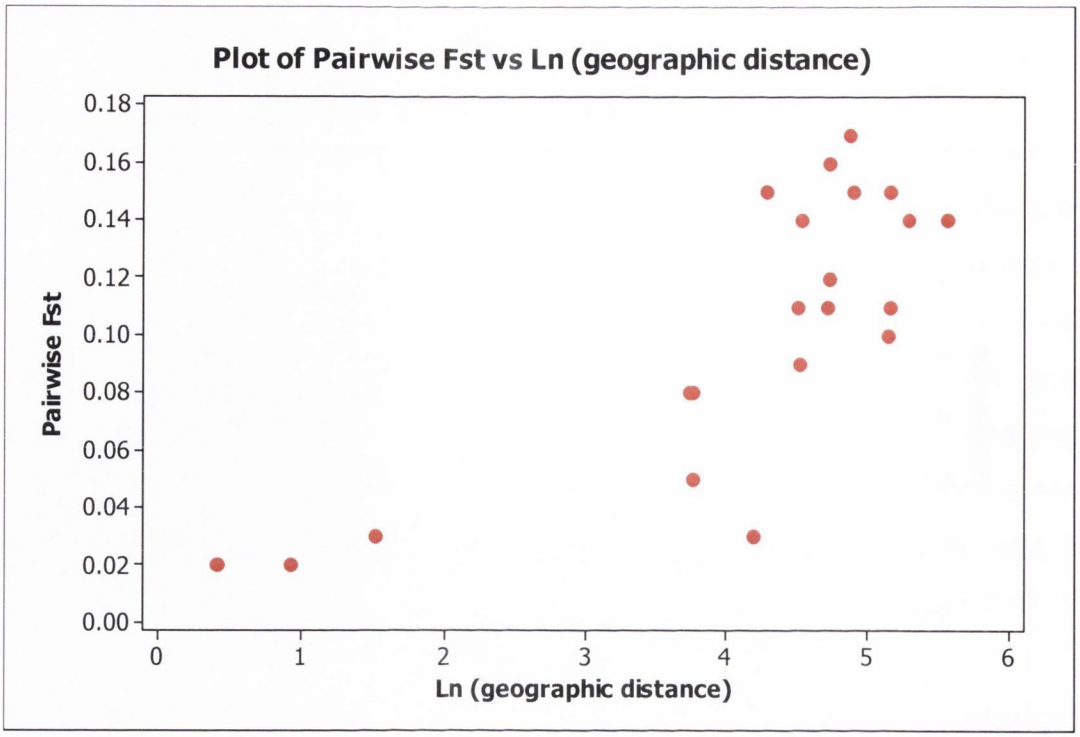


Figure 2.4 Natural logarithm of geographical distance (in km) is plotted against pairwise F_{ST} for seven population pairs - Portglenone, Castle Leslie, Beau Parc, Clongowes Wood, Mountarmstrong, the K Club and Mount Juliet to test for isolation by distance. A significant correlation between geographic distance and genetic distance was observed (Mantel test, $r < 0.79$, $P = 0.001$).

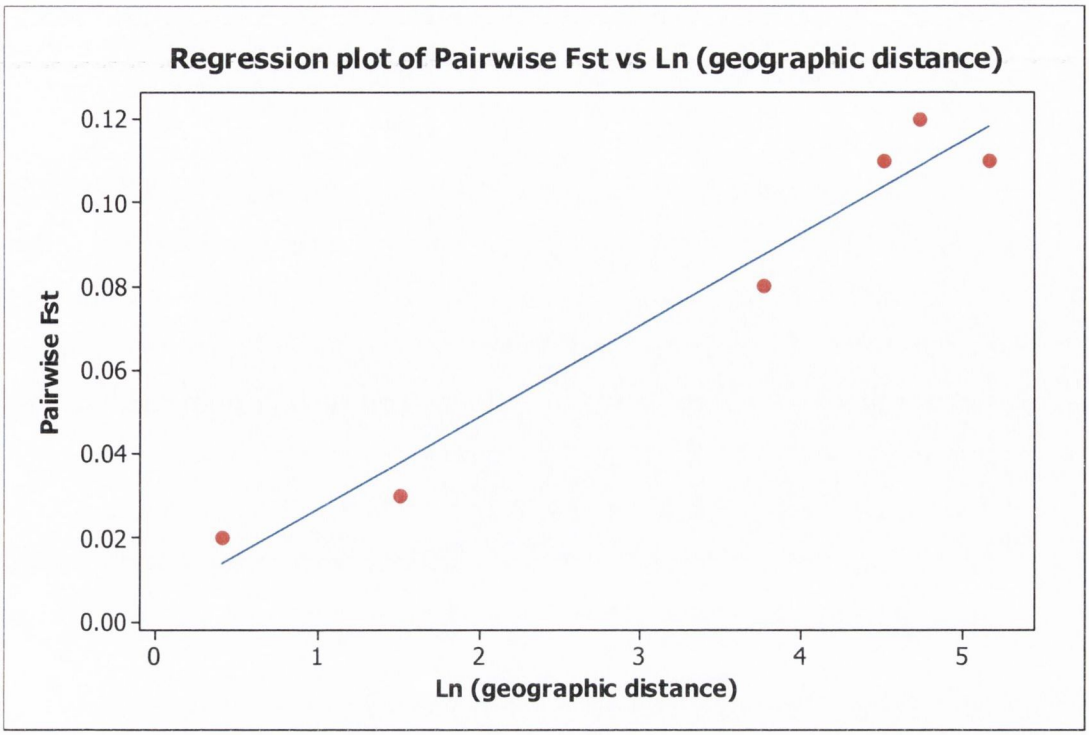


Figure 2.5 Linear regression analysis of ln (geographic distance) V pairwise F_{ST} for all pairwise comparisons between Clongowes Wood and the all other (6) populations (d.f =5, R^2=0.96, P=0.001).

2.3.5 2MOD

A 2MOD analysis on the entire data set consisting of all 7 populations indicated the gene flow model has a much higher likehood than the pure drift model (*P* gene flow model 0.935; P drift 0.065; bayes factor 14.4. A second 2MOD analysis on a data set consisting of 5 populations (excluding the K Club, and Mountarmstrong) again indicated the gene flow model also had a higher likehood than the pure drift model (*P* gene flow model 0.827; P drift 0.173; bayes factor 4.8). A Bayes factor >3.2 is conventionally referred to as a substantial support for a model likehood, >10 is strong support and >100 is decisive support (Kass and Raftery, 1995).

2.3.6 Population Bottlenecks

All grey squirrel populations in Ireland were established by a small number of individuals dispersing from other nearby populations. Therefore, all have experienced a founder effect or population bottleneck in the past.

Statistically significant evidence for recent bottlenecks was found in two populations, Castle Leslie and Mount Juliet (Table 2.4). However, the data are not consistent with respect to the age of the populations in which bottlenecks were detected. A bottleneck was found in the longest established population tested, Castle Leslie (Jackson, 1961) (Established 45 years, Wilcoxon Test, one tailed for heterozygosity excess, P=0.03). The populations of Beau Parc, Clongowes Wood, Mountarmstrong and the K Club, although established after Castle Leslie, did not show significant evidence of a recent bottleneck.

Likewise, the most recently established population, Portglenone (O'Teangana, 1999), did not have significant evidence of a recent bottleneck (Established 7 years, Wilcoxon Test, one tailed for

heterozygosity excess, P=0.08). However, as only 5 loci were used for the tests, the power of the Wilcoxon Test is low, which might account for the low *P*-value but absence of statistical significance.

Mount Juliet, which is established for a similar length of time to Portglenone, showed evidence of a recent bottleneck (Established 15 years, Wilcoxon Test, one tailed for heterozygosity excess, P=0.05).

Table 2.4 Estimated year populations were founded, age of populations when tested and results of the bottleneck detection tests for seven populations.

Site	Estimated year population was founded	Age of population in years when tested for bottleneck	P value of Wilcoxon Test
Portglenone	1994 [1]	12	0.08
Castle Leslie	1960 [2]	45	0.03
Beau Parc	1973 [3]	31	0.60
Clongowes Wood	1975 [4]	28	0.10
K Club	1975 [4]	28	0.93
Mountarmstrong	1975 [5]	28	0.40
Mount Juilet	1989 [5]	15	0.05

1. O'Teangana (1999)
2. Jackson (1961)
3. Crichton (1974)
4. Personal communication
5. O'Reilly (1997)

2.3.7 Sex biased dispersal

2.3.7.1 Sex biased breeding dispersal

To test for sex biased breeding dispersal in a total of 131 adult males and 136 adult females, sampled from seven populations (Portglenone, Castle Leslie, Beau Parc, Mount Juliet, Clongowes Wood, The K Club and Mountarmstrong), F_{IS}, mAI_C and vAI_C were calculated for each sex separately (Table 2.5). F_{IS}, a measure of heterozygosity deficiency, was significant higher in males than females (P = 0.001). This indicates that there are more immigrants in the male populations, which due to the Wahlund effect, are showing a greater heterozygote deficit and a larger F_{IS}. Dispersal leads to lower assignment indices and higher variance of assignment indices in the dispersing sex. Mean corrected assignment index (mAI_C) is significantly lower in males than females (P = 0.011). Variance of mAI_C (vAI_C) was greater for males than females, but not significantly so (P = 0.053).

F_{ST} was calculated for 51 adult males and 52 adult females, sampled from three populations, located within 5 km of each other, Clongowes Wood, the K Club and Mountarmstrong (Table 2.6). Lower interpopulation differentiation, as measured by F_{ST} is predicted for the dispersing sex. F_{ST} between males was lower than between females but not significantly so (P = 0.197).

All four test statistics indicate that breeding dispersal is male biased. F_{IS} and mAI_C are statistically significant.

Table 2.5 F_{IS}, mAI_C and vAI_C calculated for seven populations (excluding juveniles) - Portglenone, Castle Leslie, Beau Parc, Mount Juilet, Clongowes Wood, The K Club and Mountarmstrong.

	N	F_{IS}	mAI_C	vAI_C
Males	131	0.014	-0.267	3.904
Females	136	-0.103	0.256	2.791
P		0.001	0.011	0.053

Table 2.6 F_{ST} calculated for three populations (excluding juveniles) - Clongowes Wood, The K Club and Mountarmstrong.

	N	F_{ST}
Males	51	0.019
Females	52	0.041
P		0.197

2.3.7.2 Sex biased natal dispersal

The single population spatial autocorrelation analysis did not detect any genetic structure in the Beau Parc population at any distance class for males, females or the entire population. No value of r in any analysis, for any distance class, exceeded the 95% bootstrap confidence interval (obtained by permutation) about the null hypothesis of no spatial structure for the data (all $r < r_{bs}$, permutation test).

To illustrate this, results are shown for distance class 40 (200 metres), for males, females and the entire population. For distance class 40 (200 meters) there were 7 distance classes along the length of the transect (1.45 meters) (Tables 2.7-2.9, Figures 2.6-2.8). The final distance class is not shown as it contained few data and gave a very skewed value for r.
If present, genetic structure will be detected in the first, or first and second distance classes, i.e. within the natal dispersal distance and is manifest by $r > r_{bs}$. Thereafter, in subsequent distance classes it is expected that $r \leq r_{bs}$.

Table 2.7 Results of the Spatial Autocorrelation Analysis for distance class 40 (200 meters) for male grey squirrels in Beau Parc. r, U and L values are adjusted by subtracting the Mean permuted r. Upper (U) and lower (L) confidence limits bound the 95% confidence interval about the null hypothesis of no spatial structure for the combined data set as determined by permutation.

Spatial Autocorrelation Analysis for male grey squirrels in Beau Parc

No. Samples	28						
Distance Classes	40						
No. Dist. Classes	7						
No. Permutations	999						
No. Bootstraps	1000						
Distance	0-40	41-80	81-120	121-160	161-200	201-240	241-280
n	119	68	54	44	38	37	18
r	0.019	0.009	-0.043	-0.027	0.027	-0.025	0.018
U	0.047	0.059	0.065	0.076	0.079	0.077	0.117
L	-0.038	-0.063	-0.073	-0.080	-0.082	-0.082	-0.138
Prob $r > r_p$	0.171	0.378	0.885	0.749	0.271	0.724	0.398
Mean r_p	-0.037	-0.038	-0.036	-0.036	-0.039	-0.037	-0.037

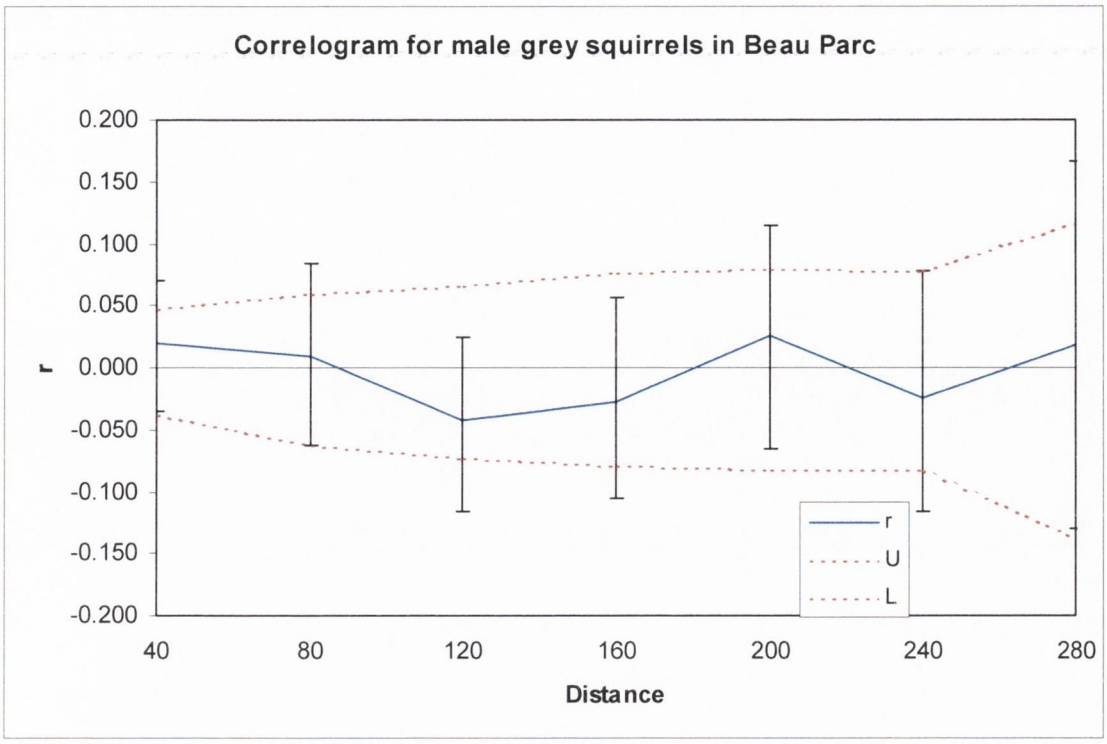


Figure 2.6. Correlogram of autocorrelation coefficient (r) v distance class 40 (200 meters) over 7 distance intervals for male grey squirrels in Beau Parc. Upper and lower error bars bound the 95% confidence interval about r as determined by bootstrap resampling. Upper (U) and lower (L) confidence limits bound the 95% confidence interval about the null hypothesis of no spatial structure for the combined data set as determined by permutation.

Table 2.8 Results of the Spatial Autocorrelation Analysis for distance class 40 (200 meters) for female grey squirrels in Beau Parc. r, U and L values are adjusted by subtracting the mean permuted r (r_p). Upper (U) and lower (L) confidence limits bound the 95% confidence interval about the null hypothesis of no spatial structure for the combined data set as determined by permutation.

Spatial Autocorrelation Analysis for female grey squirrels in Beau Parc

No. Samples	37
Distance Class	40
No. Dist. Classes	7
No. Permutations	999
No. Bootstraps	1000

Distance	0-40	41-80	81-120	121-160	161-200	201-240	241-280
n	194	130	104	91	77	50	16
r	-0.005	0.006	-0.003	0.003	0.001	0.006	-0.056
U	0.032	0.040	0.045	0.057	0.055	0.072	0.139
L	-0.028	-0.041	-0.046	-0.051	-0.060	-0.070	-0.137
Prob $r > r_p$	0.612	0.386	0.543	0.457	0.501	0.428	0.798
Mean r_p	-0.028	-0.028	-0.027	-0.029	-0.027	-0.029	-0.025

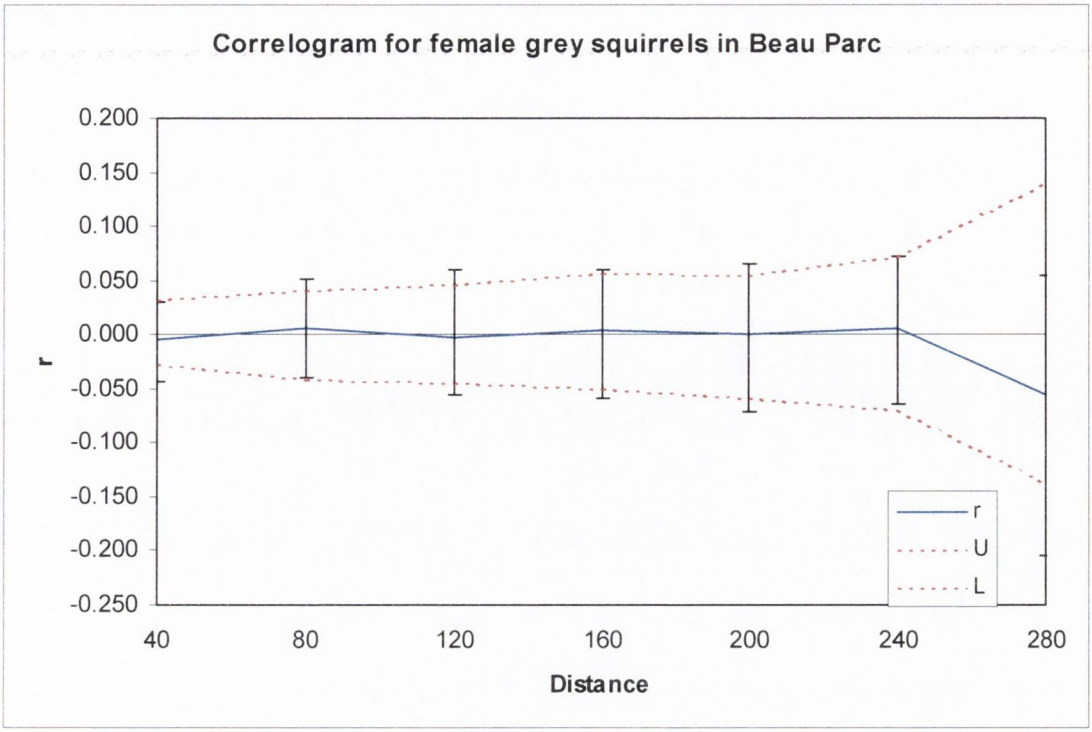


Figure 2.7 Correlogram of autocorrelation coefficient (r) v distance class 40 (200 meters) for female grey squirrels in Beau Parc. Upper and lower error bars bound the 95% confidence interval about r as determined by bootstrap resampling. Upper (U) and lower (L) confidence limits bound the 95% confidence interval about the null hypothesis of mo spatial structure for the combined data set as determined by permutation.

Table 2.9 Results of the Spatial Autocorrelation Analysis for distance class 40 (200 meters) for all grey squirrels in Beau Parc. r, U and L values are adjusted by subtracting the mean permuted r (r_p). Upper (U) and lower (L) confidence limits bound the 95% confidence interval about the null hypothesis of no spatial structure for the combined data set as determined by permutation.

Spatial Autocorrelation Analysis for all grey squirrels in Beau Parc

No. Samples	65
Distance Classes	40
No. Dist. Classes	7
No. Permutations	999
No. Bootstraps	1000

Distance	40	80	120	160	200	240	280
n	627	406	304	262	231	173	69
r	-0.007	0.004	-0.018	0.019	0.020	0.009	-0.044
U	0.016	0.023	0.026	0.030	0.032	0.038	0.063
L	-0.015	-0.024	-0.028	-0.030	-0.035	-0.039	-0.060
Prob $r > r_p$	0.799	0.363	0.899	0.121	0.116	0.318	0.921
Mean r_p	-0.016	-0.016	-0.015	-0.017	-0.016	-0.014	-0.017

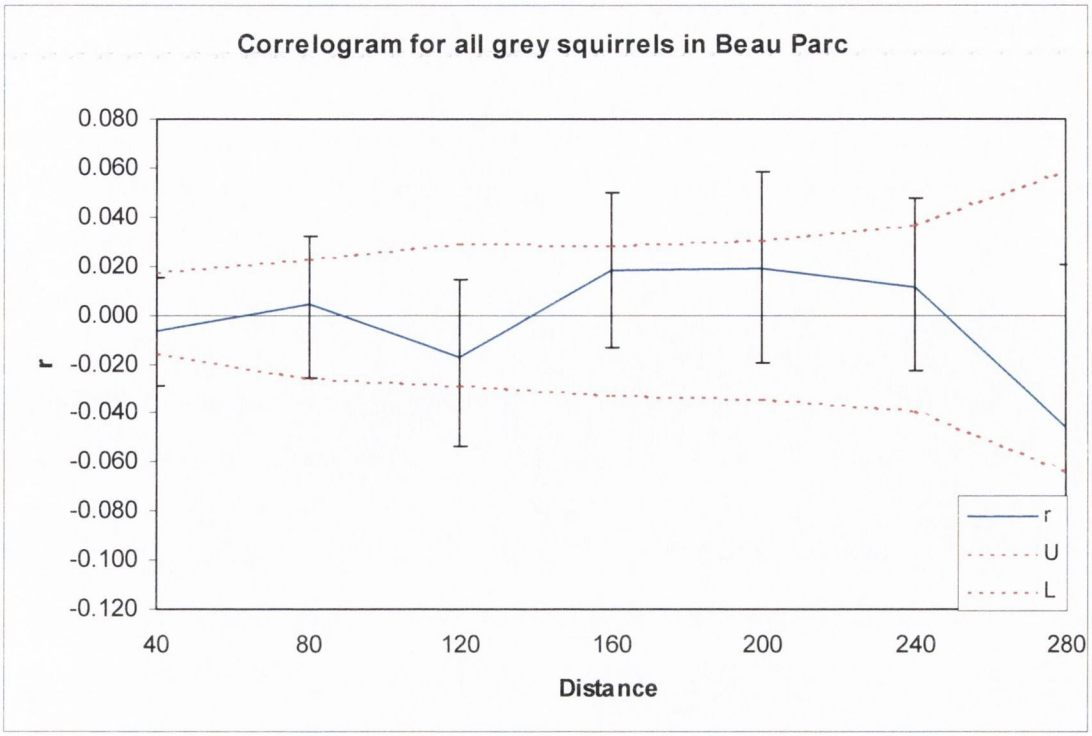


Figure 2.8 Correlogram of autocorrelation coefficient (r) v distance class 40 (200 meters) for all grey squirrels in Beau Parc. Upper and lower error bars bound the 95% confidence interval about r as determined by bootstrap resampling. Upper (U) and lower (L) confidence limits bound the 95% confidence interval about the null hypothesis of no spatial structure for the combined data set as determined by permutation.

2.4 Discussion

Populations are products of opposing evolutionary forces. Gene flow promotes homogeneity among populations, genetic drift promotes divergence, and selection may act in either direction. The relative effects of these forces shape the genetic population structure (pattern of genetic variation among and within populations) of a species. Population structure is positively associated with genetic diversity and resilience to disturbance such that large, highly structured populations have high genetic diversity and probability of persistence. In contrast, small, panmictic populations are vulnerable to inbreeding, demographic stochasticity, genetic drift and thus, reduced evolutionary potential, and increased probability of extinction (Ramstad *et al.*, 2004).

2.4.1 Genetic diversity

The Irish grey squirrel populations displayed moderate genetic diversity at the microsatellite loci studied. There is little genetic data available from grey squirrel populations in other countries, therefore this data will also be compared to data from red squirrel, Sciurid and other populations.

The 5 microsatellites used in this study were developed for red squirrels, and tested on a small number of grey squirrel samples (n=2-7) from the north of England and found to amplify well (Hale *et al.*, 2001a). The mean number of alleles per locus (MNA) for these 5 microsatellites was similar in the English samples (MNA 5.0, range 4-6) and in the Irish samples (MNA 5.2, range 4-6). However, MNA is dependent on sample size. Sample size was between 2 and 7 for the English samples and 349 for the Irish samples. Moreover, the English samples were all collected from the north of England while the Irish samples were collected nationwide. It is likely that with nationwide sampling and a comparable sample size, the MNA in the UK grey squirrel population would be

greater.

An indication of the genetic diversity of the British grey squirrel population comes from multilocus DNA fingerprinting (minisatellite) data from two grey squirrel populations in the UK, at Alice Holt Forest on the borders of Hampshire and Surrey in southern England and Thetford Forest in East Anglia. Band-sharing coefficients between individuals from Alice Holt ranged from 0.08 to 0.52 and between individuals from Thetford Forest from 0.11 to 0.51. The mean background band-sharing coefficient was 0.22 (n = 91, sd = 0.093) for the Alice Holt population and 0.26 (n = 91, sd = 0.089) for the Thetford Forest population. The results showed that there was a significant regional difference in the levels of genetic variation between the two study sites, but overall the mean background band-sharing levels found in the two populations were similar to data from other studies of outbred genetically diverse small mammal populations, for example, 0.16 in the California mouse (*Peromyscus californicus*), 0.28 in the Banner-tailed Kangaroo rat (*Dipodomys spectabilis*) and 0.10 in the Common or Eurasian shrew (*Sorex araneus*) (David-Gray *et al.,* 1999).

The grey squirrel populations now found in Britain was not established from a small single introduction, as was the case in Ireland, but from repeated introductions over several years of individuals from different source populations. Shorten (1954) lists nine introductions from the U.S.A. and two from Canada between 1876 and 1910, and 21 translocations within Britain (including eight from Woburn in Bedfordshire, the source population of the grey squirrels introduced into Ireland) between 1892 and 1929. This combination of independent introductions and translocations has mediated against any founder effects and accounts for the high levels of genetic diversity found in grey squirrels in Britain (David-Gray *et al.,* 1999). In Ireland a small number of grey squirrels (twelve) were introduced to one location (Castleforbes) with no further known translocations. Genetic drift theory predicts two

consequences for populations founded by a small number of individuals. Genetic variability will be less and allele frequencies will be different when compared to the founder population (Halliburton, 2004). The genetic diversity of the grey squirrel population in Ireland is therefore likely to be less than that in Britain, and still less than that in the eastern United States, the native range of the grey squirrel.

The mean number of alleles per locus for Irish grey squirrel populations (MNA 5.2, range 4-6) is similar to that found in red squirrel populations in other countries.

For the 5 microsatellite loci used both for grey squirrels in this study and for red squirrels in the north of England (Hale *et al.,* 2001a), MNA is higher in grey squirrels (MNA 5.2, range 4-6) than in red squirrels (MNA 4.4, range 2-6), but again the sample size was higher in Ireland (n=349) than in England (n ranges from 14-33). For 163 red squirrels sampled from 11 populations in Belgium and Germany and genotyped at 5 different microsatellite loci, MNA was higher than in this study (MNA 6.6, range 4-8) (Todd, 2000). In the Italian Alps, Trizio *et al.,* (2005) genotyped 289 red squirrels from 7 populations at 10 microsatellite loci and obtained a MNA slightly lower than in this study (MNA 4.6, no range obtainable from the published data). On the island of Anglesea, the islands remnant red squirrel population of 46 individuals had a very low MNA of 1.7 (range 0-3) across 6 micosatellite loci (Ogden *et al.,* 2005). However, the authors did not exclude monomorphic loci prior to conducting the analysis and 3 of the 6 loci studied were monomorphic.

In related Sciurid species Gunn *et al.,* (2005) isolated and tested 48 microsatellite loci and found 18 to be polymorphic in a wild population of North American red squirrels, *Tamiasciurus hudsonicus*. MNA was high at 9.0, range 6-13. These loci were investigated in 6 grey squirrels, *Sciurus carolinensis*, 12 loci amplified and 6 were polymorphic. In 12 European red squirrels, *Sciurus vulgaris*, 13 loci amplified and 12 were

polymorphic. Of the 20 microsatellite loci isolated and tested by Shibata *et al.,* (2003) for the Japanese squirrel, *Sciurus lis*, 11 loci were found to be polymorphic. MNA was 4.9, range 2-7. These microsatellites were not tested on other *Sciurus* or *Tamiasciurus* species.

Mean observed heterozygosities (H_O) and mean gene diversities (expected heterozygosities, H_E) for Irish grey squirrel populations were similar to those obtained for red squirrel populations in Belgium/Germany, Italy and Britain and also for other *Sciurus* or *Tamiasciurus* species. In this study mean H_O and mean H_E across loci were 0.63 and 0.61 respectively, in Belgium/Germany they were 0.54 and 0.57 respectively (Todd, 2000) and in Italy 0.56 and 0.55 respectively (Trizio *et al.,* 2005). In Britain mean H_O and mean H_E were 0.36 and 0.58 respectively (Hale *et al.,* 2001a). The low mean H_O relative to mean H_E in Britain was due to the Wahlund effect and sampling across populations. For the North American red squirrel, *Tamiasciurus hudsonicus,* mean H_O and mean were H_E were a little higher, 0.71 and 0.74 respectively (Gunn *et al.,* 2005) and for the Japanese squirrel, *Sciurus lis,* 0.45 and 0.62 respectively (Shibata *et al.,* 2003). In five poulations of bank voles in Denmark (n=161) gene diversity was higher (H_E = 0.75-0.83) (Redeker *et al.*, 2006) but it was similar in 8 poulations of feral pigs in south Western Australia (H_E = 0.68) (Hampton *et al.*, 2004).

To put these figures in a broader context, from a total of 524 microsatellite loci considered in nearly 40,000 individuals of 78 species, freshwater fish displayed levels of population genetic variation (mean H_E = 0·46, and MNA =7·5) roughly similar to those of non-piscine animals (mean H_E = 0·58 and MNA = 7·1). In contrast, local population samples of marine fish displayed on average significantly higher heterozygosities (mean H_E = 0·79) and nearly three times the number of alleles per locus (MNA = 20·6). Anadromous fish were intermediate to marine and freshwater fish (h=0·68 and a=11·3) (Dewoody and Avise, 2000).

Loss of genetic diversity is a process that occurs between generations, even in cases of a single colonising event,. As long as the founding population consisted of more than a few individuals, and providing the population rapidly increased in size, relatively little genetic diversity is lost (Austerlitz *et al.*, 1997). As with grey squirrels, the introduction of the marsh frog, *Rana ridibunda*, into England from Hungary involved only 12 individuals; yet due to rapid population growth, the established non-native population exhibited a level of genetic diversity similar to that of the source population (Zeisset and Beebee, 2003). Based on a review of the relevant literature, Wares *et al.*, (2005) concluded that introduced populations often retain as much as 80% of the genetic variation of the source population, or even more.

2.4.2 Inbreeding

No evidence of inbreeding was detected in any population. This is somewhat surprising given that the population is derived from just 12 individuals from a single source population and that some mating between related individuals was probable in the small, early population (see Gurnell *et al.,* 2001).

However, for inbreeding to accumulate in a population over generations, that population must remain completely isolated. Inbreeding can be substantially reduced by the introduction of individuals from other populations. This occurs even when the immigrants themselves are inbred, as long as they come from genetically distinct populations (Frankham *et al.,* 2002). Theoretically, one or more migrants per generation can alleviate the deleterious effects of inbreeding on reproductive fitness and extinction risk (Frankham *et al.,* 2002). Once introduced to Castleforbes, the grey squirrel population increased in size and range rapidly (Watt, 1923). As its range expanded populations became more isolated and differentiated from each other due to the founder effect and genetic drift (see section 1.5). Immigration of

individuals with atypical genotypes between populations would then tend reduce inbreeding and prevent the build-up of homozygous genotypes within a population. When dispersal is sex biased, as observed in grey squirrels (Koprowski, 1996), this also functions as a means of inbreeding avoidance in populations (Lawson Handley *et al.,* 2007). Finally, if population size recovers rapidly following a severe bottleneck, as was the case for the Irish grey squirrel population (Watt, 1923) individuals which are highly inbred will suffer higher levels of inbreeding depression, have reduced fitness and tend to be removed from populations by natural selection (Frankham *et al.,* 2002).

2.4.3 Genetic differentiation and population structure

Population differentiation was significant at both a national and local scale. At a national level the Irish grey squirrel population is not a panmixic population but is differentiated into subpopulations, with moderate to great genetic differentiation between some subpopulations (Table 2.2) (for example, between Beau Parc and Mount Juliet). The seven populations can be divided into four main groups, a northern group, consisting of Portglenone and Castle Leslie, a Co. Kildare group consisting of Clongowes Wood, the K Club and Mountarmstrong and Beau Parc and Mount Juliet as separate groups (Figure 2.2)

Overall differentiation between the seven grey squirrel populations was moderate (F_{ST} = 0.10; 95% confidence limits 0.07-0.13). Trizio *et al.,* (2005) genotyped 289 red squirrels at 10 loci in 6 populations in continuous conifer woodland in the Italian Alps, over a similar scale to this study (this study 1.5km-263km; in the Italian Alps 1.1km-276km). Overall differentiation for red squirrel populations in continuous forest was significant but lower (F_{ST} = 0.04; 95% confidence limits 0.03-0.06) than for grey squirrels in fragmented woodland in Ireland (F_{ST} = 0.10; 95% confidence limits 0.07-0.13). All pairwise comparisons of F_{ST} were significant for red squirrel populations in the Italian Alps, ranging from

0.02-0.06 but again lower than those obtained for grey squirrel populations in Ireland, ranging from 0.02-0.17. In both the Italian Alps and Ireland, population differentiation was significant at both the regional/national and local scale. Barratt *et al.,* (1999) found an 'extraordinary' level of genetic differentiation among fragmented populations of red squirrels in the UK using mitochondrial DNA control region sequence. They concluded that, within the UK, the genetic patterns observed were most likely explained by the effects of genetic drift which has occurred since the isolation of populations in the past few hundred years, hence no underlying phylogenetic pattern could be detected. A microsatellite (Hale *et al.,* 2001a) and mitochondrial DNA (Hale *et al.,* 2004) analysis of red squirrel museum pelt specimens in the UK also showed that British red squirrel populations are strongly differentiated with high F_{ST} values (0.04-0.57; (Hale *et al.,* 2004)). The mitochondrial variation appeared random with no reflection of geographic distribution in the phylogenetic analysis.

A hierarchial analysis of molecular variance showed the majority of molecular variance to be partitioned within populations (85.99%, $P<0.001$), variance among populations within groups is low but significant (7.90%, $P<0.001$), as is variance between groups (6.12%, $P=0.048$) (Table 2.3). Trizio *et al.,* (2005) obtained similar results for red squirrels in the Alpine Alps but a higher percentage of variation (99.5%, $P<0.001$) was found for the within population component and lower percentage for the within group (0.3%, $P<0.001$) and between group (0.2%, P= 0.069) components.

For grey squirrels in Ireland and red squirrels in the UK habitat fragmentation, with lower levels of gene flow between habitat fragments, would appear to be responsible for the greater genetic differentiation seen in comparison with red squirrels in continuous, conifer forest in the Alpine Alps.

The significant population differentiation between the three grey squirrel populations at a local level (Clongowes Wood, the K Club and Mountarmstrong) is more unexpected, as it implies restricted gene flow between these populations, despite the fact that each population is within the dispersal range of the other. Significant population differentiation was also recorded for two red squirrels populations 1.1km apart in the Alpine Alps (F_{ST} = 0.018) (Trizio *et al.*, 2005).

Therefore, assuming that dispersal of grey squirrels is sufficiently low and over sufficiently short distances to allow population differentiation exist over both national and local scales, a question that arises is what number of individuals dispersing (and then successfully breeding) between adjacent woodlands would give F_{ST} values of 0.02-0.03, as found between Clongowes Wood/Mountarmstrong/The K Club.

Wright (1969) using an 'infinite island model' of population structure determined that migration rates of one migrant per generation are theoretically sufficient to prevent fixation of alleles and that 5 migrants per generation are enough to prevent substantial differentiation and keep all individual subpopulation allele frequencies near the mean allele frequencies over all subpopulations. This effect is independent of population size. In a large population genetic drift is weak so it takes less gene flow (expressed as the proportion of immigrants in each generation) to counteract it and vice versa.

The OMPG rule results from a simple model of population structure based upon a host of unrealistic assumptions including: an island model of migration, selective neutrality and no mutation, ideal populations, demographic equality and gene frequency equilibrium (Mills and Allendoff, 1996).

Despite these unrealistic assumptions, the one migrant per generation rule has been tested and found to reduce inbreeding depression in

experiments involving fruit flies (*Drosophlia melanogaster*) (Spielman and Frankham 1992) houseflies (*Musca domestica*) (Bryant *et al.*, 1999) and mustard (*Brassica campestris*) (Newman & Tallmon 2001) and in a population genetic model which predicts the frequency of deleterious mutations and the viability of the individuals in a set of populations with varying numbers of migrants (Couvet, 2002).

An estimate of the average number of dispersers (N_M) per subpopulation per generation can be obtained from Wright's F_{ST} (Wright 1969). However, the relationship between N_M and F_{ST} is based on the idealized island model of population structure so its applicability to natural populations is suspect (Whitlock and McCauley, 1999). Moreover, the island model does not yields the local, population-specific estimates required to study the underlying mechanisms of dispersal (Ims and Yoccoz, 1997).

Mills and Allendoff (1996) noted that one of the most realistic tests of the effects of migration on genetic variability and differentiation in subpopulations involved simulations by Lindenmayer and Lacy (1995) with Leadbeaters possum (Gymnobelideus leadbeateri). Lindenmayer and Lacy (1995) used the computer program VORTEX to model the viability of metapopulations with subpopulations occupying a small number of habitat patches of varying size. They used values for life-history parameters drawn from extensive field studies, allowed local subpopulations to become extinct, and included the effects of inbreeding depression. Leadbeater's possums have a similar life history to grey squirrels. They are an an arboreal marsupial, live in old-growth montane ash forest in Victoria, south east Australia, weigh about 150g, have a home range of 1.5ha, breed at age 2 years, have a maximum 4 young in two litters per year, a maximum longevity of 8 years and a generation length of 3.71 years (Lindenmayer and Lacy, 1995). Annual migration rate was varied in the model. In their largest metapopulation, which consisted of 5 subpopulations of 40 individuals each, an annual

migration rate of 0.01 (1.5 migrants per generation (3.71 years)) produced an overall F_{ST} of 0.07 between subpopulations and an annual migration rate of 0.05 (7.4 migrants per generation (3.71 years)) produced an overall F_{ST} of 0.02.

In real populations, Lacy (1987) suggested that around 5 migrants per generation were required to prevent population differentiation. Mills and Allendoff (1996) suggest a minimum of 1 and a maximum of 10 migrants per generation in order to maintain genetic variability within real subpopulations without causing uniformity of allele frequencies across populations. Therefore, given a generation length of 2.07 years in grey squirrels (Millar and Zammuto, 1983), and based on the above estimates, an exchange of maximum 2.5-5 migrants annually between grey squirrel populations i.e. migrants who successfully breed would be possible without still allowing for population differentiation.

As the above estimate only refers to individuals successfully dispersing and breeding, the next question is how many individuals disperse but fail to breed, and so fail to contribute to gene flow between the populations. In ideal populations, immigrants have the same demographic attributes, in terms of survival and reproduction probabilities, as resident individuals. However, it is generally considered that immigrants have lower survival. Thompson (1978a) studied a grey squirrel population on a 28.7ha site in Mount Pleasant Cemetery, Toronto. He found that only 14.3% of immigrants in autumn and 7.85% of immigrants in spring who were trapped on his study site became established in the population. Once established the rate of loss of immigrants was similar to the rate of loss of residents. This would suggest high mortality in immigrants attempting to enter a new population. However, as Thompson's study site was part of continuous woodland some individuals who failed to become established on the study site may also have moved off it and became established elsewhere. In contrast, Wauters *et al.*, (1994) studied survival and lifetime

reproductive success in dispersing and resident red squirrels. Body mass, longevity, lifetime reproductive success and dominance rank of 34 resident offspring (settling within 400m of the natal range) and 70 immigrants (not born on the study site and established a home range more than 400m away from a potential birth place i.e.> 400m from those edges of the study sites that bordered the rest of the forest) were compared and found to be similar for residents and immigrants. Survival in the period prior to settling on a home range was also found to be similar.

Another characteristic of the pattern of migration is the interval between migrants. In its simplest form the island model assumes that probability of migration per generation is constant. Will 1 migrant per generation have the same effect as 10 migrants every 10 generations? Grey squirrel dispersal is likely to be high in autumns when population density is high and food availability is low, and vice versa (Gurnell, 1987). Lacy (1987) concludes that sporadic immigration should be just as effective as steady supplementation provided that the same long-term average rate is maintained. On the other hand Levin (1988) concludes that a variable migration rate homogenizes less than a uniform rate with the same mean. Mills and Allendoff (1996) note that that is no empirical data to offer an insight into this question.

In conclusion, it is difficult to translate measures of population differentiation, for example F_{ST}, into numbers of migrants per generation, and these measures may be best considered as an indicator of the historical genetic connectivity between populations (Lowe *et al.*, 2004). Given that the woodlands of Clongowes Wood, Mountarmstrong and the K Club are within a range of 1.5-4.5km of each other, that at these distances dispersal is infrequent, particularly when dispersing through a matrix between two woodlands (Flyger, 1960; Mosby, 1969; Fitzgibbon, 1993; Taylor *et al.*, 1971; Hale *et al.*, 2001b, for red squirrels; this study, section 3.3.1), it is logical that differentiation between populations could

be evident and significant at both a national and local scale.

2.4.4 Isolation by Distance

According to Slatkin (1993), during the evolution of a gene flow-drift equilibrium system, IBD should be evident first over small geographical distances, then over larger ones. However, physical barriers to gene flow may prevent a pattern of IBD from emerging (Garnier *et al.,* 2004).

Data was available to test for IBD mainly over larger geographic distances (up to 263km, Table 2.2). IBD was evident when all 7 populations were used in the Mantel test, but not when only 5 populations were used (excluding the K Club and Mountarmstrong). The difference in the two analyses can be attributed to the effect of incorporating the cluster of three populations in Co. Kildare, Clongowes Wood, the K Club and Mountarmstrong as independent samples in the larger scale analysis. The cluster of three populations in Co. Kildare, Clongowes Wood, the K Club and Mountarmstrong, are separated by 1.5-4.5km and have pairwise F_{ST} values ranging from 0.02-0.03 and similar pairwise F_{ST} values with each of the other populations (Table 2.2). Consequently, when included in an analysis the isolation by distance relationship is significant.

Two factors may be responsible for the absence of IBD and gene flow-drift equilibrium in the 5 population analysis. First, there is a sharp discontinuity in allele frequencies between Castle Leslie and Beau Parc. Beau Parc is located on the south bank of the River Boyne. Rivers have been shown to be a significant barrier to dispersal in grey squirrels. Grey squirrels were introduced to Castleforbes on the east bank of the River Shannon in 1911 and have failed to become established on the western side to date (but see section 4.3). O'Teangana (1999) notes that "the Lower Bann river with only seven bridges crossing it, has proved a considerable barrier to grey squirrel entry into Antrim", Grey squirrel

distribution was continuous on the west bank of the Lower Bann when O'Teangana (1999) conducted his survey. On the east bank there were only two "recent reports [of grey squirrels]..at Toome and Portglenone suggesting a breakthrough," both locations being adjacent to bridges. In the south of Ireland, grey squirrels distribution is continuous along the north bank of the River Suir, with only one breakthrough in Gurteen, south of the bridge in Kilsheelin (section 4.3). In order for significant population differentiation to occur between populations either side of a river, it is not necessary for dispersal to be entirely prevented. If it is significantly reduced, (perhaps to between 1 and 10 migrants per generation (Mills and Allendorf, 1996)) then population differentiation can occur. Major rivers have also been noted as a barrier to gene flow in Eastern Chipmunks (*Tamias striatus*) in Canada (Chambers and Garant, 2010).

Noting pairwise comparisons between Clongowes Wood, immediately south of Beau Parc, and other populations, there is further evidence of IBD emerging. The pairwise comparisons are Clongowes Wood-Mountarmstrong, F_{ST} = 0.02, distance apart 1.5km, Clongowes Wood-K Club, F_{ST} = 0.03, distance apart 4.5km, Clongowes Wood-Beau Parc, F_{ST} = 0.08, distance apart 43km, Clongowes Wood-Mount Juliet F_{ST} = 0.11, distance apart 91km, Clongowes Wood-Castle Leslie F_{ST} = 0.12, distance apart 113km. A linear regression plot of ln pairwise distances v pairwise F_{ST} shows a significant relationship (R^2=0.96, P=0.001) (Figure 2.5).

The study sites in this analysis are aligned roughly on a north-south axis. The River Boyne, with its tributaries the River Blackwater, Tremblestown River, Stoneyford River, River Deel and Knightsbrook River, is the only river system to flow west to east through the study sites (see Figure 4.1). Populations further south of Beau Parc, for example Clongowes Wood, are less differentiated from Castle Leslie and Portglenone than Beau Parc itself. This suggests that while gene flow directly across the River Boyne at Beau Parc may be reduced, greater

gene flow may be taking place around the western edge of the River Boyne system, and across those western parts of the River Boyne system with more rural bridges to allow greater grey squirrel dispersal. The distance, from Beau Parc, Clongowes Wood or Mount Juliet to Castle Leslie or Portglenone, via the western end of the River Boyne system, might better reflect the distance over which gene flow is occurring and IBD should be measured. To investigate this it would be necessary to analyse grey squirrel populations near the western end of the River Boyne system, and on the north bank of the River Boyne opposite Beau Parc, and see how they fit into the IBD analysis.

Second, all grey squirrel populations in Ireland have been newly established in the fairly recent past (Table 2.4), each presumably by a small number of founders. Insufficient time may have elapsed for gene flow-drift equilibrium to be established in these populations. For example, the population at Portglenone was founded in about 1994 or slightly prior to this (O'Teangana, 1999). This population had been established for about 12 years prior to sampling and genetic analysis. The genetic distance between Portglenone and Castle Leslie was small (F_{ST} = 0.03) even though the populations were separated by 67km. The genetic distance between longer established populations a similar distance apart is larger (Beau Parc-Clongowes Wood, F_{ST} = 0.08, 43km apart; Clongowes Wood-Mount Juliet, F_{ST} = 0.11, 91km apart). The bottleneck detection test for Portglenone (Wilcoxon Test, P=0.08) was not significant, but does suggest that the effects of a bottleneck or founder effect are still present in the population. The bottleneck detection test for Castle Leslie was significant (Wilcoxon Test, P=0.03), approximately 45 years after becoming established. Stochastic founder effects are likely to be responsible for the similarity between Portglenone and Castle Leslie and insufficient time may have elapsed for significant divergence to occur due to genetic drift.

2.4.5 2MOD

The 2MOD analysis supports the existence of an equilibrium between gene flow and drift when compared to an equilibrium of (pure) drift (*P* gene flow model 0.935; P drift 0.065; Bayes factor 14.4 gene flow model. Support for the gene flow model is not as strong as found for red squirrel populations in continuous forest in the Alpine Alps (Trizio *et al.,* 2005), where *P* gene flow model = 0.99, *P* drift = 0.02, Bayes factor = 499 gene flow model, but is nonetheless substantial. It suggests that an overall equilibrium between gene flow and drift exists in the Irish grey squirrel population with further investigation around the River Boyne merited.

2.4.6 Bottleneck detection

Grey squirrels populations in Ireland have potentially undergone a myriad of bottlenecks or founder effects in the past 100 or so years. The first was when a number of individuals were introduced from North America to Woburn Abbey in the UK, the second when 12 of this population were used to found a new population at Castleforbes and, thereafter, every new population was founded by a small number of individuals dispersing from other nearby populations.

Therefore, it is surprising that all populations did not show a heterozygosity excess. The tests for heterozygosity excess can detect bottlenecks for only a given 'window of time' after a bottleneck has been initiated (Cornuet and Luikart, 1996). Power analysis and theoretical models in suggest that a bottleneck of N_e is likely to be detectable for ~25-250 generations (0.25-2.5 times $2N_e$ generations) after the initiation of a population reduction (Cornuet and Luikart, 1996). Piry *et al.,* (1999) later narrowed this suggestion to 1-2 times $2N_e$ generations. Thus, only 'recent' historical population declines are detectable (Cornuet and Luikart, 1996). As the longest established population in this study, Castle Leslie (45 years), showed evidence of a bottleneck it is reasonable

to suggest that the 'window of time' has not passed during which bottlenecks could be detected in other populations. To achieve reasonably high statistical power (>0.8), Piry *et al.,* (1999) recommend genotyping at least 10 polymorphic loci and sampling at least 30 individuals per population. It is possible that there was insufficient power in the 5 loci used in this study to detect bottlenecks in all other populations.

However other studies have also failed to detect population bottlenecks in rodents where they were known to exist. Busch *et al.*, (2007) tested three methods of detecting population bottlenecks, heterozygosity excess, mode shift and M-ratio tests, using two banner-tailed kangaroo rat, *Diplodomys spectabilis*, populations that were known to have experienced recent demographic reductions. Neither population showed a genetic bottleneck signature. No evidence of heterozygosity excess or mode shift was uncovered in mainland populations of snowshoe hares or voles, despite the regular reductions in these periodically cycling species (Burton *et al.*, 2002; Berthier *et al.*, 2006; Redeker *et al.*, 2006) or in red squirrels in recently fragmented forest (Trizio *et al.*, 2005). This is probably because immigration, even at a very low rate, and possibly an elevated rate of mutation, can quickly erase a bottleneck signature (Busch *et al.*, 2007).

2.4.7 Sex biased dispersal

2.4.7.1 Breeding Dispersal

All the indices estimated in this study suggest that in the grey squirrel breeding (inter-population) dispersal rates are higher among males than females. Significant differences between the sexes were observed in the mean assignment index and in the F_{IS}, whereas differences in the assignment index variance and F_{ST} were not significant.

These tests have been implemented for a variety of species. In the greater white toothed shrew (*Crocidura russula*) (Favre *et al.,* 1997) direct field observations and genetic data found dispersal to be highly female biased which is unusual among mammals. In the red-billed quelea (*Quelea quelea*) (Dallimer *et al.,* 2002) dispersal was male biased, in the bullfrog (*Rana catesbeiana*) (Austin *et al.,* 2003) and the common frog (*Rana temporaria*) (Palo *et al.,* 2004) female biased, in brook charr (Salvelinus fontinalis) (Fraser *et al.,* 2004) male biased and in White's skink (*Egernia whitii*) (Chapple and Keogh, 2005) no clear and consistant evidence was found.

Where possible genetic data on sex-biased dispersal should be compared to direct field data. Taylor *et al.,* (1971) noted that the maximum recorded distances moved by male and female squirrels were different. Males travelled longer distances more frequently than females. For example, slightly over 10% of females caught more than once were recorded at points more than 360m apart, whereas the corresponding portion for adult males was 40%. Mosby (1969) reported intermittent live trapping was carried out in many of the woodlots on Virginia Polytechnic Institute Farm (VPIF) between 1947-1966. Out of a total of 812 grey squirrels, there were 45 records (5.5%) of between woods movements involving 30 males and 15 females. The average distance travelled between woods by the males was 880m; the females averaged 608m. Another study on VPIF focused on two woodlots separated by 200m at their nearest points (Mosby, 1969). There were 25 between woods movements. The sex-age of the individuals involved were 15 males, 10 females; 10 adults (>16 months), 10 subadults (6-16 months) 5 juveniles (<6 months). Other studies indicate more juvenile males than juvenile females make long distance dispersal movements (Gull 1977 in Gurnell, 1987; Don 1981, in Gurnell, 1987). Therefore, both genetic data and direct field data are in agreement predicting male biased breeding (long distance) dispersal in grey squirrels.

2.4.7.2 Sex biased natal dispersal

The single population spatial autocorrelation analysis did not detect any genetic structure (i.e. any evidence of sex biased natal dispersal) in the Beau Parc population at any distance class for males, females or the entire population. Neither Thompson (1978a) (grey squirrels) nor Wauters *et al.,* (1994) (red squirrels) detected any sex bias in dispersal in their studies. However, sex biased natal philopatry has been directly observed in grey squirrels by Koprowski (1996) and Gurnell *et al.,* (2001).

Koprowski (1996) found that natal philopatry was female-biased in grey squirrels studied for 4 years in a 4.2ha parkland on the University of Kansas campus. He considered squirrels to be philopatric if continuous residence was maintained within any portion of the natal area until an age of $\geq$1.25 years, the age at which adult body size and reproduction may be achieved. In actuality, philopatric individuals used the same dens and home range before and after maturation. Of 43 females captured as juveniles, 16 (37%) remained in their natal area until at least 1.25 years of age while only two of 34 (6%) males remained in their natal area. These philopatric males remained in the natal area following their mothers' death immediately after weaning.

Gurnell *et al.,* (2001) studied spacing behaviour, kinship and population dynamics of grey squirrels in a newly colonized broadleaved woodland in Italy. Juvenile female grey squirrels were philopatric, while must juvenile males settled off the mothers home range, resulting in the formation of female kin groups.

Assuming evidence of sex biased natal dispersal was present in Beau Parc there are a number of reasons why it may have not been detected.

The sex bias in natal philopatry might not have been very strong, for

example, in Koprowski (1996) it was 37% female philopatry v 6% male philopatry. Consequently, even in Koprowski's study, considerably more females dispersed away from the natal area than remained. This would weaken any genetic structure in the population and make a sex bias in natal philopatry difficult to detect.

The number of squirrels trapped from individual kin groups may have been too small. Given a weak bias for natal philopatry, unless most, or all, members of those kin groups which did exist were trapped, genetic structure in the population would be difficult to detect.

Radiotracking data in Gurnell *et al.,* (2001) showed the kin group were located within an area of about 200m X 100m. No radiotracking data was obtained on core areas of individuals in Beau Parc. The location of capture of individuals may not have reflected their core areas with sufficient accuracy and precision to allow a kin group be distinguished from unrelated neighbouring individuals.

Too few markers with too little polymorphism may have been used to adequately distinguish kin from non-kin.

2.5 Conclusions

The Irish grey squirrel populations displayed moderate genetic diversity at the microsatellite loci studied, generally similar to other sciurid populations.

There is no evidence of inbreeding in Irish grey squirrel populations.

Population differentiation is significant at both a local and national scale suggesting restricted gene flow between populations.

There is some evidence of an equilibrium between gene flow and genetic

drift, as seen in the pattern of isolation by distance, but the River Boyne may present a significant barrier to gene flow.

There is genetic evidence of male bias in breeding dispersal.

3. Population size recovery in grey squirrels following control

3.1 Introduction

The introduced American grey squirrel, *Sciurus carolinensis*, is responsible for the decline in population size and range of the red squirrel, *Sciurus vulgaris*, in Ireland (O'Teangana *et al.,* 2000) and the U.K (Gurnell and Pepper, 1993). The grey squirrel also damages many species of broadleaved tree by barkstripping (Lawton, 1999). Control of grey squirrel populations is therefore desirable. Previous work has shown that grey squirrel population size recovers following culling within 4 weeks (Rowe, 1980) or 6-10 weeks (Lawton, 1999). A better understanding of the factors driving population size recovery i.e the effectiveness of culling at reducing population size, and subsequent recruitment and immigration to the population, would aid the design of more efficient control programmes.

3.1.1 Control of invasive vertebrate pests

Control of an invasive pest species is likely to be needed in perpetuity unless their pest status changes or they are completely eradicated (Nugent *et al.*, 2007). A wide array of methods is used to control invasive terrestrial vertebrates and these vary somewhat by taxonomic group (Witmer *et al.*, 2007). Complete eradication of invasive terrestrial vertebrates, is more difficult but is increasingly successful on islands with chemical poisons and hunting (Myers *et al.*, 2000).

Management of invasive rodents most often utilizes rodenticides, and primarily anticoagulants. These methods are supplemented, especially in and around buildings, with trapping, exclusion and sanitation, and habitat modification (Witmer *et al.*, 2007). Most island eradications have

utilized anticoagulant rodenticides - hand broadcast, in bait stations, or aerially broadcast (Howald *et al.*, 2007). Examples of successful eradications from islands in New Zealand have included the house mouse (*Mus musculus*), the black rat (*Rattus rattus*) and the Norway rat (*Rattus norvegicus*). Norway rats were removed from Langara Island (3100 ha) on the northwest tip of Queen Charlotte Islands, British Columbia, in a matter of months through an intensive poisoning program (Myers *et al.*, 2000).

Carnivores are mainly trapped and shot. Hunting with dogs and toxic bait (monofluoracetate in fish baits) has been used to control feral cats (*Felis catus*) on islands (Nogales *et al.*, 2004). Introduced foxes have been eradicated from several Aleutian Islands with the use of shooting, traps, and toxic baits (Ebbert, 2000).

Ungulates are controlled by shooting, trapping, pursuit with dogs, exclusion, food removal, and Judas animals (Witmer *et al.*, 2007). Goats (*Capra hircus*) have been eradicated from Isla Santiago (58,000ha) in the Galapagos Islands, with 66,329 killed in 37 hours flying time and 59 forays. Man dog teams and Judas goats were used when numbers were at their lowest (Sherley, 2007).

Methods used to manage invasive bird populations are similar to those used for mammals: traps, shooting, exclusion, and limited use of toxicants (Witmer *et al.*, 2007). Millett *et al.*, (2004) used shooting and toxicants to eradicate invasive common mynahs (*Acridotheres tristis*) from several small islands in the Seychelles. An attempt is underway to eradicate the ruddy duck (*Oxyura jamaicensis*) from the U.K., although full eradication will require the control of other European populations and the management of existing captive stocks (Henderson and Robertson, 2007).

Trapping, hand-capture, pit fall traps and toxicants are most commonly used with amphibians and reptiles. Most method development has occurred for brown treesnakes (*Boiga irregularis*) in Guam (80mg acetaminophen in dead neonatal mice, delivered in polyvinyl chloride tubes or aerially via biodegradable parachutes (Savarie *et al.*, 2007) and for coqui frogs (*Eleutherodactylus coqui*) in Hawaii (16% citric acid or 3% hydrated lime) (Sin and Radford, 2007).

Eradication of vertebrate pests from mainland sites has been attempted within areas bounded by multiple species exclusion fencing. Exclusion fencing can exclude rodents (including mice), lagomorphs, mustelids, hedgehogs, brushtail possums, cats, dogs, feral pigs, goats, deer, and domestic livestock. Supporting components and technology, such as pest-free pedestrian and vehicle gates, waterway gates and remote surveillance systems to mitigate reinvasion risks have enabled some projects to succeed. Over 20 exclusion barrier systems have now been constructed in areas up to 3,400 ha in size and have allowed the reintroduction of threatened wildlife species to mainland sites (Day and MacGibbon, 2007).

Grey squirrels are controlled by shooting (alone or combined with drey-poking), trapping and poisoning (Shorten, 1962; Marstrand, 1974; Taylor, 1977; Rowe, 1980; Rowe, 1983; Pepper and Currie, 1998). As far back as records show squirrels have been hunted for their fur and as food (Gurnell, 1987). Today, they are shot for sport and culled to prevent bark stripping damage to broadleaved trees (Pepper and Currie, 1998) or as part of red squirrel conservation programmes, for example on the island of Anglesey (Ogden *et al.,* 2005). Immunocontraceptive vaccines are also being investigated as a means of inhibiting the fecundity of a variety of mammalian species (Miller, 2002).

Shooting is inexpensive, but inefficient as a means of controlling grey squirrel population size (Rowe, 1980). It is now usually carried out for

recreational purposes, with a secondary aim of controlling grey squirrel numbers. Mosby (1969) found that that shooting did not adversely affect recruitment, has no significant influence on the average annual mortality rate and probably only removed a segment of the population that would normally be lost to 'natural losses'.

Trapping, and subsequent culling, is considered an effective method of removing grey squirrels (Shorten, 1962; Rowe, 1980; Rowe, 1983; Pepper and Currie, 1998). It is the method of choice when controlling grey squirrels in woodlands where both red and grey squirrels are present. although traps are expensive and trapping is very labour intensive (Marstrand, 1974; Taylor, 1977)

Traps must be set to coincide with the activity periods of grey squirrels and during months when the probability of capture is highest. Grey squirrels do not hibernate and are diurnal (Reynolds, 1985). During winter activity begins at dawn and peaks in the late morning. In summer activity begins at dawn, peaking 2-4 hours later, and begins again in the afternoon, peaking 2-4 hours before sunset. Spring and autumn are intermediate between summer and winter (Tonkin, 1983; Reynolds, 1985). Therefore, traps should be set at dawn and must be checked at least once, or ideally twice, daily. As grey squirrels spend much of their foraging time on the ground (86% compared to 33% for red squirrels in comparable habitats (Kenward and Tonkin, 1986), traps may be placed on the ground.

Gurnell (1996) noted that the mean probability of capture of grey squirrels in an oak wood in southern England over a 12 year period, was 0.51 in January, 0.66 in April and 0.84 in June/July. In April the probability of capture varied from 0.19 in 1986 to 0.91 in 1987. Capture probabilities in winter and to a lesser extend in spring were inversely related to food availability. Following a large autumn seed crop in 1987, Gurnell (1996) noted that it was difficult to catch squirrels at any time

during 1998. Therefore, unless a cull is conducted during June/July or in a year following a very poor autumn tree seed crop, it is likely to miss a proportion of the grey squirrel population.

Even when trapping during the summer there can be considerable difficulty in capturing grey squirrels. Prior to a summer cull of grey squirrels in Thetford Forest, Gurnell and Steele (2002) radio-collared 6 individuals in 2000 and 7 in 2001 to reveal the approximate positions of the animals before, during and after control. None of the radiocollared animals were trapped during the 7 days control trapping in 2000 and only 1 in 2001. Most of the animals stayed within a small area, with ranges adjacent to or overlapping control compartments throughout the study, but avoided capture.

In Britain, poisoning using Warfarin 0.02% on whole wheat bait, placed in specially constructed hoppers at density of 1 per 1-5 hectares, between 15 March and 15 August, is the method of choice to control grey squirrel population size for the purpose of preventing bark stripping damage. Warfarin may only be used where red squirrels and pine martens are absent (Pepper and Currie, 1998).

3.1.2 Grey squirrel population recovery following control

In studies to date, grey squirrel population recovery following control generally occurs within a matter of months. Taylor (1977) reported that a 10 acre woodland, isolated by 800 yards from any other woodland, was repopulated in 3 months. In a second example, 40 acres of oak within a large area of forest were systematically cleared of grey squirrels by shooting and trapping with 36 adult grey squirrels bring killed. Two months later 17 grey squirrels were trapped. 1 month later a further 15 were killed and eight months later 39 more grey squirrels were killed (Taylor, 1977). Rowe (1980) notes that grey squirrels can migrate into an area cleared of squirrels in less than 4 weeks during the May-July

period, but provides no further details on the size of the woodland or method of control.

Lawton and Rochford (2007) investigated the recovery of grey squirrel populations after intensive control programmes. They conducted 5 culls in broadleaved woodland in Co. Meath. In three out of four springtime culls, the populations recovered to pre-cull levels within ten weeks. In the four springtime culls, greater numbers of squirrels were removed than had previously been recorded as present in the woodland immediately before the culls. Following the springtime culls the squirrels captured in the post-cull samples were generally unmarked adults.

The authors concluded that: a cull of five days was sufficient to remove most, if not all, of the squirrels within the woodlands concerned; this was evident from the very low numbers of previously marked animals in the postcull samples; post cull populations tended to consist of unmarked adult animals which had entered from outside the woodland.

3.1.3 Hypotheses behind the removal experiment

Previous work has shown that grey squirrel populations recover rapidly following culling within 4 weeks (Rowe, 1980) or 6-10 weeks (Lawton, 1999). Following on from this work the following three hypotheses were examined, and are discussed below.

1. Culling removes mainly dominant squirrels but fails to remove a cohort of subordinate squirrels, which are then responsible for the population recovery described above.

2. Culling removes mainly larger, heavier squirrels but fails to remove a cohort of smaller, lighter squirrels, which are then responsible for the population recovery described above.

3. Grey squirrels from neighbouring woodlands disperse to the study site post cull and contribute significantly to the re-establishment of the population

The concept of dominance has contributed greatly to the understanding of social structure in animals (Drews, 1993). Dominance is defined as an attribute of the pattern of repeated, agonistic interactions between two individuals, characterised by a consistent outcome in favour of the same dyad member (the dominant individual) and a default yielding response of its opponent (the subordinate individual) rather than escalation (Schjelderupp-Ebbe, 1922 in Drews, 1993). Dominance rank refers to the position of an individual in a hierarchy and is a relative measure, not an absolute property of individuals, and may change as group composition changes (Drews, 1993). Problems inherent in determining dominance ranking of individuals include choosing measurements, response variables and statistical techniques. Individual dominance measurements may only assess situation- or resource-specific responses (Lanctot and Best, 2000) and there has been relatively little work on the factors determining dominance rank and its importance in free-ranging grey squirrels.

Sex, age, body size, group size, type of society and interval of study are important determinants of dominance rank. In Townsend's chipmunks large females are more frequently dominant over small females and always dominant over small males. Larger males were dominant over smaller females. Large body size was a good indicator of female dominance during the non-breeding season (Edelman and Koprowski, 2006). In the highveld mole-rat, *Cryptomys hottentotos pretoriae*, dominance rank could not be predicted on the basis of body mass, sex or assumed reproductive status (Malherbe and Bennett, 2007). In banded mongoose, *Mungos mungo*, dominance hierarchies were linear but were not correlated with age (De Luca and Ginsberg, 2001). Social

groups of European badgers have dominance hierarchies, with an effect of sex but not age, on dominance rank (Hewitt *et al.*, 2009).

In grey squirrels a social structure exists based on a dominance hierarchy formed through agonistic encounters (Gurnell, 1987). For example, dominant grey squirrels fight for and defend proximity to the oestrus female during mating bouts and account for more copulations than subordinates (0.83 v 0.23 copulations/male/mating bout) (Koprowski, 1993). Pack *et al.*, (1967) described linear hierarchies between male and female grey squirrels at artificial feeders in two woodlots. Males were dominant over females. Social rank also increased with age in both woodlot populations. Dominant grey squirrels appeared to restrict subordinate individuals from gaining access to food resources Pack *et al.*, (1967) do not provide any data on squirrel weight but they noted that greater size, experience and hormonal development might explain the increase in rank with age. In grey squirrels generally, males dominate females, adults dominate juveniles and dominant squirrels are normally heavier than subordinates (Pack *et al.*, 1967; Allen and Aspey, 1986). The integrity of the hierarchy appears to be maintained by visual and/or olfactory cues (Taylor, 1968; Thompson, 1978a,b).

The first hypothesis assumes that dominant grey squirrels learn to use baited traps as a food source during the prebaiting phase of trapping (and during capture-mark-recapture studies, if applicable) and exclude subordinate squirrels, mainly young adults and juveniles, from the traps during the early stages of a cull. To test this hypothesis the sex and weights of squirrels trapped and their order of capture was analysed.

The second hypothesis is based on the finding that larger grey squirrels have a higher daily energy expenditure (DEE) than smaller grey squirrels (Bryce *et al.*, 2001), and so must consume more food. Therefore, larger grey squirrels may enter baited traps more readily than smaller grey squirrels. This hypothesis was tested using the analysis of the weights of

squirrels trapped and their order of capture above. Several studies have also reported difficulties in trapping red squirrels, which have a smaller body mass than grey squirrels, where grey squirrels are present (Kenward and Hodder, 1998, Bryce and MacDonald, 2000). The difference in trappability between red and grey squirrels may be partly due to the higher DEE of grey squirrels compared to red squirrels. When comparing DEE there is no evidence of a species effect other than that which could be attributed to the difference in body mass between the species (Bryce *et al.*, 2001).

The third hypothesis is that grey squirrels from neighbouring woodlands disperse to the study site post cull and contribute significantly to the re-establishment of the population. The alternative hypothesis is that the population re-establishes from survivors of the cull and gradually increases in size as their offspring are born. This hypothesis will be tested in three ways.

Firstly, if it can be determined that an individual found on the study site post cull is the offspring of adults resident on that study site then the most logical explanation is that it was missed by the cull and is a local recruit. Likewise an individual trapped on the study site but born to parents resident on a different site must have dispersed to the study site. In this way it should be possible to assess the role of local recruitment and long distance dispersal (immigration) on the re-establishment of the population.

The genetic distance between the populations from 2003-2005 will also be examined to determine if an influx of immigrants from neighbouring populations following the cull reduced the genetic distance between the study site population and the neighbouring populations.

Finally, a population model will constructed to determine what set of population parameters could explain the observed recovery in the study site population.

3.1.4 Objectives

Determine if dominant/larger squirrels are more likely to be trapped in the early stages of a cull in comparison with subordinate/smaller squirrels.

Understand the processes involved in the reestablishment of a grey squirrel population following a cull by determining the origin of individuals found on the study site post cull

Conduct a parentage analysis on juveniles found on the study site postcull to identify their parents and determine their woodland of origin

Compare the genetic distance between the study site and neighbouring populations from 2003-2005

Construct a population model to determine what set of population parameters could explain the observed rate and extent of recovery in population size

Compare genetic and observational estimates of dispersal/gene flow

3.2. Methods

3.2.1 Study sites

The study area contained four broadleaved woodland study sites: Clongowes Wood, Mountarmstrong and the K Club, as described in Section 2.2.1, and Loughbollard, located in Co. Kildare (Figure 3.1a-d).

Loughbollard is a small 1 hectare woodland, located 500m southeast of Clongowes Wood, composed almost entirely of mature beech, with just a few mature sycamore and oak. The canopy is open and the understory is composed mainly of grass.

3.2.2 Trapping

In order to deal with a clearly defined population, and to ensure the cull is as complete as possible an 'island' study site of manageable size (between 10-15 ha), and having a number of surrounding woodlands within 3km, was chosen. The grey squirrel populations of the woodlands were then studied for 3 years to quantify the extent of dispersal from each woodland into the study site post cull.

It was decided not to estimate the size of the study population in the study site prior to conducting the cull, for example by using a capture-mark-recapture method. The study aimed to examine the effects of a cull on a population of grey squirrels not previously exposed to traps, bait etc., as would be the case if a cull was conducted in practice by a forester or wildlife manager.

It is important to deal with a clearly defined population when conducting culls and studying long distance dispersal. If a cull is carried out in one part of a woodland then it could be expected that the surviving squirrels in the remainder of the woodland would rearrange or shift their home

ranges over time to take advantage of the space and resources newly available following the cull (the vaccum effect). This is not long distance dispersal and such movements are not the focus of this study.

Prior to the first cull and in early spring of each subsequent year, grey squirrels were trapped and tagged in the woodland sites surrounding the main study site. Following the spring dispersal (April/May) the main study site was monitored in June or July and following the autumn dispersal (September-mid-December) the study site was monitored in February of the following year. As the population of the study site was greatly reduced following the cull it was expected that the survival of any immigrants would be greatly increased. Genetic parentage analysis was used with the aim of determining the parentage of untagged juveniles found on the study site following the cull.

It is believed that once adult squirrels have become established residents in a population, they remain in the same general area of the forest for life, unless a shortage of food forces them to leave (Gurnell, 1987). Studies indicate that juveniles and young adults are responsible for almost all dispersal (Thompson, 1978b; Gurnell, 1987).

Trapping, weighing, ear tagging, culling and sampling ear tissue of all grey squirrels trapped was conducted as described in Section 2.2.2. All grey squirrels in Clongowes Wood had tissue samples taken from the right ear only. All squirrel samples from Loughbollard, Mountarmstrong and the K Club had tissue samples taken from the left ear only. In this way when one, or occasionally both, tags were lost from a grey squirrel, the woodland of origin of the individual could still be identified. Trap density was high in all sites, approximately 2.5-3.3 traps/ha.

Juvenile males are less than 10-12 months old, with undescended testes and small scrotal sacks. Juvenile females are less than 12 months old

and have a very small vulva and still invisible nipples (Wauters and Dhondt, 1992; Wauters and Lens, 1995).

The reproductive condition of adult males was based on the size and position of the testes. Breeding males had large scrotal testes; non-breeding animals had small testes. Females were recorded as non–breeding or breeding. Breeding was defined as pregnant (swollen abdomen), lactating (large swollen nipples, milk secretion sometimes noted) or recently finished lactating (nipples still prominent but becoming smaller). Grey squirrels were handled in a wire mesh handling cone and it was not always easy to decide if a female was pregnant. Females were only classified as pregnant if it was clear from their body mass and size of abdomen. Consequently, the number of females pregnant is likely to be underestimated (Gurnell, 1996).

During spring trapping, pregnancies which occurred early in the breeding season (December-January) were likely to be detected, but pregnancies which occurred later were not. During summer trapping, females who were pregnant early in the breeding season (December-January) would have stopped lactating about 4-6 weeks previously and would be recorded as non-breeding, providing they were not pregnant again. Females who were pregnant later in the breeding season, and whose pregnancy escaped detection, would have stopped lactating more recently and would be recorded as breeding (recently finished lactating). Therefore, spring estimates of breeding females are underestimates and summer estimates are overestimates. However, the overall estimate of the number of breeding females would be accurate. In summer second pregnancies among the early breeding females were detected on the basis of body mass and enlarged nipples.

From February-March 2003 grey squirrels were trapped for 12 days in Mountarmstrong, 8 days in the K Club and 5 days in Loughbollard. All grey squirrels trapped were weighed, tagged and released. Small samples

of ear tissue were taken from all squirrels trapped, placed in 100% ethanol and stored at $4C^0$. Trapping was conducted until all grey squirrels were captured and tagged. Throughout the study, this was considered to have been achieved when there were no new captures (Gurnell, 1996) or just 1 new capture for three consecutive days.

Figure 3.2 details the months and years in which trapping occurred in all study sites from 2003-2005.

In April 2003, grey squirrels were trapped and culled in Clongowes Wood for 14 consecutive days (Cull 1). It was apparent that this first cull failed to remove the entire population as small numbers of squirrels were caught during the latter days of the cull. Culls conducted by woodland managers tend to be carried out over relatively short periods and then repeated at intervals, described by Gurnell and Steele (2002) as a pulsed removal method. It was decided to follow this pattern of culling and examine its impact on the population. A second 14 day cull was therefore planned for May 2003 but damage to the traps forced its postponement.

In June 2003, grey squirrels were trapped in Clongowes Wood for 14 consecutive days (hereafter called 'Cull 2'). Grey squirrels were not culled but were released unharmed at various points of a broadleaved wood, approximately 10km from Clongowes Wood at its nearest point. In July 2003, trapping and removal resumed for 9 consecutive days in this fashion (hereafter called 'Cull 3'). It was not until the cull at the end of July 2003, when trapping success fell to zero for 3 days, that it was felt that no, or very few, further grey squirrels would be trapped in Clongowes Wood that year.

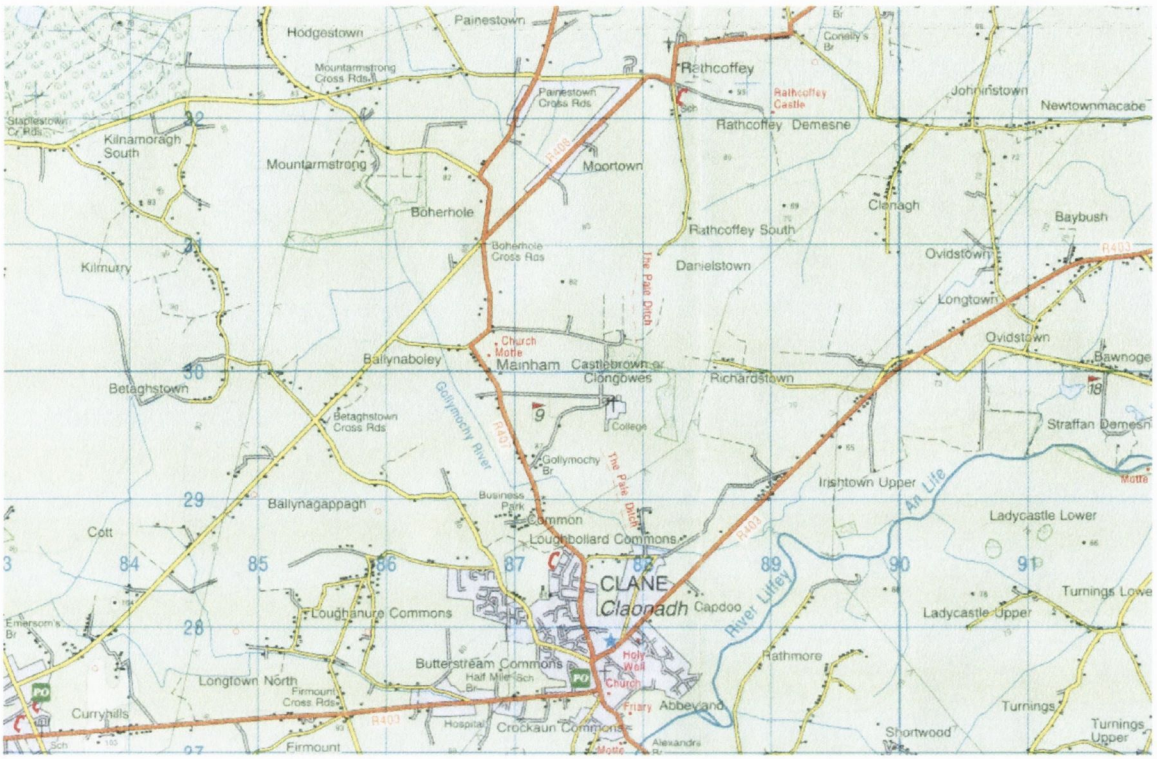


Figure 3.1a. 1km square grid map showing Clongowes Wood, Mountarmstrong, K Club (Straffan Demesne) and Loughbollard study sites and Donadea Forest (top left of picture) in Co. Kildare. Source: Discovery Series Ordnance Survey Map of Ireland No. 49. 2nd Edition, 2003.

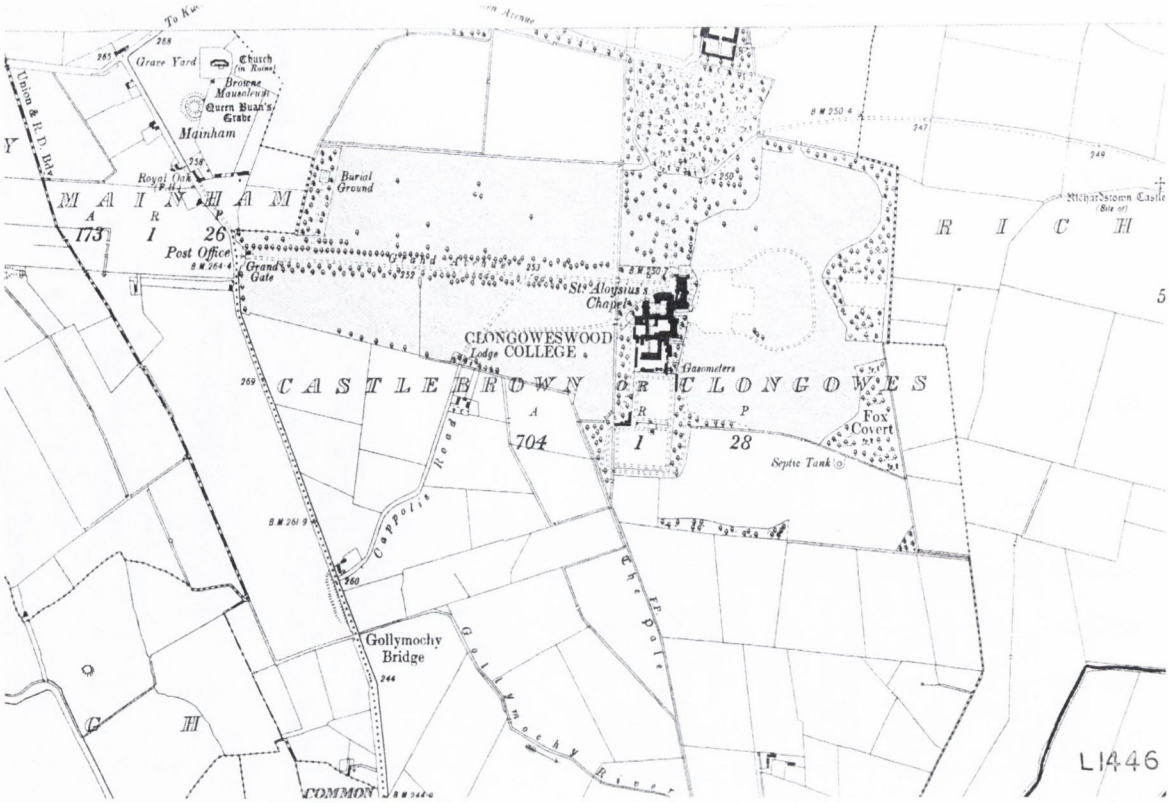


Figure 3.1b. Map of Clongowes Wood study site. Two lines of lime trees can be seen along the avenue leading to the college. Source: Ordnance survey map of County Kildare. Sheet 10. Revised 1908-9. Published 1911. Scale 1:10,560



Figure 3.1c. Map of Mountarmstrong study site. Source: Ordnance survey map of County Kildare. Sheet 14. Revised 1908-9. Published 1911. Scale 1:10,560

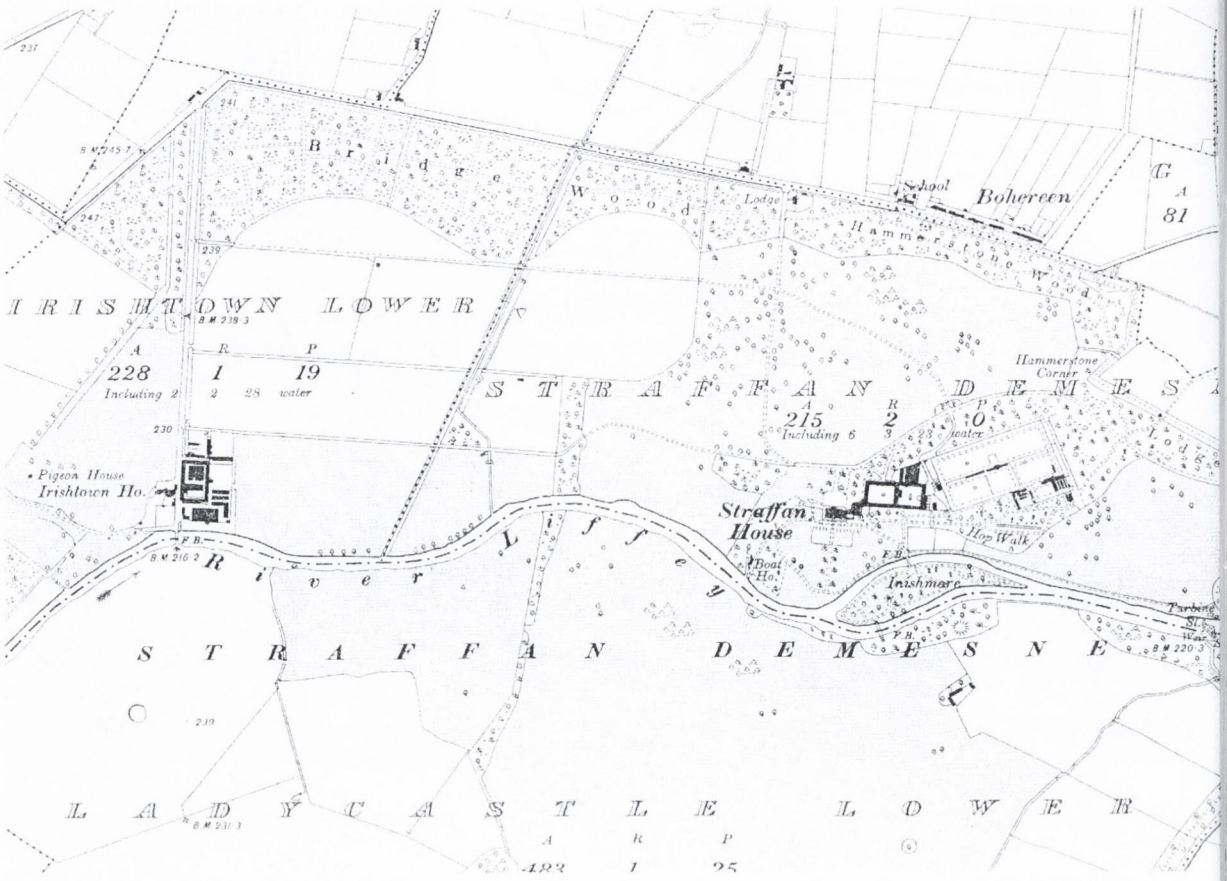


Figure 3.1d. Map of the K Club study site. Source: Ordnance survey map of County Kildare. Sheet 14. Revised 1908-9. Published 1911. Scale 1:10,560

Culls 1-3 were carried out to determine the grey squirrel population size and the success of culling as a means of controlling population size. Culls 2-3 were also carried out to determine the extent of immigration to the woodland during the spring dispersal of 2003 and the origin of colonising animals.

In February 2004, trapping was conducted in Clongowes Wood for 13 days until all grey squirrels in the population were tagged. This was to determine the population size, the extent of immigration to the woodland during the autumn dispersal of 2003 and the origin of the colonising animals. These and all subsequent grey squirrels trapped in Clongowes Wood were released unharmed to allow the population re-establish.

In March 2004, trapping was conducted in the K Club for 9 days and in Loughbollard for 5 days until all grey squirrels were tagged. This was to determine the size of each population, which will act as a control in assessing the recolonisation of Clongowes Wood, and to tag/sample any unmarked animals. Permission was not obtained to resume trapping in Mountarmstrong in 2004.

In July 2004, trapping was conducted in Clongowes Wood for 12 days until all grey squirrels were tagged. This was to determine the population size, the extent of immigration to the woodland during the spring dispersal of 2004 and the origin of the colonising animals.

In February 2005 trapping was conducted in Clongowes Wood for 12 days until all grey squirrels in the population were tagged. This was to determine the population size, the extent of immigration to the woodland during the autumn dispersal of 2004 and the origin of the colonising animals.

In March 2005 trapping was conducted in the K Club for 9 days, in Loughbollard for 5 days and in Mountarmstrong for 8 days until all grey

squirrels were tagged to determine the size of the populations, which will act as a control in assessing the recolonisation of Clongowes Wood, and to tag/sample any unmarked animals which might subsequently disperse to Clongowes Wood.

In June-July 2005 trapping was conducted in Clongowes Wood for 7 days, there was a break for 7 days, traps were prebaited again for 5 days and then trapping was conducted again for 7 days to determine the final population size in the study site.

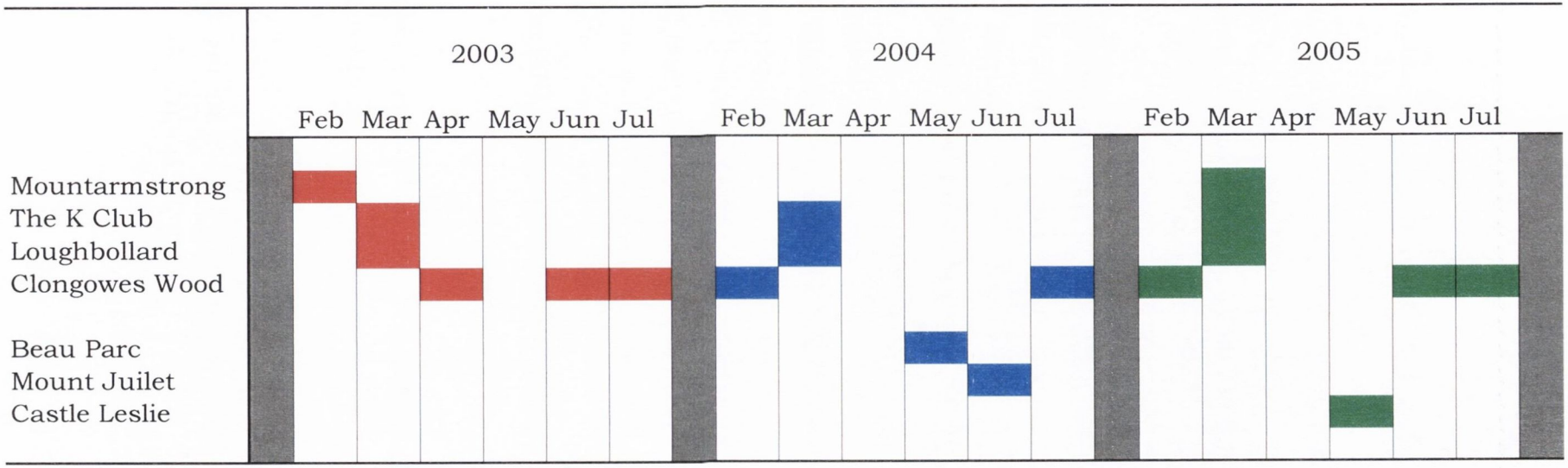


Figure 3.2. Gnatt diagram detailing the month(s) in which trapping occurred in each study site from 2003-2005

3.2.3 Food availability

A vegetation survey was conducted in four woodlands: Clongowes Wood, Mountarmstrong, the K Club and Loughbollard. Line transects were marked in each woodland along straight line paths or tracks. All tree species of seed bearing age visible from the transects were identified. This data was used to estimate the number and percentage of each tree species of seed bearing age present in the woodland (Table 3.5a,b).

Beech is the dominant food source for grey squirrels in the adjacent study sites of Clongowes Wood, Mountarmstrong, the K Club and Loughbollard. Therefore food availability for grey squirrels was determined primarily by the size of the beech mast. 10 beech trees were selected for study in Clongowes Wood, 10 in Mountarmstrong, 8 in the K Club and 4 in Loughbollard. Trees were not selected at random but because they shed onto a suitable substrate, for example a road or path or open ground which could be cleared of fallen leaves and old pericarps. Beechnut seed fall occurs between week 30 and week 42 (Gurnell, 1993). The area under each tree was swept clean in mid July 2003 and 2004. In mid October of 2003 and 2004 the ground underneath each tree was searched for a standard period of 7 minutes. Nuts were collected whether large or small, full or empty, whole or damaged (Hilton and Packham, 1997).

Nuts were sorted into full nuts containing seed, even if damaged by insects or mould, and nuts that were empty or contained shrivelled seed (Hilton and Packham, 1997).

Masting was described in terms of five categories defined by the mean number of full nuts per tree, collected in a 7 minute sample, for each site following Hilton and Packham (1997).

1	very bad	<11
2	poor	<11-50
3	moderate	<51-100
4	good	<101-150
5	very good	<150

3.2.4 Microsatellite analysis

All individuals trapped were genotyped at 5 microsatellites Scv15, Scv19, Scv4, Scv18, Scv6 as described in Section 2.2.3

3.2.5 Trapping data analysis

Grey squirrel population densities were calculated as the Minimum Number known to be Alive (MNA) per hectare. This is the total number of individual squirrels caught in one woodland during one continuous trapping period and assumes there were no losses due to emigration or death (Gurnell, 1996). In Clongowes Wood, grey squirrels trapped on the study site in spring 2004, not trapped in summer 2004, but trapped again in spring 2005 were assumed to have been present on the study site in summer 2004 but not trapped.

The Petersen/Lincoln index was used to determine the final population size (N) in Clongowes Wood in summer 2005. It was calculated as

$$N = \frac{(M + 1)(C + 1)}{(R + 1)} - 1$$

M = number of grey squirrels marked and released in the first session
C = total number of grey squirrels captured in the second session
R = number of marked individuals trapped in the second session.

The standard error (SE) was calculated as:

$$(SE)^2 = \frac{M^2 (C + 1) (C - R)}{(R + 1)^2 (R + 2)}$$

A 95% Confidence Interval was calculated as:

$$N \pm 1.96 (SE)$$

Trapping success (TS) was calculated as the total number of squirrels captured per trap-day for each session and expressed as a percentage.

$$TS = (n_T/(t*d))*100$$

TS = Trapping success
n_T = Total number of grey squirrels trapped during the trapping session
t = Number of traps set each day
d = Number of days traps were set for in the trapping session

Percentage survival of adults, juveniles and overall was calculated for those populations where, as far as possible, all individuals were trapped before and after the time interval specified.

Fishers Exact Test was used to determine if the sex ratios in each cohort of squirrels culled conformed to 1:1. A Kruskal-Wallis analysis of variance was conducted to determine if the body weights of squirrels trapped during each cull were the same. Fishers Exact Test was used to test for an association between body weight and sex in each cohort of squirrels culled (Zar, 1999).

3.2.6 Parentage analysis

The parentage of 19 individuals trapped in Clongowes Wood in spring 2004 was investigated using Cervus 2.0 (Marshall *et al.,* 1998). This was to determine the natal woodland of the founder individuals of the

population in Clongowes Wood following the culls in 2003. 102 out of 112 adults trapped in Clongowes Wood, the K Club, Mountarmstrong and Loughbollard in 2003 were investigated as candidate parents. Tissue samples were misplaced for the remaining individuals. Candidate parents and potential offspring were genotyped at 5 microsatellite loci Scv15, Scv19, Scv4, Scv18, Scv6 as described in Section 2.2.3. The number of simulation cycles implemented in Cervus 2.0 was 10,000, the proportion of candidate parents was sampled was 1.00, the proportion of loci typed was 1.00 and the proportion of loci mistyped was 0.10.

3.2.7 Genetic distance between populations

Previous research has suggested that grey squirrel population recovery postcull occurs in 6-10 weeks as a result of immigration (Lawton, 1999). In Clongowes Wood, Cull 2 and Cull 3 were conducted 8 and 12 weeks respectively after Cull 1. If immigration is primarily responsible for population recovery it would be expected that the genetic distance between the Clongowes Wood Cull 1 population and the Clongowes Wood Cull 2 or Cull 3 population would be similar to the genetic distance between the Clongowes Wood Cull 1 population and the immigrant source populations i.e. Mountarmstrong or the K Club. As the Cull 3 population was small (n=12) the populations from Cull 2 and Cull 3 were combined. This maximised the potential for immigrants to be present in this sample.

The genetic distance between the populations sampled in Clongowes Wood Cull 1 (n=54) and in Clongowes Wood Cull 2 (n=38) and Cull 3 (n=12) combined (total combined population n=50) was calculated using GENALEX (Peakall and Smouse, 2006). A graphical representation of the distance matrix using pairwise Nei's standard genetic distance, Ds (Nei, 1978) was then constructed using the UPGMA algorithm of PHYLIP version 3.5c (Felsenstein, 2004) as described in section 2.2.4. To provide

support for the nodes in each tree 1000 bootstrap replicates of the original gene frequency data set were generated (Felsenstein 2004).

For scale and comparison purposes only, populations from other parts of Ireland were also included in the analysis. As only 6 individuals were present in the Loughbollard population, this population was not considered as the population size was too small to have a consistent allele frequency 'signature' or have a significant effect on the recovery of the Clongowes Wood population postcull.

Pairwise Nei's genetic distance, Ds, (Nei, 1978) was then calculated between the Clongowes Wood, K Club and Mountarmstrong populations from 2003-2005 using GENALEX (Peakall and Smouse, 2006). This was to determine if the populations present in Clongowes Wood in 2004 and 2005 were more closely related to the original Clongowes Wood population of 2003 or to neighbouring populations. All individuals sampled in Cull 1, Cull 2 and Cull 3 in Clongowes Wood in 2003 were now considered to belong to one Clongowes Wood population. A graphical representation of the distance matrix using pairwise Nei's standard genetic distance, Ds (Nei, 1978) was constructed using the UPGMA algorithm of PHYLIP version 3.5c (Felsenstein, 2004). To provide support for the nodes in each tree 1000 bootstrap replicates of the original gene frequency data set were generated (Felsenstein 2004). As before, populations from other parts of Ireland were included for scale and comparison purposes only and the Loughbollard population was excluded.

3.2.8 Modelling grey squirrel population growth in Clongowes Wood

It was hypothesised that the population growth noted from 2004-2006 could be explained by high fecundity/low mortality without the need for immigration.

Microsoft Excel was used to construct a simple, individual based, deterministic model of grey squirrel population growth in Clongowes Wood (Figure 3.3) from 2004-2006 using data from the literature on grey squirrel fecundity and mortality (Gurnell, 1987; Gurnell, 1990; Koprowski, 1994; see Rushton *et al.*, 2002) and data obtained from 2003-2005 in Clongowes Wood (Table 3.1a).

Three sets of population parameters were modelled; high fecundity/low mortality (High Fecundity), moderate fecundity/moderate mortality (Moderate Fecundity), low fecundity/high mortality (Low Fecundity), each with high or no immigration.

The starting point for all three models was the actual population trapped in Clongowes Wood in the spring of 2003. Juveniles trapped on the study site in spring 2003 were assumed to be summer born juveniles from the previous year.

There were two periods of breeding and litter production, spring and summer. Spring born juveniles and summer born juveniles entered the breeding population the following spring and summer respectively. A proportion of the adult female population bred twice, in spring and summer. The remainder bred either once, in summer, or not at all. The sex ratio of all individuals which were born, died or immigrated to the study site in any one time period was assumed to be 1:1. Mortality was assumed to occur in autumn/winter only. The model therefore shows a population peak in late summer and a population trough in winter, as seen in natural populations (Gurnell, 1996).

The proportion of adult males and adult females breeding in the spring and summer of 2004 and 2005 was taken from actual data recorded in Clongowes Wood. Thereafter, the parameters that varied were the mean litter at birth, the proportion of adults dying and the proportion of juveniles dying.

The 'High Fecundity' model began with high fecundity/low mortality in 2004, when the grey squirrel population size in Clongowes Wood was very low, and gradually changed to more average values as the population size increased in 2005 and 2006. Mean litter at birth decreased from 3.5 in 2004 to 3.0 in 2005 and 2006. The proportion of juveniles dying increased from 0.4 in 2004 to 0.7 in 2005 and to 0.8 in 2006. The proportion of adults dying increased from 0.2 in 2004 to 0.3 in 2005 and to 0.4 in 2006.

In the 'Moderate Fecundity' model mean litter at birth was 2.5 from 2004-6. The proportion of juveniles dying increased from 0.65 in 2004 to 0.75 in 2005 and to 0.8 in 2006. The proportion of adults dying increased from 0.3 in 2004 and 2005 and to 0.5 in 2006.

In the 'Low Fecundity' model mean litter at birth was 2.0, the proportion of juveniles dying was 0.8 and the proportion of adults dying was 0.5 from 2004-2006.

These parameters are summarised in Table 3.1a.

Immigration occurred in the autumn, immigrants being spring born young of that year. Two levels of immigration were modelled for each of the three scenarios above; the number of immigrants was set at either zero (Low Immigration) or 10 (High Immigration). In section 2.4.3, immigration was estimated very generally at 2.5-5 individuals annually. Consequently to allow for inaccuracies in this estimate, High Immigration was set at 10. These individuals disperse, successfully breed and are not subject to mortality until the following year.

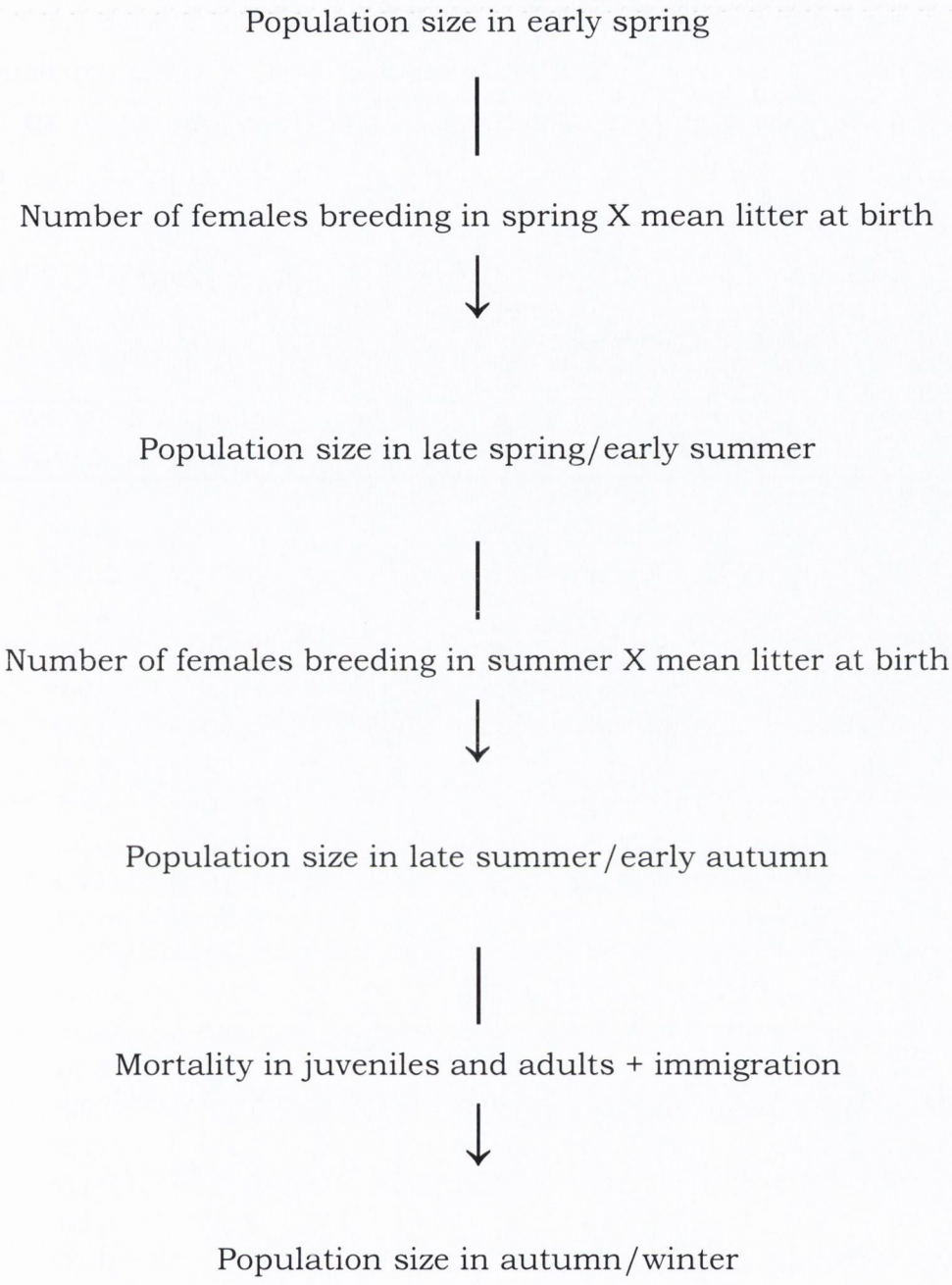


Figure 3.3 Flow diagram depicting the different stages in the population model over 1 year.

Table 3.1 Table of population parameters used in each population model. Immigration was set at zero individuals (Low Immigration) or 10 individuals (High Immigration) and occurred once a year in the autumn. Figures marked with an * indicates data obtained from 2003-05 in Clongowes Wood. Other data obtained from Gurnell (1987), Gurnell (1990), Koprowski (1994) and see Rushton *et al.*, (2002).

Year/Season	Parameter	High Fecundity	Moderate Fecundity	Low Fecundity
2004				
Spring	Prop. of adult males breeding	0.85*	0.85*	0.85*
	Prop. of adult females breeding	0.55*	0.55*	0.55*
	Mean litter at birth	3.50	2.50	2.00
Summer	Prop. of adult males breeding	1.00*	1.00*	1.00*
	Prop. of adult females breeding	1.00*	1.00*	1.00*
	Prop. of adult females not breeding	0.00	0.00	0.00
	Mean litter at birth	3.50	2.50	2.00
Autumn	Prop. of juveniles dying	0.40	0.65	0.80
	Prop. of juveniles surviving	0.60	0.35	0.20
	Prop. of adults dying	0.20	0.30	0.50
	Prop. of adults surviving	0.80	0.70	0.50
2005				
Spring	Prop. of adult males breeding	0.77*	0.77*	0.77*
	Prop. of adult females breeding	0.50*	0.50*	0.50*
	Mean litter at birth	3.00	2.50	2.00
Summer	Prop. of adult males breeding	0.90*	0.90*	0.90*
	Prop. of adult females breeding	0.75*	0.75*	0.75*
	Prop. of adult females not breeding	0.25	0.25	0.25
	Mean litter at birth	3.00	2.50	2.00
Autumn	Prop. of juveniles dying	0.70	0.75	0.80
	Prop. of juveniles surviving	0.30	0.25	0.20
	Prop. of adults dying	0.30	0.30	0.50
	Prop. of adults surviving	0.70	0.70	0.50
2006				
Spring	Prop. of adult males breeding	0.75	0.75	0.75
	Prop. of adult females breeding	0.40	0.30	0.20
	Mean litter at birth	3.00	2.50	2.00
Summer	Prop of adult males breeding	0.90	0.90	0.90
	Prop. of adult females breeding	0.90	0.90	0.90
	Prop. of adult females not breeding	0.10	0.10	0.10
	Mean litter at birth	3.00	2.50	2.00
Autumn	Prop. of juveniles dying	0.80	0.80	0.80
	Prop. of juveniles surviving	0.20	0.20	0.20
	Prop. of adults dying	0.40	0.50	0.50
	Prop. of adults surviving	0.60	0.50	0.50

3.3 Results

3.3.1 Trapping data and population density estimates

A total of 17 grey squirrels in the K Club, 34 grey squirrels in Mountarmstrong and 6 grey squirrels in Loughbollard, the three woodlands surrounding Clongowes Wood, were tagged and released during February-March 2003. 15 of these squirrels were juveniles. 104 grey squirrels were later trapped in Clongowes Wood and removed from the population in three stages, 54 in April 2004, 38 in June 2004 and 12 in July 2004 (Table 3.2).

During the first and second cull the number of grey squirrels trapped each day declined over the course of the cull without every reaching zero for three consecutive days. During the third cull no further squirrels were trapped after day 6.

Grey squirrel population density in the three woodlands surrounding Clongowes Wood ranged from of 2.8–6.0 squirrels per hectare (Table 3.3).

As the cull in Clongowes Wood took place over a four month period it is difficult to calculate the population density for a single point in time. A total of 104 squirrels were trapped in Clongowes Wood in 2003. This would give an estimated population density estimate of 8.7/squirrels ha for Clongowes Wood at the start of the first cull in April 2003 (assuming all squirrels trapped in 2003 were present on the study site in April 2003).

In 2004, the population density in Clongowes Wood increased only slowly from 1.7 grey squirrels per hectare in February (20 individuals) to 2.3 squirrels per hectare in June (27 individuals). 3 adult males were not trapped in Clongowes Wood in June 2004 but were later trapped there in

February 2005. It was assumed that these individuals were present in Clongowes Wood in June 2004 but not trapped.

In March 2003, the population density in the K Club was 2.8 squirrels/ha and in Loughbollard it was 6.0 squirrels/ha. In March 2004 both had decreased slightly to 2.0 squirrels/ha (12 individuals) in the KClub and 4.0 squirrels/ha (4 individuals) in Loughbollard. Unfortunately as permission was not granted to survey Mountarmstrong in 2004 the population density in 2004 is unknown. Therefore, only surviving members of the 2003 population were tagged.

By February 2005 the population density in Clongowes Wood had increased significantly - from 2.3 grey squirrels per hectare in June 2004 (27 individuals) to 4.6 grey squirrels per hectare (55 individuals).

In March 2005 the population densities in the woodlands surrounding Clongowes Wood were similar to previous spring population densities – Mountarmstrong had 5.2 grey squirrels per hectare (31 individuals), the K Club 2.5 grey squirrels per hectare (15 individuals) and Loughbollard 4.0 grey squirrels per hectare (4 individuals) (Table 3.3).

In June-July 2005, a capture-mark-recapture study indicated that the grey squirrel population of Clongowes Wood, using the Petersen/Lincoln index, was 8.1 squirrels per hectare (SE 1.3; 95% CI = 5.6-10.6 squirrels per hectare). 41 individual grey squirrels were trapped in the first trapping session - 20 grey squirrels tagged from spring 2005 and 21 untagged grey squirrels. Of these 41 grey squirrels, 19 were trapped in the subsequent trapping session and 26 untagged grey squirrels were trapped. The precull population density in 2003 was 8.6 squirrels per hectare. The population recovery, therefore, took 2 years to complete (Figure 3.4).

Table 3.2 Grey squirrel life history data for each study site

Site	n	Male				Female			
		Breeding Adult	Non Breeding Adult	Juvenile	Total	Breeding Adult	Non Breeding Adult	Juvenile	Total
2003									
K Club	17	6	1	2	9	3	1	4	8
Mountarmstrong	34	9	4	3	16	7	6	4	18*
Loughbollard	6	0	3	0	3	1	0	2	3
Clongowes Cull 1	54	14	3	11	28	10	8	8	26
Clongowes Cull 2	38	2	4	13	19	6	2	11	19
Clongowes Cull 3	12	3	1	1	5	2	4	1	7
2004									
K Club	12	5	0	1	6	4	2	0	6
Loughbollard	4	2	0	0	2	2	0	0	2
Clongowes Spring	20	6	1	1	8	5	4	3	12
Clongowes Summer	27**	8	0	2	10	12	0	2	14
2005									
K Club	15	5	1	2	8	3	2	2	7
Mountarmstrong	31	5	9	3	14	6	9	4	19
Loughbollard	4	2	0	0	2	2	0	0	2
Clongowes Spring	55	17	5	9	31	9	9	6	24
Clongowes Summer	97***	23	4	10	37	20	7	9	30

*1 adult female escaped before it's breeding status could be determined

**3 adult males not trapped in summer 2004, but later trapped in spring 2005, were assumed to be present in summer 2004 but not trapped

***population size determined by capture-mark-recapture study

Table 3.3. Grey squirrel population density and trapping success for each study site

Site	n	Population density	Trapping success
2003			
K Club	17	2.8	30
Mountarmstrong	34	5.7	42
Loughbollard	6	6.0	35
Clongowes Cull 1	54	--	13
Clongowes Cull 2	38	--	9
Clongowes Cull 3	12	8.7	3
2004			
K Club	12	2.0	32
Loughbollard	4	4.0	40
Beau Parc	67	--	32
Mount Juilet	32	--	11
Clongowes Spring	20	1.7	16
Clongowes Summer	27	2.3	23
2005			
K Club	15	2.5	24
Mountarmstrong	31	5.2	54
Loughbollard	4	4.0	35
Castle Leslie	64	--	27
Clongowes Spring	55	4.8	34
Clongowes Summer	97**	8.1	41

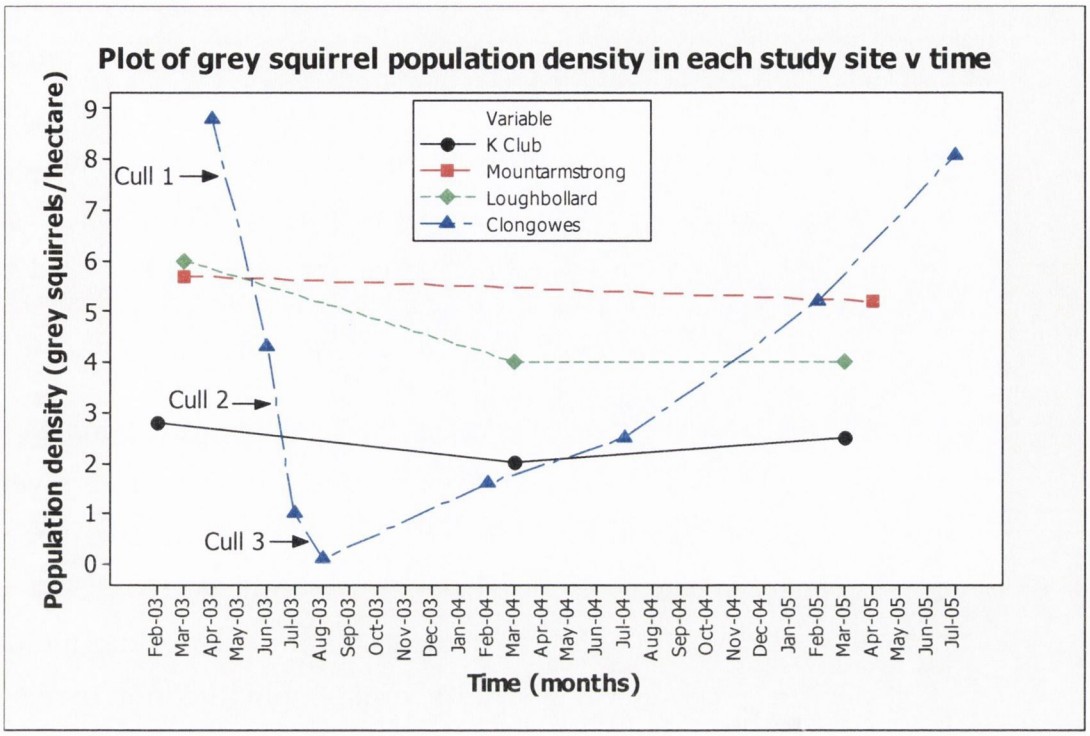


Figure 3.4 A plot of grey squirrel population density (number of grey squirrels/hectare) in each study site v time (months). Population densities were calculated in spring of each year for the K Club, Mountarmstrong and Loughbollard and, following the cull, in spring and summer in Clongowes Wood.

Fecundity was high in Clongowes Wood in 2004 following the cull in 2003. 4/9 (44%) of adult females were pregnant in February 2004 and 1/9 (11%) was lactating. 4 adult females and 3 juveniles were not breeding. In June 2005, these 7 females were lactating or had recently finished lactating. Of the 5 females pregnant in spring, 3 were definitely pregnant again and 2 appeared to be pregnant again. Therefore, in 2004 all females 12/12 (100%) bred at least once and 5/12 (42%) bred twice (Table 3.2).

In February 2005, in Clongowes Wood, 7/18 (39%) adult females were pregnant and 2/18 (11%) were lactating. 9 adult females and 6 juveniles were not breeding. In June 2005, 16/27 (60%) adult females were lactating or had recently finished lactating and 4/27 (15%) were pregnant for a second time. 7 adult females were not breeding including 5 that had been pregnant in February and 2 that did not breed that year. Therefore, in 2005 21/27 (78%) bred once, 4/27 (15 %) bred twice and (2/27) 7% did not breed at all (Table 3.2).

In March 2004, 6/8 (75%) adult females in The K Club/Loughbollard were breeding and in 2005 11/22 (50%) of adult females in The K Club/Loughbollard/Mountarmstrong were breeding (Table 3.2).

In general a very high percentage of males were breeding in all study sites and in all years throughout the breeding period. Overall 117/154 (76%) adult males were breeding during the three years of the study (Table 3.2).

Trapping success was low in Clongowes Wood in 2003. For example during cull 1 in spring 2003, trapping success was 13% in Clongowes Wood and 42% in the neighbouring woodland of Mountarmstrong. In summer 2005, when the population density of Clongowes Wood had increased to 2003 levels, and the probability of capture of grey squirrels is at its highest, trapping success was high at 41%. However, in spring

2005, trapping success was higher still in Mountarmstrong at 54%. Trapping success for the K Club was relatively low ranging from 24-32%. Trapping success for Beau Parc, Mount Juilet and Castle Leslie are shown for comparative purposes. Trapping success in Mount Juilet in summer 2004 was also very low at 11% (Table 3.3).

Data on grey squirrel survival was gathered for different time periods and under different circumstances in each study site. Survival was 100% in Clongowes Wood for both adults and juveniles from spring 2004 to summer 2004, when the population was small, and overall survival remained high at 59% from summer 2004 to spring 2005. In Mountarmstrong, overall survival was also high, from spring 2003-2005, at 23%. This suggests an annual overall survival of about 46%. Overall annual survival in the K Club varied form 33-41% (Table 3.4).

Only 1 tagged individuals from the surrounding study sites, Mountarmstrong, the K Club and Loughbollard was detected in the main study site, Clongowes Wood during the three years of the study. This was a juvenile female from Loughbollard, trapped in Clongowes Wood in February 2004, following the autumn dispersal of 2003.

Table 3.4. Grey squirrel survival in the K Club, Mountarmstrong and Clongowes Wood

Site	Time period	Percentage survival		
		Adult	Juvenile	Total
K Club	Spring 2003-Spring 2004	45	33	41
K Club	Spring 2004-Spring 2005	36	0	33
Mountarmstrong	Spring 2003-Spring 2005	28	22	23
Clongowes Wood	Spring 2004-Summer 2004	100	100	100
Clongowes Wood	Summer 2004-Spring 2005	50	61	59

3.3.2 Trapping of dominant and subordinate squirrels

Social rank is related to sex, age and weight. Males tend to dominate females (although old females may dominate young males) Adults dominate juveniles and dominant squirrels are normally heavier than subordinates. (Pack, 1967; Gurnell, 1987).

It was hypothesised that dominant squirrels may exclude subordinates squirrels from traps during a short cull of 5 days. These subordinate individuals may then be trapped during the latter stages of a longer cull, 14 days in this study, or during subsequent culls.

An analysis of the order in which squirrels were trapped relative to their social status and home ranges i.e. did dominant squirrels access the bait in traps before subordinate squirrels would be needed to answer this question conclusively. Ideally, the social rank and home range of each squirrel would be known. Then, it could be studied if subordinate squirrels avoided those traps which were within their home range, and the home range of a dominant squirrel, until after the dominant squirrel was trapped. These complete data are not available and would be very difficult to gather. There will be a continuum of dominance relationships between a squirrel and its neighbours in different parts of its home range so that an individual will have a different social rank with its neighbours in a different part of its home range. Hierarchical relationships only become clear when squirrels congregate together, for example at feeding aggregations or at the time of mating (Gurnell, 1987). Despite the absence of this complete dataset, different aspects of the data gathered could be analysed to determine if the above hypothesis was true.

Males dominate females, although adult females may dominate juvenile males. Therefore, the sex ratio of each cohort of squirrels was first investigated. The sex ratio in each of the cohorts of squirrels culled did

not differ from 1:1 (Chi-squared GOF Test (Zar, 1999) - Cull 1: n = 54, df = 1, $0.75<P<0.90$; Cull 2: n = 38, df = 1, $0.75<P<0.90$; Cull 3: n = 12, df = 1, $0.10<P<0.25$). Therefore females, which are generally considered to have a lower social rank than males, had equal access to traps during the culls.

Dominant squirrels are usually 2 years and older (Gurnell, 1987). Juvenile squirrels tend to weigh about 500g or less (Lawton, 1999). Over autumn and early winter, grey squirrels can increase their body weight by to 20%. Therefore, with a good supply of food, dominant grey squirrels would weigh about 600g and above. For the purposes of this study this weight (600g) was taken to be the cutoff between 'dominant' and 'subordinate' grey squirrels.

Squirrel body weight data was examined to determine if heavier squirrels were trapped before lighter squirrels. The squirrel body weight data was not normal and could not be transformed. The median body weights of squirrels captured during each cull were; cull 1- 580g, cull 2- 510g and cull 3 - 550g. A Kruskal-Wallis analysis of variance showed that there was a significant difference in the body weights of squirrels trapped during the three culls (df = 2, $P = 0.001$).

Squirrels were subdivided into two social ranks: 'dominant' (600g or greater) and 'subordinate' (less than 600g). Figure 3.5 shows an individual value plot of body weight for culls 1,2, and 3. For cull 1 (n=54), 23 grey squirrels have a body weight equal to or greater than 600g, for cull 2 (n=38), only 3 grey squirrels have a body weight equal to or greater than 600g and for cull 3 (n=12) no grey squirrels had a body weight greater than 600g.

For culls 1 and 2, there was a significant association between the proportion of 'dominant' individuals trapped and the cull (Fisher Exact test, $P = 0.0004$).

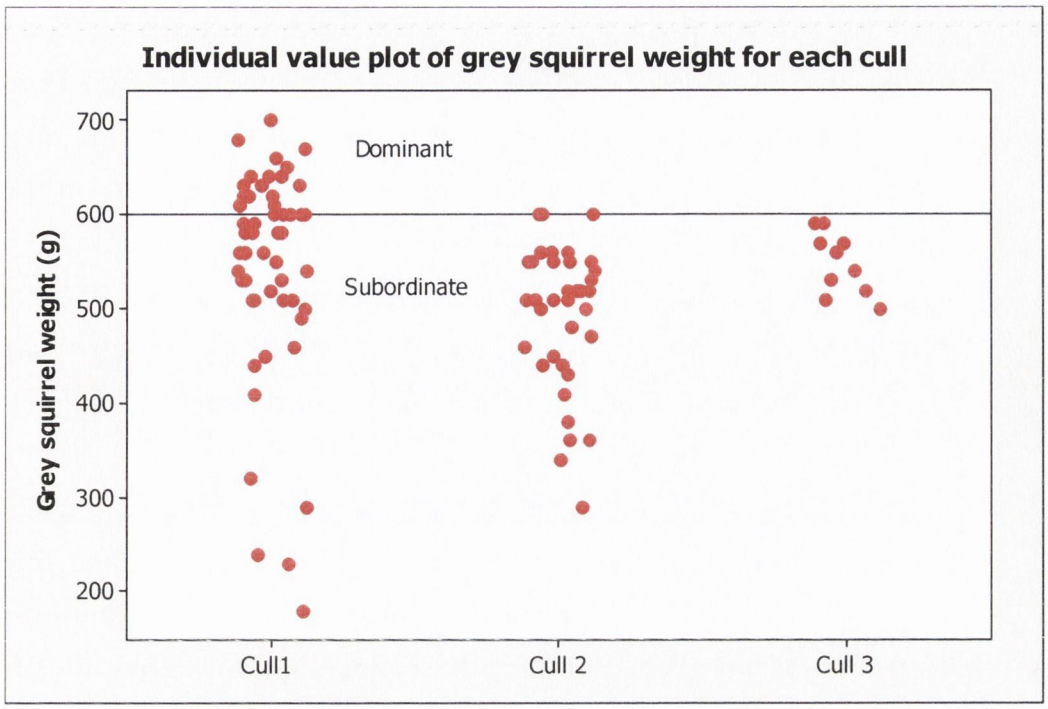


Figure 3.5. An individual value plot of body weight for culls 1,2, and 3. Squirrels were subdivided into two social ranks: 'dominant' (600g or greater) and 'subordinate' (less than 600g). For cull 1 (n=54), 23 grey squirrels have a body weight equal to or greater than 600g, for cull 2 (n=38), only 3 grey squirrels have a body weight greater than 600g and for cull 3 (n=12) no grey squirrels had a body weight greater than 600g.

It was hypothesized that, for cull 1, heavier male squirrels (body weight greater than or equal to median body weight) might be over-represented in the cohort culled. A Fisher Exact Test, to test for an association between sex and weight, was performed on this cohort and on the cohort obtained following cull 2. No association was found for either cohort (Cull 1: P=1; Cull 2: P=0.746). This again indicates that while sex is a factor in social rank, it is not a factor in probability of early capture of grey squirrels, either alone or in association with weight.

A plot of social rank v day of capture for all squirrels captured during the three culls (Figure 3.6) is shown. Squirrels were ranked linearly by weight with the lightest ranked as 1. Day of capture is the cumulative number of days on which culling took place i.e. day 1 of cull 2 is day of capture number 15. There is a relatively weak correlation between social rank and day of capture (Spearmans rank correlation, n=104, rs statistic –0.32, P = 0.004).

Cull 1 was in April 2003, cull 2 in June 2003 and cull 3 in July 2003. Grey squirrel body weights may decline during the summer if food is scarce. However, as this population was greatly reduced in size during the first cull in April 2003 this is likely to offset any significant loss in body weights over the summer.

'Dominant' squirrels were trapped throughout the 14 days of Cull 1 in different parts of the woodland. If the above hypothesis were true, then some 'subordinate' squirrels would have access to traps earlier than others, and before some 'dominant' squirrel were trapped. This could result in a pattern of a gradual capture of 'dominant' squirrels followed, with a lag time of a few days, by a gradual capture of 'subordinate' squirrels. As the home ranges of the squirrels are unknown it is difficult to know if such a pattern exists. This could account for the weak correlation described above.

However, the above analysis can also be carried out in one 2ha compartment Compartment A in Clongowes Wood. (See Figure 3.1b. Compartment A is the compartment at the start of the avenue leading to the college and is marked Burial Ground on the map). This compartment is isolated from the main woodland by a tree lined avenue of about 300m. It could be assumed that the squirrels resident in this part of the wood would be somewhat isolated from the remainder of the woodland and that the home ranges of these squirrels would encompass all the available traps in the compartment. 25 squirrels, out of a total of 54, were trapped in Compartment A during cull 1. Trapping success was 26%.

A plot of social rank v day of capture for all squirrels captured in Compartment A during the three culls (Figure 3.7) is shown. Squirrels were ranked linearly by weight with the lightest ranked as 1. Day of capture is the cumulative number of days on which culling took place i.e. day 1 of cull 2 is day of capture number 15. There was a similar, weak correlation between social rank and day of capture (Spearmans rank correlation, n=34, rs statistic –0.30, $P = 0.001$).

Therefore, within the above compartment squirrels of lower social rank (weight) did not avoid the traps until after squirrels of higher social rank (weight) were trapped. 'Dominant' squirrels were more likely to be trapped in this compartment during cull 1 rather than cull 2 (Fisher Exact Test, $P = 0.03$) but not to the exclusion of 'subordinate' squirrels.

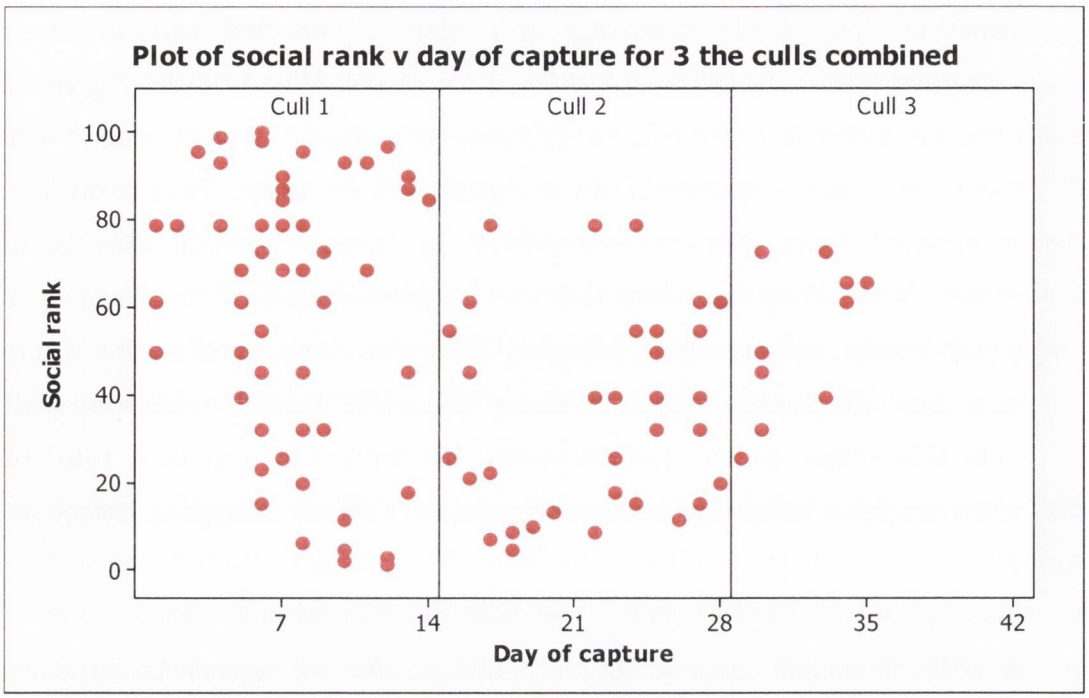


Figure 3.6 A plot of social rank v day of capture for all squirrels captured in Clongowes Wood during the three culls is shown. Squirrels were ranked linearly by weight with the lightest ranked as 1. There is a relatively weak correlation between social rank and day of capture (Spearmans rank correlation, n=104, rs statistic –0.32, $P = 0.004$).

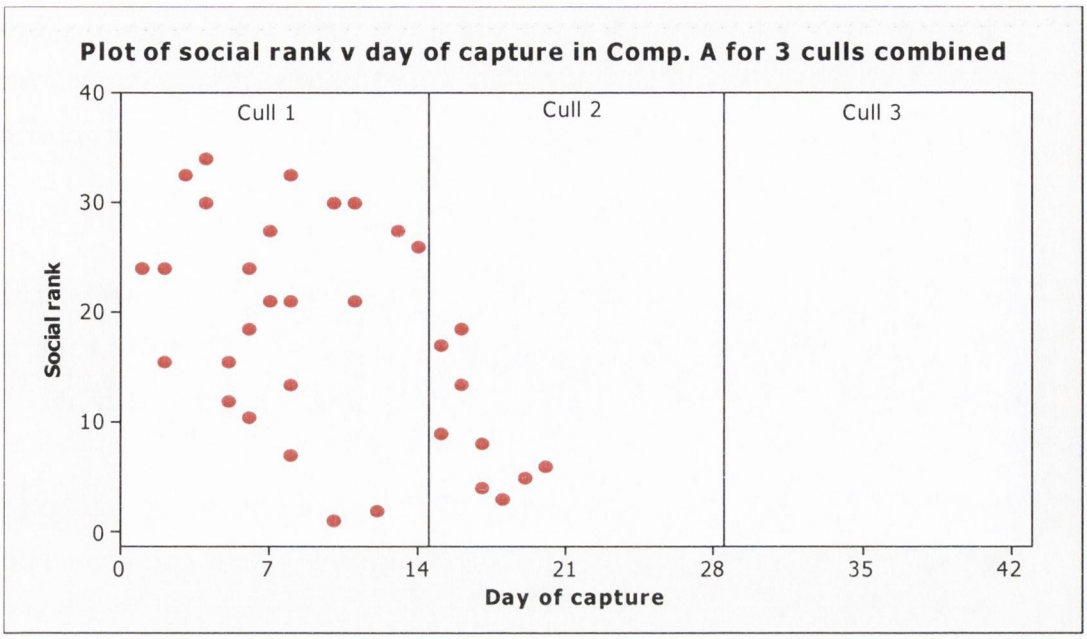


Figure 3.7. A plot of social rank v day of capture for all squirrels captured in Clongowes Wood, Compartment A during the three culls is shown. Squirrels were ranked linearly by weight with the lightest ranked as 1. There was a similar, weak correlation between social rank and day of capture (Spearmans rank correlation, n=34, rs statistic –0.30, P = 0.001).

It was hypothesised that, post cull, in the absence of 'dominant' adults, juveniles and young adults could assume higher quality home ranges, have greater access to traps and would later form a greater proportion of the trapped population.

The differences in age class (adult v juvenile) between squirrels trapped during cull 1 and cull 2 were investigated. It is assumed that these differences would reflect differences in dominance and social rank.

From cull 1 to cull 2, the percentage of juveniles trapped increased from 31% (17/54) to 63% (24/38). The association between the proportion of juveniles trapped and the cull (i.e. cull 1 or cull 2) was significant (Fisher Exact Test P= 0.03). However, during cull 3, the percentage of juveniles trapped was only 17% (2/12) and the remainder of the population trapped was adult. Therefore, no consistent increase in the proportion of juveniles trapped over the course of the culling programme was observed.

Overall, these data demonstrate that male squirrels were not more readily trapped than female squirrels in the early stages of this culling programme. Heavier squirrels, particularly 600g or greater in weight, were more readily trapped in the early stages but not to the exclusion of lighter squirrels. As dominance rank in grey squirrels is determined primarily by sex and age and to a lesser extend weight, dominance rank was not a factor in the order of capture of grey squirrels during this cull.

The second hypothesis to test is that larger grey squirrels enter baited traps more readily than smaller grey squirrels. Larger grey squirrels have a higher daily energy expenditure (see Bryce *et al.*, 2001) and must consume more food, regardless of their sex or dominance rank. The above data on body weight, although analysed in terms of dominance and social rank, provide some support for this hypothesis. Heavier squirrels, particularly 600g or greater in weight, were more readily

trapped in the early stages of the culling programme but not to the exclusion of lighter squirrels.

3.3.3 Food availability

Beech was the dominant food source for grey squirrels in all woodlands (Table 3.5a,b).

Lime trees make up 39% of trees of seed bearing age in Clongowes Wood, standing two abreast on both sides of the avenue leading to Clongowes Wood College and in one patch adjacent to the avenue, covering a total area of approximately 1ha. Lime seeds are small, about 0.5cm in diameter. 1000 seeds weigh 70-100g (Tree Factsheet, Forest Ecology and Management Group, Wageningen University). The smaller size and weight of lime seeds would require longer foraging and handling time to achieve a similar rate of energy intake, relative to beech nuts. The use of lime seeds by grey squirrels, their energy, nutrient and tannin content and their contribution to food availability in Clongowes Wood is unknown.

2003 was a moderate-good year for beech mast in Clongowes Wood, Mountarmstrong, a very good year in Loughbollard and a moderate year in the K Club. 2004 was a poor year for beech mast in all study sites (Table 3.6).

Table 3.5a Number of each tree species of seed bearing age identified from line transects in each woodland.

	Transect distance (m)	Beech	Oak	Lime	Yew	Horse Chestnut	Sycamore	Hazel	Palm	Ash	Scots Pine	Sitka Spruce	Total
Clongowes Wood	1689	176	12	135	10	8	1	3	2	2	0	0	349
The K Club	1056	33	8	0	0	1	9	1	0	6	5	0	63
Mountarmstrong	1161	198	0	0	0	0	0	1	0	0	0	40	239
Loughbollard	90	10	1	0	0	4	0	0	0	0	0	0	15

Table 3.5b Percentage of each tree species of seed bearing age identified from line transects in each woodland.

	Transect distance (m)	Beech	Oak	Lime	Yew	Horse Chestnut	Sycamore	Hazel	Palm	Ash	Scots Pine	Sitka Spruce	Total
Clongowes Wood	1689	50	3	39	3	2	0	1	1	1	0	0	100
The K Club	1056	52	13	0	0	2	14	2	0	10	8	0	100
Mountarmstrong	1161	83	0	0	0	0	0	0	0	0	0	17	100
Loughbollard	90	67	7	0	0	27	0	0	0	0	0	0	100

Table 3.6 Mean and standard deviations for numbers of total nuts and full nuts per tree collected in 7 minute samples in each site in mid October in 2003 and 2004.

		2003 Total Nuts		2003 Full Nuts		2004 Total Nuts		2004 Full Nuts	
	n	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Clongowes Wood	10	149	59	101	40	75	48	47	38
Mountarmstrong	10	132	42	95	37	60	46	40	37
The K Club	8	100	31	70	28	58	52	40	39
Loughbollard	4	175	32	159	36	34	16	11	6

3.3.4 Microsatellite analysis

All 5 microsatellites loci, Scv15, Scv19, Scv4, Scv18, Scv6, were in Hardy-Weinberg equilibrium and all pairs of loci were in linkage disequilibrium as described in Section 2.2.3

3.3.5 Parentage analysis

The third hypothesis to test was that grey squirrels from the woodlands surrounding Clongowes Wood migrate there post cull and contribute significantly to the re-establishment of the population.

The parentage analysis was unable to assign any of the 19 individuals trapped in Clongowes Wood in spring 2004 to one of the 102 potential parents from Clongowes Wood, Mountarmstrong, the K Club or Loughbollard with a level of confidence of 80% or above (Table 3.7).

In the genotype file 9 individuals out of 121 had exactly matching genotypes. This illustrates that number of loci analysed and the level of polymorphism encountered was insufficient for the purposes of a parentage analysis.

Table 3.7 Results of the parentage analysis of 19 individuals trapped in Clongowes Wood in spring 2004. 102 adults trapped in Clongowes Wood, the K Club, Mountarmstrong and Loughbollard in 2003 were investigated as candidate parents. Candidate parents and potential offspring were genotyped at 5 microsatellite loci Scv15, Scv19, Scv4, Scv18, Scv6 as described in Section 2.2.3. The number of simulation cycles implemented in Cervus 2.0 was 10,000, the proportion of candidate parents was sampled was 1.00, the proportion of loci typed was 1.00 and the proportion of loci mistyped was 0.10.

Level	Confidence (%)	Delta Criterion	Number of offspring assigned	Percentage of offspring assigned
Strict	95.00	2.28	0 (0)	0 (0)
Relaxed	80.00	1.99	0 (0)	0 (1)
Unresolved			19 (19)	100 (99)

(expected valves predicted by the simulation are shown in brackets)

3.3.6 Genetic distance between populations

In the absence of data from the parentage analysis, the genetic distance between populations was analysed to determine the influence of neighbouring populations on the gene frequencies of the Clongowes Wood population post cull.

The populations of Clongowes Wood, the K Club, Mountarmstrong were all significantly differentiated from each other at the start of the study in 2003 (Adjusted P Value = 0.02, all $p < 0.02$). In 2004 and 2005 none of the populations pairs were significantly differentiated from each other (2004; all $p > 0.05$, 2005; all $p>0.02$). As the genetic distance between these three populations is small (pairwise F_{ST} = 0.02-0.03) it is possible that these populations are at a boundary where population differentiation may or may not be statistically significant in a given year depending on random genetic drift, immigration and sample size analysed. In 2003 the sample size was high (Clongowes Wood n=94, The K Club n=17, Mountarmstrong n=31), in 2004 the sample size was low (Clongowes Wood n=24, The K Club n=12, no sample for Mountarmstrong) and in 2005 the sample size was intermediate (Clongowes Wood n=53, The K Club n=17, Mountarmstrong n=29).

Pairwise Nei's genetic distance, Ds, between the Clongowes Wood Cull 1 population and the combined Clongowes Wood Cull 2 and Cull 3 population was 0.003. The genetic distance between the Clongowes Wood Cull 1 population and the K Club and Mountarmstrong populations of that year (2003) was 0.061 and 0.049 respectively. Following 1000 bootstraps of the original gene frequency data set and the application of the UPGMA algorithm of PHYLIP version 3.5c (Felsenstein, 2004), the Clongowes Wood Cull 1 population (CG1a) and the combined Clongowes Wood Cull 2 and Cull 3 population (CG1b) partitioned together 100 percent of the time (Figure 3.8). This strongly suggests that the Clongowes Wood Cull 1 population (CG1a) and the

combined Clongowes Wood Cull 2 and Cull 3 populations are derived from a single population and not different populations.

Pairwise Nei's genetic distance, Ds, for Clongowes Wood, K Club and Mountarmstrong populations from 2003-2005, calculated using GENALEX (Peakall and Smouse, 2006), are shown in Table 3.8.

The populations from Clongowes Wood in 2003, 2004 and 2005 are more closely related to each other than to any other population in any year. (Table 3.8) The largest pairwise Nei's genetic distance between the three Clongowes Wood populations from 2003-2005 is 0.018 while the smallest pairwise Nei's genetic distance, Ds, between any of these three populations and any other population is 0.034.

Following 1000 bootstrap replicates and the application of the UPGMA algorithm of PHYLIP version 3.5c (Felsenstein, 2004), the Clongowes Wood populations of 2003, 2004 and 2005 partitioned together 86 percent of the time (Figure 3.9). This suggests that the Clongowes Wood populations of 2004 and 2005 are derived largely from survivors of the cull in 2003 and not from immigrants from the K Club, Mountarmstrong or elsewhere.

The K Club populations partitioned together 94 percent of the time and also showed low pairwise Nei's genetic distance (0.015-0.022). However, the Mountarmstrong populations only partitioned together 36 percent of the time and displayed the largest pairwise Nei's genetic distance of any pair of populations from one woodland (0.038). This may be due to genetic drift or to the immigration of grey squirrels from Donadea Forest Park, a broadleaved woodland of about 225 hectares with a large grey squirrel population which is about 1.5km from Mountarmstrong at their nearest point, and the effect these grey squirrels have on allele frequencies in Mountarmstrong.

Table 3.8 Pairwise Nei's genetic distance Ds (Nei, 1978) for Clongowes Wood, K Club and Mountarmstrong populations from 2003-2005 calculated using GENALEX (Peakall and Smouse, 2006).

Pop	CG2003	CG2004	CG2005	KC2003	KC2004	KC2005	MA2003
CG2003	0.000						
CG2004	0.012	0.000					
CG2005	0.016	0.018	0.000				
KC2003	0.061	0.059	0.061	0.000			
KC2004	0.059	0.073	0.078	0.015	0.000		
KC2005	0.087	0.092	0.095	0.020	0.022	0.000	
MA2003	0.049	0.037	0.070	0.075	0.088	0.090	0.000
MA2005	0.047	0.034	0.042	0.056	0.076	0.060	0.038

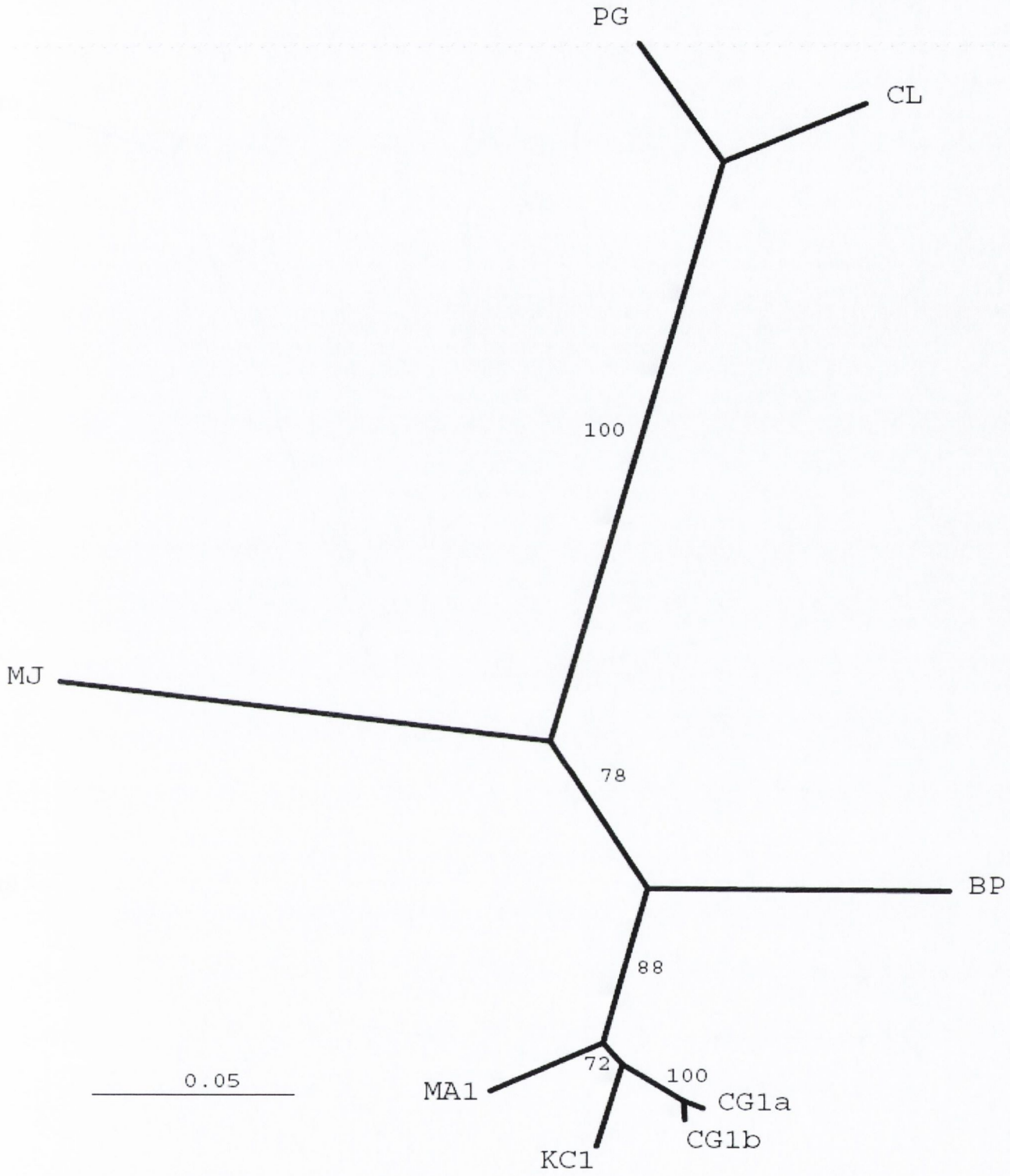


Figure 3.8 Unrooted UPGMA tree of Nei (1978) genetic distance obtained using PHYLIP v 3.5 for Clongowes Wood Cull 1 (CG1a) Clongowes Wood Cull 2 and Cull 3 populations combined (CG1b) Mountarmstrong (MA) K Club (KC) Beau Parc (BP) Mount Juilet (MJ) Castle Leslie (CL) and Portgleneone (PG). The numbers on the branches indicate the number of times (in percentage) the partition of the species into the two sets which are separated by that branch occurred out of 1000 bootstrapped trees.

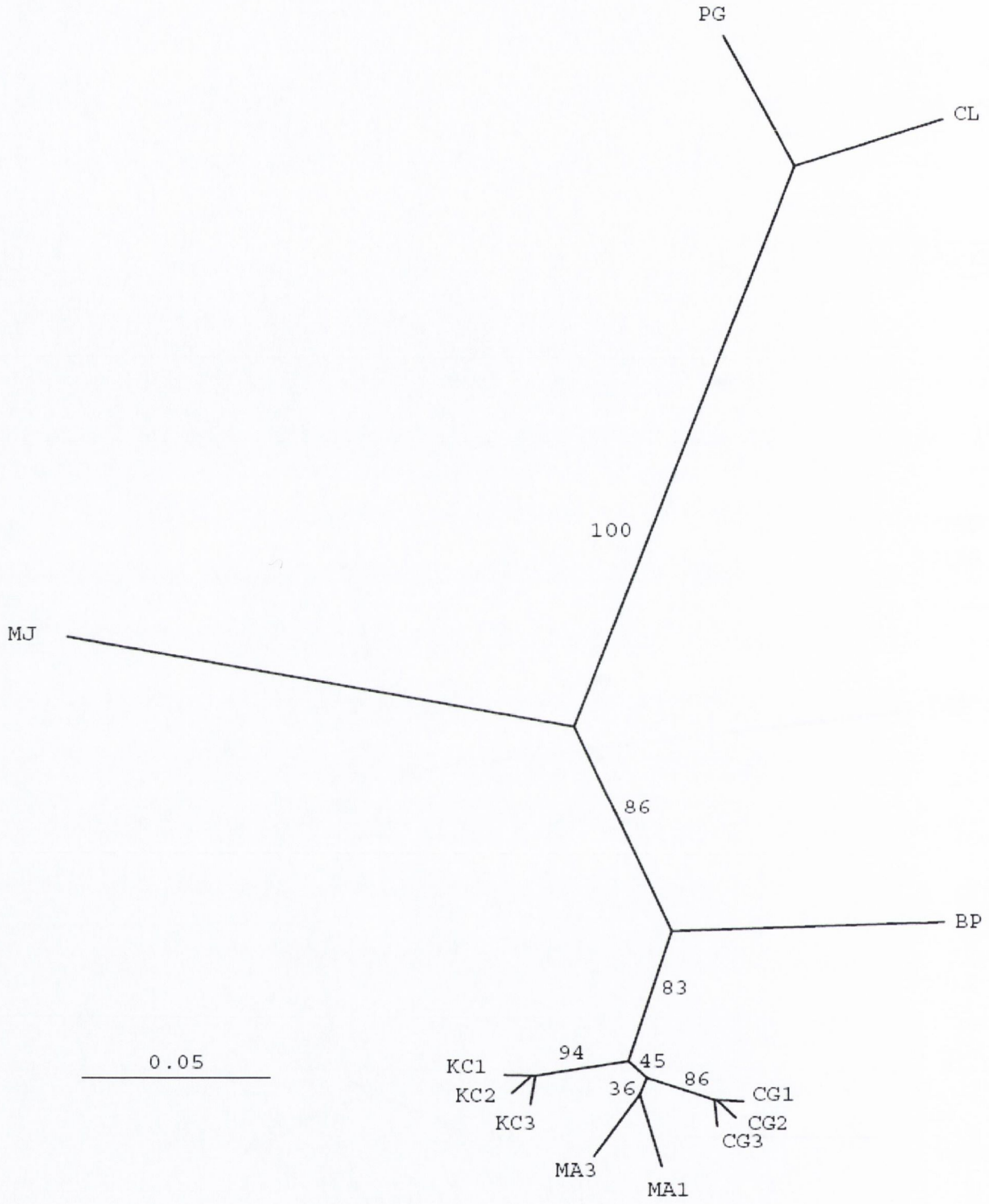


Figure 3.9 Unrooted UPGMA tree of Nei (1978) genetic distance obtained using PHYLIP v 3.5 for Clongowes Wood 2003 (CG1) 2004 (CG2) Spring 2005 (CG3), Mountarmstrong 2003 (MA1) 2005 (MA3), K Club 2003 (KC1) 2004 (KC2) 2005 (KC3), Beau Parc (BP) Mount Juilet (MJ) Castle Leslie (CL) and Portgleneone (PG). The numbers on the branches indicate the number of times (in percentage) the partition of the species into the two sets which are separated by that branch occurred out of 1000 bootstrapped trees.

3.3.7 Modelling grey squirrel population growth in Clongowes Wood

The population growth in Clongowes Wood was modelled to determine what paramaters could explain the observed population growth observed. The results from the individual based population models for Clongowes Wood from 2004-2006 are shown in Figures 3.10 - 3.12.

The models indicate that high fecundity and low mortality are required for the rate of population size recovery observed and that this may be achieved with or without immigration from neighbouring woodlands.

The predictions of the population model and the trapping study should coincide most accurately in late winter/early spring. At this time, all juveniles from the previous breeding season have been born and entered the trappable population, the autumn dispersal period is finished and winter mortality has impacted on the population.

The 'High Fecundity/No Immigration' model predicts a grey squirrel population in early spring 2005 of 52 individuals, in early summer of 67 individuals and in late summer 2005 of 130 individuals. This compares favourably with the trapping estimates in early spring 2005 of 55 individuals and in mid-summer of 97 individuals. Not all juveniles predicted to be born in summertime by the population model will have be born in midsummer at the time of trapping. Litters from summer breeding can be born in the autumn (Gurnell, 1987). Of those juveniles born, some will not have entered the trappable population at the time of the trapping, which occurs at about 3 months of age. This model indicates that the rate of population recovery observed in the Clongowes Wood grey squirrel population could be explained by high fecundity and low mortality alone, arising from greatly reduced intraspecific competition following the three culls (Figure 3.10).

The 'High Fecundity/High Immigration' model predicts a grey squirrel population in early spring 2005 of 62 individuals and in late summer 2005 of 149 individuals. The predicted spring 2005 population of 64 individuals is comparable with the estimate from the trapping data of 55 individuals. The late summer 2005 predicted figure of 149 individuals is somewhat higher than the midsummer capture-mark-recapture estimate of 97 individuals from the trapping data, although this may again be explained as above (Figure 3.10).

With no other model, Moderate Fecundity/High Immigration, Moderate Fecundity/Low Immigration, Low Fecundity/High Immigration, Low Fecundity/Low Immigration were the predicted population sizes at the beginning of spring or at the end of summer as high as the observed population sizes (Figure 3.11-3.12).

For example, in the 'Low Fecundity/No Immigration' scenario the summer populations predicted (37 in 2005, 31 in 2006) do not approach the actual populations in Clongowes Wood (104 in 2003, 97 in summer 2005) suggesting that the population parameters are too conservative and do not equate the population parameters existing in Clongowes at the time of the culls and thereafter.

Consequently, the models indicate that high fecundity and low mortality are a prerequisite for the rate of population size recovery observed and that this may be achieved with or without the assistance of immigration.

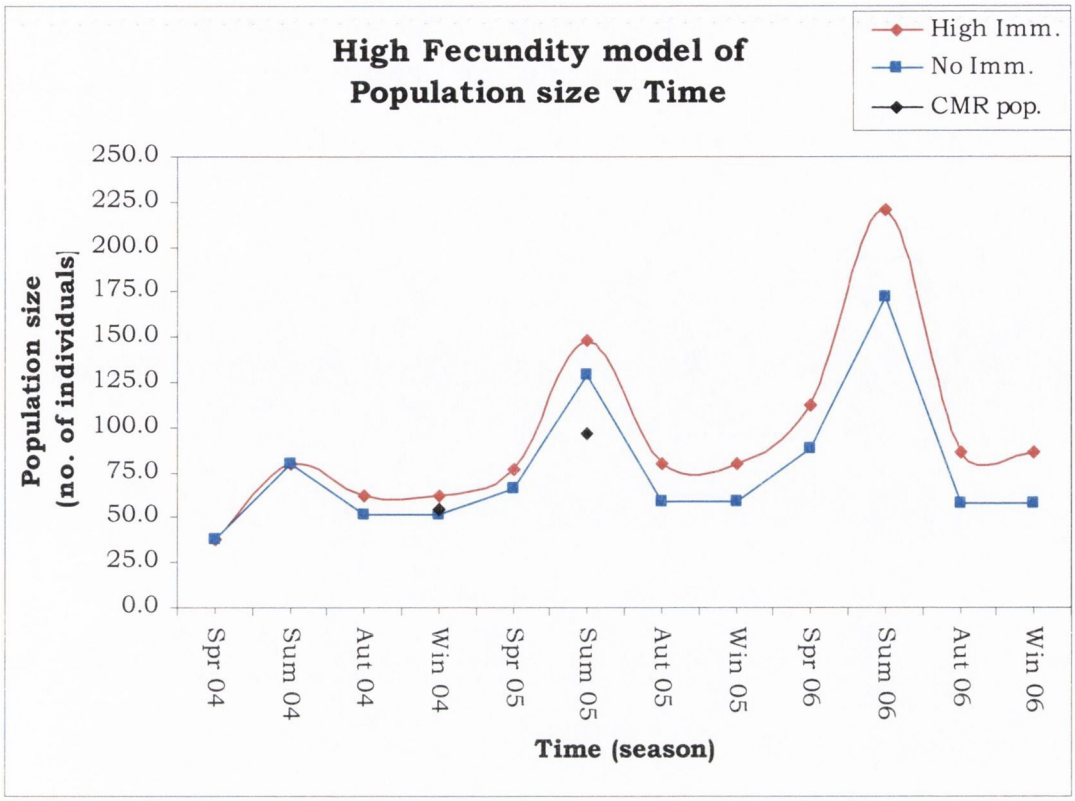


Figure 3.10 Plot of population size (number of individuals) versus time (season-year) for projected population growth in Clongowes Wood 2004-2006 assuming high fecundity. The starting population in Spring 2004 was 20 individuals. The data shows the projected population at the end of each season. High Imm. = High Immigration (10 individuals each autumn). No Imm. = No Immigration. CMR pop = Capture-Mark-Recapture population estimate. Spr = Spring. Sum = Summer. Aut = Autumn. Win = Winter. 04 = 2004. 05 = 2005. 06 = 2006.

The population size predicted by the model for late summer is expected to be significantly higher that the trapping estimate obtained in mid-summer for 2 reasons. Of those juveniles born by mid-summer, some will not have entered the trappable population, which occurs at about 3 months of age. Also, litters from summer breeding can be born well into the autumn (Gurnell, 1987), however the model assumes all such litters are born by late summer.

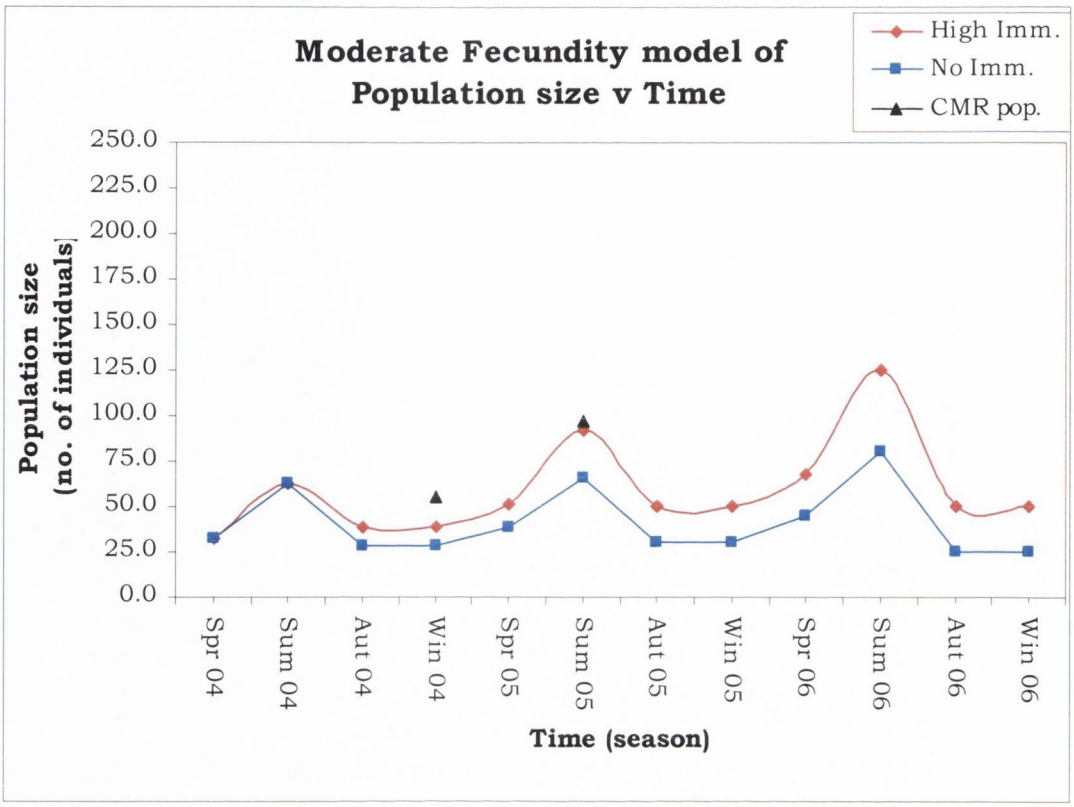


Figure 3.11 Plot of population size (number of individuals) versus time (season-year) for projected population growth in Clongowes Wood 2004-2006 assuming moderate fecundity. The starting population in Spring 2004 was 20 individuals. The data shows the projected population at the end of each season. High Imm. = High Immigration (10 individuals each autumn). No Imm. = No Immigration. CMR pop = Capture-Mark-Recapture population estimate. Spr = Spring. Sum = Summer. Aut = Autumn. Win = Winter. 04 = 2004. 05 = 2005. 06 = 2006.

The population size predicted by the model for late summer is expected to be significantly higher that the trapping estimate obtained in mid-summer for 2 reasons. Of those juveniles born by mid-summer, some will not have entered the trappable population, which occurs at about 3 months of age. Also, litters from summer breeding can be born well into the autumn (Gurnell, 1987), however the model assumes all such litters are born by late summer.

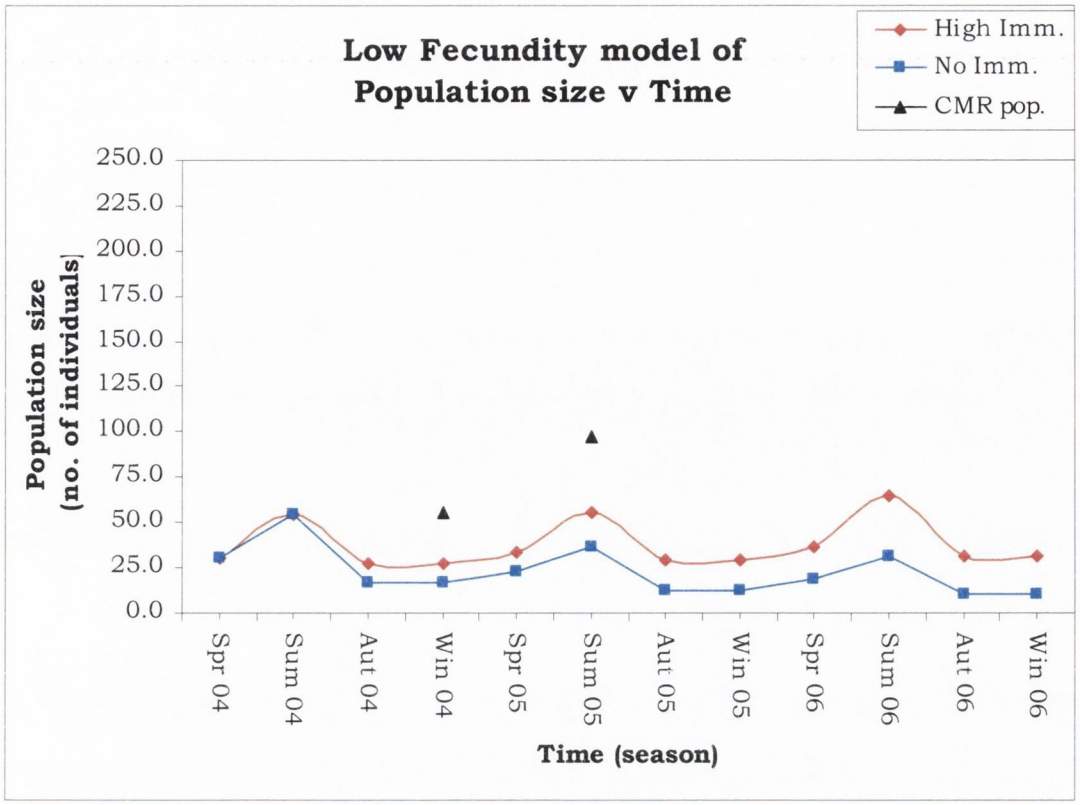


Figure 3.12. Plot of population size (number of individuals) versus time (season-year) for projected population growth in Clongowes Wood 2004-2006 assuming low fecundity. The starting population in Spring 2004 was 20 individuals. The data shows the projected population at the end of each season. High Imm. = High Immigration (10 individuals each autumn). No Imm. = No Immigration. CMR pop = Capture-Mark-Recapture population estimate. Spr = Spring. Sum = Summer. Aut = Autumn. Win = Winter. 04 = 2004. 05 = 2005. 06 = 2006.

The population size predicted by the model for late summer is expected to be significantly higher that the trapping estimate obtained in mid-summer for 2 reasons. Of those juveniles born by mid-summer, some will not have entered the trappable population, which occurs at about 3 months of age. Also, litters from summer breeding can be born well into the autumn (Gurnell, 1987), however the model assumes all such litters are born by late summer.

3.4 Discussion

3.4.1 Population data

Population size estimates were based on the minimum number of grey squirrels alive (MNA) in a woodland or on the Petersen-Lincoln method. The Petersen-Lincoln relies on the following assumptions (Begon, 1979) discussed below.

The population is closed.

A closed population is one that does not change in size during the study period; that is the effects of births, deaths, and movements are negligible and do not affect the ratio of marked to unmarked animals Thus populations are typically closed over only a short period of time. As this capture-mark-recapture study took place over a 4 week period the opportunity for large changes in population size was reduced. The most likely violation of this assumption would be the emergence of spring born young into the trappable population, but the absolute number would be small. As the study was conducted outside the main mortality period of winter, and outside the main dispersal periods of spring and autumn, deaths and movements would be low.

For the first sample, all individuals have an equal probability of being captured.

In mark-recapture estimation, the population size (N) actually refers to the trappable population. If there are animals which cannot be trapped, then mathematically, they are not part of the population, although biologically they are. Some individuals may simply be difficult to capture or 'trap-shy' leading to an underestimation of the population size. Also, trapping grey squirrels in early summer will miss spring/summer born young that have not emerged from the nest into the trappable

population. This will lead to an underestimation of the population size, the extent of which is difficult to quantify. As juvenile mortality is typically 50-75% annually and trapping is difficult in autumn and winter, many of these undetected individuals will die before detection in subsequent seasons and before entering the breeding population. However, if autumn/winter food availability is high, mortality and dispersal of these juveniles will be low and the trappable population in the spring and summer of the subsequent year will appear to have increased markedly over the previous summer estimate due to the survival of these individuals.

Trapping and/or marking does not alter catchability

This is an extremely important assumption. If trapping and/or marking an animal alter it's behaviour or survival, such that it is more or less catchable in the next sampling period, then this can over- or underestimate the population. If a marked individual becomes more difficult to catch the population size will be overestimated and if a marked individual becomes easier to catch the population size will be underestimated. Following initial trapping, some grey squirrels appear to become 'trap-happy', and are caught repeatedly in the same or adjacent traps, while others appear to become 'trap-shy' become are only occasionally trapped again. The overall effect on the estimation of population size is difficult to quantify.

Marks are not lost, or missed between sampling periods.

If a mark falls off or disappears, or the mark is missed, then the size of the marked population has effectively been reduced, and the population size will be overestimated. As the study was conducted over a short period of time (4 weeks) and both grey squirrel ears were tagged with durable, easy to see metal tags it is thought that this assumption was met.

Population densities in all study sites indicate reasonably large populations of grey squirrels were present in the study area. The population density in Clongowes Wood ranged from 8.1-8.7 grey squirrels per hectare in summer, in Mountarmstrong it was 5.2-5.7 grey squirrels per hectare in spring and in the K Club 2.0-2.8 grey squirrels per hectare in spring. Gurnell (1996) recorded a mean mid-winter density of 4.5 squirrels/ha, a mean mid-summer density of 8.1 squirrels/ha and an overall mean of 6.3 squirrels/ha over a 10 year period in an oak wood in southern England.

It proved much more difficult to remove the entire grey squirrel population of Clongowes Wood than anticipated. Culling usually takes place over a period of 5 days (Lawton, 1999; Gurnell and Steele, 2002) but was extended in this study to 14 days to improve control efficiency. In addition trap density was approximately 1 trap /0.4 hectares increased from the norm of 1 trap/1-2 hectares approx. A total of 3 separate culls, each lasting 14 days, were required before it was felt that the population of grey squirrels in Clongowes Wood had been reduced to zero or near zero. This difficulty in trapping all the grey squirrels in the population was particularly evident in April 2003, most likely due to good food availability in Clongowes Wood. No information was available on the autumn tree seed crop of 2002 prior to the commencement of the study.

Following the cull in Clongowes Wood in 2003 all individuals trapped on the study site in spring 2003 survived to summer 2004. However, 3 adult males were not trapped on the study site in summer 2004, but were subsequently trapped on the study site in spring 2005. It must be assumed that these individuals were present on the study site in summer 2004 but were not trapped. The beech mast crop was estimated to be moderate-good in Clongowes in 2003, which would make trapping grey squirrels more difficult in 2004. This again illustrates the difficulty of attempting to capture and cull an entire population of grey squirrels,

even in the summertime when natural food availability is at its lowest and grey squirrels are most readily trapped.

Fecundity was high in Clongowes Wood in 2004, 42% of adult females bred twice and 100% bred once. Dispersal was low. Only 1 tagged individual from the surrounding study sites of Mountarmstrong, the K Club and Loughbollard was detected in the main study site, Clongowes Wood, following the autumn 2003 dispersal. This was a juvenile female from Loughbollard, trapped in Clongowes Wood in February 2004.

2003 was a moderate-good year for beech mast in Clongowes Wood and Mountarmstrong, a very good year in Loughbollard and a moderate year in the K Club. When food supplies are good, survival, fecundity and local recruitment is higher and dispersal is lower (Gurnell, 1983; 1987). The availability of tree seed to each individual in Clongowes Wood in would also be greater than normal due to the reduced population size.

2004 was a poor year for beech mast in all study sites. In 2005, fecundity was lower in Clongowes Wood, 15% of adult females bred twice, 78% once and 7% not at all. However, despite 2004 being a poor year for tree seeds, no dispersal into Clongowes Wood was detected for either the autumn 2004 or spring 2005 dispersal periods.

Population data are not available for summer for Mountarmstrong, the K Club and Loughbollard. The data available for spring is in general agreement with the influence of food availability on fecundity described above: in March 2004, 6/8 (75%) adult females in The K Club/Loughbollard were breeding and in 2005 11/22 (50%) of adult females in The K Club/Loughbollard/Mountarmstrong were breeding.

Nixon and McClain (1975) found that 36% of females produced two litters in a year following a mast crop, 30% did so in a good seed year while none did when the seed crop the previous autumn failed. Gurnell

(1983) notes that only about a third of the adult female squirrels in a population produce two litters during a season, although the figure may be higher following a good seed crop. The data on adult females breeding in Clongowes Wood is similar to the above.

There is relatively little data quantifying the extent of dispersal between two woodlands. Taylor *et al.,* (1971) monitored both short distance and long distance movements of grey squirrels in continuous woodland by trapping from June 1954-July 1960. Traps were laid in the 'Centre' and within all woodland within 2 miles of the 'Centre'. At the end of the study squirrels were cleared from the entire area by shooting and trapping. 922 squirrels were marked and released, squirrels were handled 3453 times and 1655 squirrels were killed at the end of the study, of which 236 were young of the year and 310 had been previously marked. Taylor *et al.,* (1971) found that in all 104 squirrels occurred in the Centre and in outside trap groups. Over 1500 recapture records were made in traps outside the Centre but only 23 journeys in excess of 1.6km and 1 over 3.2 km were recorded. No marked squirrels were recorded amongst 60 squirrels in woodland 6.4 km from the Centre.

Fitzgibbon (1993) studied grey squirrel presence in 68 deciduous woodlands, ranging in size from 0.2-12.5ha and separated by an average of 300m in East Anglia, UK. The probability of woodlands containing grey squirrels declined rapidly with 'distance to the nearest wood greater than 5ha' from 0.5 at a distance of roughly 50m to 0.05 at a distance of 1.5km. (Only woodlands not containing hazel or beech were used in the analysis). Grey squirrels were also more likely to occur in woods surrounded by a greater density of hedgerows. Mosby (1969) reported intermittent live trapping was carried out in many of the woodlots on Virginia Polytechnic Institute Farm (VPIF) between 1947-1966. Out of a total of 812 grey squirrels, there were 45 records (5.5%) of between woods movements involving 30 males and 15 females. The average distance travelled between woods by the males was 880m; the females

averaged 608m. Another study on VPIF focused on two 10 acre woodlots separated by 200m at their nearest points (Mosby, 1969). There were 25 between woods movements over 6 years. Flyger (1960) studied the movements of grey squirrels in two 10 acre woodlots of oak, hickory, beech and walnut located in the grounds of a Baltimore hospital separated by 1/3 of a mile (533m) over a three year period. None of the 177 marked squirrels moved between the two woodlots during the course of the study. Hale *et al.*, (2001b), studying the genetic structure of red squirrel populations found that significant genetic subdivision existed between populations in woodlots at linking distances of 1.5km, 2km 2.5km and 3km and concluded that the maximum dispersal distance of red squirrels over unfavourable ground was probably less than or equal to 1.5km.

Dispersal between woodland is probably both relatively uncommon and also difficult to detect. A limitation of the study is that there were too few juveniles and young adults tagged in the study sites surrounding Clongowes Wood to get good estimates of dispersal at a population level. In 2003 13 juveniles and 44 adults were tagged in total. Only 1 juvenile dispersed to Clongowes Wood. In 2004, 1 juvenile and 31 adults were tagged (assuming 50% overall survival in Mountarmstrong from 2003-2004) and in 2005 11 juveniles and 39 adults were tagged. No further tagged individuals were detected in Clongowes Wood. Given that the number of grey squirrels dispersing varies from year to year, that not all juveniles disperse, that dispersing individuals can move in any direction from their woodland of origin and that the perceptual range of the grey squirrel has been estimated at 300 metres (Zollner, 2000) it is not surprising that so few immigrants were detected in Clongowes Wood. A more comprehensive study of grey squirrel dispersal would require a much longer time period and much larger numbers of tagged individuals.

3.4.2 Trapping of dominant and subordinate squirrels

The data obtained in this study suggest that dominance rank was not a factor in the order of capture of grey squirrels during this cull. The data on body weight provide some support for the hypothesis that larger grey squirrels will enter traps more readily as they have a higher daily energy expenditure (see Bryce *et al.*, 2001) and must consume more food, regardless of their sex or dominance rank. Heavier squirrels, particularly 600g or greater in weight, were more readily trapped in the early stages of the culling programme but not to the exclusion of lighter squirrels. Similarily, Pack *et al.,* (1967) noted that subordinate grey squirrels were not denied access to feeder hoppers.

Trapping success was low during each cull; 13% for cull 1, 9% for cull 2 and 3% for cull 3. Therefore, a mean of 2.0 traps/ha for cull 1 and 2.3 traps/ha for cull 2 were unoccupied at all times. Male home ranges and core areas overlap with other males and females. Female grey squirrels maintain small core areas with little or no overlap with other females, particularly when lactating. Female home ranges show greater overlap. Given the large numbers traps unoccupied traps at all times, over the entire woodland, and the virtual absence of any territoriality in grey squirrels, it is difficult to see how dominant squirrels could effectively exclude subordinate squirrels from unoccupied traps. Therefore it is thought that other factors, such as a larger absolute energy requirement per day, arising particularly during the breeding season as dominant individuals attempt to maximize their reproductive success, must be responsible for the higher proportion of dominant individuals trapped during cull 1.

The increase in the numbers of juveniles trapped between Cull 1 in April (17/54) to Cull 2 in June (24/38) may also be partly explained by the emergence of spring born young from the nest. 5 of the juveniles trapped in June were less than 400g in weight and another 4 were

between 400-450g in weight. The former were certainly early spring born young and most likely the latter also. Gestation in grey squirrels is about 44-46 days and entry of juveniles into the trappable population occurs at about 3 months of age. As breeding begins from mid-December to February, depending on food availability, there was therefore less opportunity to catch spring born young in April, during Cull 1, than in June, during cull 2.

3.4.3 Food availability

Beech is the dominant woodland tree species in the study area. Annual production of mast by the beech is highly variable. In a sixteen-year record of regional and temporal variation in the fruiting of beech in 10 sites throughout England from 1980-1995 the best sites (Killerton, Woodbury and Patcham) had numerous well spaced trees with domes of branches exposed to sunlight (Hilton and Packham, 1997). This is generally similar to the type woodland found in Clongowes Wood, Mountarmstrong, the K Club and Loughbollard.

Good or very good masting was generally always followed by very bad. This justifies the general opinion that good masting does not occur in consecutive years. There were exceptions to this however. For three years from 1982-84 Himley Hall was rated moderate. Ripon had two good years in 1989/90. The closest sites came to masting successfully in consecutive years was 1989/90, when moderate was followed by good for five sites. However, the next year, 1991, was the second worst recorded during the whole survey (Hilton and Packham, 1997).

There was no information available on the beech mast crop or other tree seed crops for Clongowes Wood in 2002. 2003 was a moderate-good year for beech mast in Clongowes Wood and Mountarmstrong, a very good year in Loughbollard and a moderate year in the K Club. 2004 was a poor year for beech mast in all study sites.

This data gives only a guide to food availability in the study sites as it does not quantify food availability in terms of seed energy (joules) per unit area (see Gurnell, 1993; Gurnell *et al.,* 1991)) which would be preferable.

Hilton and Packham, (1997) did not count nuts which had been eaten by predators in their count of total nuts or full nuts. Gysel (1971) and Gurnell (1993) both reconstructed whole nuts from parts of nuts utilised by animals, categorised them as eaten and included them in their count of total nuts. As the grey squirrel population size in Clongowes wood was greatly reduced in the autumn of 2003 fewer nuts would have been predated this year than normal and this would elevate the count of total and full nuts in Clongowes Wood relative to the other study sites.

The avenue leading to Clongowes Wood college in bounded by two lines of mature *Tilia X Europeae*, the common lime. Unlike beech, oak, hazel and chestnut tree seeds, common lime seeds are small, about 0.5cm in diameter. 1000 seeds weigh 70-100g (Tree Factsheet, Forest Ecology and Management Group, Wageningen University). The use of lime seeds by grey squirrels and their contribution to food availability in the woodland is unknown.

3.4.4 Parentage analysis

The parentage analysis in CERVUS was unable to identify any parent/offspring combination for three main reasons. Firstly, too few microsatellite loci were analysed and those analysed were found to have low variability, most likely due to the small founder population of 12 grey squirrels from which the Irish grey squirrel population originated. Secondly, the presence of family members, other than the parents of the offspring, in the pool of candidate parents can present a serious challenge to parentage assignment (Jones and Ardren, 2003) and this is

likely to be the case for all grey squirrel populations. Finally, neither parent was identified in the field. Where it is possible to identify a parent through field observations it reduces the complexity of the parentage analysis. In retrospect this should have been attempted by radiotagging breeding females, tracking them to their nest and identifying the juveniles therein. Even then it is unlikely that the parentage analysis would have been successful with just 5 microsatellite loci given the above difficulties.

3.4.5 Modelling grey squirrel population growth in Clongowes Wood

There are a number of factors to consider when comparing the results i.e. population size data calculated by a capture-mark-recapture method and by population modelling. Firstly, not all juveniles predicted to be born in a given season by the population model will be born at the time of the capture-mark-recapture study. Gestation is approximately 44-46 days in grey squirrels, so, for example, litters from summer breeding can be born in the autumn (Gurnell, 1987) and will not be available for capture in the summer, as predicted by the model. Of those juveniles born, some will not have entered the trappable population at the time of the capture-mark-recapture study. Juvenile grey squirrels leave the nest at about 2 months of age and slowly increase their range. As they become independent, from about 3 months of age upwards their home ranges expand rapidly (Gurnell, 1987) and it is from about this time they will enter the trappable population. Of those grey squirrels which can be trapped, the probability of capture is then inversely related to food availability (Gurnell, 1996). Therefore, a capture-mark-recapture method may tend to underestimate the size of the true population. The population model in turn relies on several simplifications which affects the accuracy of the population size estimate at any point in time. Mortality is assumed to occur only in autumn/winter rather than all year round. Immigration is assumed to occur only in autumn but also occurs, to a lesser extent in spring. Also, some young adults may also

disperse in autumn, not just spring born young. The predictions of the population model and the capture-mark-recapture study should coincide most accurately in late winter/early spring. At this time, all juveniles from the previous breeding season have been born and entered the trappable population, the autumn dispersal period is finished and winter mortality has impacted on the population. However, high food availability can reduce the probability of capture at this time. In conclusion, the usefulness of the population model is to determine what broad set or sets of population parameters can explain the rate of population size increase obtained from the trapping studies rather than attempting to predict the exact population size for a given point in time.

3.4.6 Population recovery

There are four pieces of evidence which indicate that the population recovery in Clongowes Wood following control was driven by local recruitment and not dispersal. The populations of Clongowes Wood, the K Club, Mountarmstrong were significantly differentiated from each other at the start of the study in 2003, when the largest sample sizes were obtained for each population, indicating that gene flow between the study sites was somewhat restricted (see Section 2.4.3). Only 1 tagged immigrant grey squirrel was detected on the study site from 2003-2005. The Clongowes Wood populations of 2003, 2004 and 2005 partitioned together 86 percent of the time following 1000 bootstrap replicates of the allele frequencies data and the application of the UPGMA algorithm of PHYLIP version 3.5c (Felsenstein, 2004). This suggests that the Clongowes Wood populations of 2004 and 2005 are derived largely from survivors of the cull in 2003 and not from immigrants from the K Club, Mountarmstrong or elsewhere. Finally, population recovery over a two year period was predicted by a model incorporating high fecundity and low mortality, and could be achieved with or without immigration.

This finding is in contrast with the findings of Lawton and Rochford (2007) in study sites in Ardmulchan and Beau Parc who concluded that: a cull of five days was sufficient to remove most, if not all, of the grey squirrels within the woodlands concerned; this was evident from the very low numbers of previously marked animals in the postcull samples; post cull populations tended to consist of unmarked adult animals which had entered from outside the woodland.

However, Lawton and Rochford (2007) also note that higher numbers of grey squirrels were removed during the four springtime culls than had previously been recorded in the woods. This suggests that during the culls a combination of greater effort (5 days trapping in comparison with 2-4 days trapping), the removal of individuals which may have been 'trap happy' and the disruption of the social structure was yielding a greater overall return, (in terms of numbers of grey squirrels trapped) than before. It is possible that a greater trapping effort, in the form of a longer trapping schedule and a higher trap density, may also have yielded further returns. As outlined in section 3.4.1 above, data from Gurnell (1996), Gurnell and Steele (2002), Cull 5 of Lawton and Rochford (2007), where 8 marked individuals were not trapped during the cull, and this study suggest that it is very difficult to trap all the grey squirrels in a population during a single trapping session.

Lawton and Rochford (2007) did not gather any data to directly demonstrate that population recovery was occurring as a result of immigration, as this was not the focus of their study. Some of their findings suggest that other factors may be involved. First, post cull populations tended to consist of unmarked adult animals, but juvenile animals are responsible for most dispersal (Thompson, 1978a; Gurnell, 1987). Secondly, the highest rate of population recovery was in the more isolated woodland. This occurred after Cull 1 in Ardmulchan (0.31 squirrels per hectare per week), which is relatively isolated from other woodlands, the nearest being Hayes House woodland, 2km to the east

across the River Boyne. In contrast after Cull 2 and Cull 3 in the Beau Parc study site, which is continuous with 150 hectares of broadleaved woodland, the rates of population recovery were lower at 0.15 and 0.08 squirrels per hectare per week respectively (although the ongoing control in this 150 hectares in Beau Parc during the course of the study could have affected the population recovery). In the case of Ardmulchan, the rapid recoveries reported (i.e. 6-10 weeks for Cull 1 and Cull 5) would require that relatively large numbers of grey squirrels (24 individuals for Cull 1) were moving over a considerable distance (>2km) in a short time period to effect the population recovery. Dispersal of this nature has not been reported previously in the literature (see Flyger, 1960; Mosby, 1969; Taylor *et al*, 1971; Fitzgibbon 1993) apart from very occasional, large scale dispersal in autumn (Flyger, 1969).

Donadea Forest is a 225 hectare mixed broadleaved-conifer wood, 3km from Clongowes Wood, at their nearest points. It was not included in the study area and it could be suggested that perhaps many grey squirrels moved from Donadea Forest to Clongowes Wood. However, the number of grey squirrels successfully dispersing to an adjacent woodland declines with distance to that woodland (Taylor *et al.,* 1971; Fitzgibbon, 1993; Hale *et al.,* 2001 for red squirrels). 3km is at the upper limit of reported grey squirrel dispersal movement, with only occasional grey squirrels reported moving this distance (Flyger, 1960; Mosby, 1969; Taylor *et al.,* 1971; Fitzgibbon, 1993).

Also, Mountarmstrong lies midway between Donadea Forest and Clongowes Wood, 1.5km from each (Figure 3.1). Therefore, the number of grey squirrels dispersing from Donadea Forest to Mountarmstrong will be greater than the number dispersing from Donadea Forest to Clongowes Wood (Taylor *et al.,* 1971; Fitzgibbon, 1993). If we assume the number of grey squirrels dispersing from Donadea Forest to Clongowes Wood could play a significant role in population size recovery (say between 10-20 per year), then an even greater number must disperse the

shorter distance from Donadea Forest to Mountarmstrong (say between 15-30 each year). This would have the effect of completely homogenising allele frequencies between Donadea, Mountarmstong and Clongowes Wood. The fact that allele frequencies were significantly different between Mountarmstrong and Clongowes Wood in 2003 indicates that such immigration on a large scale from Donadea Forest to Clongowes Wood was not occurring.

3.4.7 Comparison of methods of estimating dispersal

Direct observation methods and and parentage assignment based on microsatellite data have been used to increase our understanding of dispersal in species such as water vole (*Arvicola terrestris*) (Telfer *et al.*, 2003) and banner-tailed kangaroo rats (*Dipodomys spectabilis)* (Waser *et al.*, 2006). A comparison of the estimates of dispersal obtained by these methods for grey squirrels was not possible due to the inability of the parentage analysis to identify parent-offspring combination.

The use of F_{ST} to infer the overall scale of dispersal across local populations was discussed in Section 2.4.3. An exchange of about 2.5-5 migrants annually between grey squirrel populations would be possible without causing uniformity of allele frequencies across populations. However this is not a local, time specific estimate of dispersal which can be compared to the capture-mark-recapture data of 2003-2005. Rather it is a more general estimate of the historical genetic connectivity between the populations (Lowe *et al.*, 1994) and should be treated with some caution.

3.4.8 Conclusions

A large effort is required to cull most, or all, the grey squirrels in a population by trapping.

In the woodland studied, population size recovery following a cull was driven primilary by local recruitment and not by immigration.

Heavier squirrels tend to access traps earlier than many lighter squirrels, but not to the exclusion of lighter squirrels.

A parentage analysis in grey squirrels would require a large number of highly polymorphic microsatellite loci to be successful.

4. A survey of range expansion by the grey squirrel in the Republic of Ireland since 1996

4.1 Introduction

At one extreme, the range of a species may be defined by the worldwide extend of its occurrence while, at the other, the precise location and habitat of a species may be identified (Forman, 1964). The scale of the analysis chosen may influence if individuals of a particular species are found in some places and absent from others (Krebs, 2001).

Within a group, for example in North American birds (De Graaf *et al.*, 1985), most species have small geographic ranges and a few have medium or large ranges. Geographic range sizes tend to decrease from polar to equatorial latitudes for many species of mammals, birds, reptiles and fish (Rapoport's Rule) (Pagel *et al.*, 1991; Gaston *et al.*, 1998) but several exceptions also exist (Gaston *et al.*, 1998). The boundaries of geographic ranges can be abrupt or gradual. In many cases a species geographic distribution is a contour map with a gradual falloff in density as one approaches the edges of the range, as with the western grey kangaroo (*Macropus fuliginosus*) in Australia (Caughley *et al.*, 1988). There is also a correlation between distribution and abundance such that widespread species are typically more abundant (Hanski's rule) (Hanski, 1982; Gaston *et al.*, 1997).

Distribution patterns are important as they allow ecologists produce testable hypotheses to investigate underlying ecological mechanisms. For example, Snyder and Weathers (1975) suggested that species have a broad tolerance to climatic variability at high latitudes and therefore can occupy larger ranges. Brown (1995) suggested that species with high dispersal capacity may have repopulated northern areas first, following the retreat of glaciers, and may therefore have large geographic ranges. Regarding distribution and abundance, the ecological specialisation model argues that species that can

exploit a wide range of resources will become both widespread and abundant (Brown, 1984). Hanski *et al.*, (1993) proposed the metapopulation model suggesting that species with a greater capacity to disperse will occupy more suitable patches of habitat and be more widespread and more abundant. There is data to provide some support for all of these hypotheses, for example, Snyder and Weathers (1975), but many of the critical predictions have still to be tested (Gaston *et al.*, 1997).

Distribution patterns may also provide insights into historical events and dispersal ability. The isolation of Ireland from Britain, and Britain from Europe, led to a skewed and limited range of native mammals on both islands. Species with greater dispersal ability were more likely reach these islands before the loss of the land bridges (Yalden, 1982).

Many species are limited in their local distribution by the presence of other species – as predator, competitor, disease or food species - and distribution surveys can reflect this. The distribution of the burrowing rat kangaroo (*Bettongia lesueur*) declined from half the Australian mainland to just three islands off Western Australia paralleling an increase in distribution of an introduced predator - the red fox (Short and Smith, 1994). Similar data have been obtained from studies of rock wallabies and red foxes in Australia (Kinnear *et al.*, 1998) and red and grey squirrels in Ireland, Britain and Italy (O'Teangana *et al*, 2000). Following the isolation of a peninsula in Shark Bay, Western Australia with an electric fence and the poisoning of the red fox, an introduction of the burrowing rat kangaroo was successful and their range expanded in this area (Sinclair *et al.*, 1998). On a smaller scale, habitat preference can result in an individual occupying a more restricted distribution than theoretical possible. In North America and Asia large desert rodents, for example the kangaroo rat (*Dipodomys merriami*), prefer open desert habitat while smaller species, for example pocket mice *Chaetodipus spp.*, prefer bush microhabitats, due to different predation risk and avoidance mechanisms (Kotler and Brown, 1988).

As ecology has developed as a discipline, surveys of the distribution patterns of species have become more rigorous and comprehensive. For example, there were nine major distribution surveys of red and grey squirrels undertaken in the UK between 1930-1983 (Lloyd, 1983). The earlier surveys by Middleton relied mainly on voluntary observers and in 1935 he gathered 1385 records. The later four surveys, planned by Shorten and conducted from 1953-1971, utilised the large number of field staff employed by the Ministry of Agriculture, Fisheries and Food and the Forestry Commission and the public to obtain a larger number of reports. The parish was used as the basic unit for recording purposes, with, if possible the name of the location where the squirrel was seen. Independent confirmation was sought where sightings of either species isolated from their main distribution occurred or to confirm the apparent absence of one species from an area where they had occurred previously. In 1946, Shorten obtained reports from 9781 parishes and 120 Forestry Commission forests, while in 1962 Lloyd obtained a total of 12,141 reports (Lloyd, 1983). Large scale surveys involving voluntary recorders now frequently require that physical evidence of an animal's presence is sent to the researcher. In the Mammal Society Pick up a Polecat survey in the U.K., records are only accepted if accompanied with photographic evidence or the body of a polecat (The Mammal Society, 2003).

4.1.2 Surveying squirrels

There are a range of methods for surveying for the presence of grey or red squirrels and to monitor population trends through time. These include live trapping and five main indirect methods (i.e. not involving trapping or handling): visual counts along line transects, the use of hair tubes, counts of dreys, cone line feeding transects in conifer forests and feeding signs on whole maize bait at feeding stations. A review of the accuracy and precision with which these indirect methods can detect population change and the effort required is presented in Gurnell *et al.,* (2004b). Observations of squirrel tracks or feeding remains in fresh snowfall (Andren & Lemnell, 1992) and the number of specimens delivered to a taxidermist and the number of road kill

have also been used (Brandl *et al.,* 1991). Only live trapping, visual observations or counts, the use of hairtubes and recording dead specimens can distinguish between grey and red squirrels.

The focus in this survey was on grey squirrels and, consequently, broadleaved woodland. Grey squirrel population density in broadleaved woodland is high (for example, 3-18 per hectare, (Gurnell, 1996), 6-8 per hectare, this study) relative to red squirrels (roughly 0.5-1.5 per hectare in both broadleaved and coniferous habitat) (Gurnell, 1990) In areas of range overlap between red and grey squirrels, as is the case in this survey, red squirrels are likely to be absent from broadleaved woodland with well-established populations of grey squirrels, but may be present in conifer woodland. In mixed conifer woodland the population density of red squirrels is low, for example 0.17–1.41 red squirrels/hectare (Lurz *et al.,* 1995). Visibility in conifer woodlands tends to be limited. Red squirrels are much more arboreal than grey squirrels irrespective of the time of the year or habitat, for example, in Lawns Wood, Cumbria red squirrels spent 67% of foraging time throughout the year in trees (Gurnell, 1990). For these reasons, red squirrels are difficult to detect in conifer plantations. In three well-thinned spruce woodlands in Co. Wicklow, McGoldrick (2002) found that the probability of detecting a red squirrel was low and declined rapidly with distance from the observer.

As expected, previous surveys of mammals indicate that grey squirrels are readily detectable by voluntary recorders. For example, the Breeding Bird Survey (BBS) in the UK now includes mammal recording by BBS volunteers. Grey squirrel was the second most frequent mammal noted after the rabbit. Through local knowledge, grey squirrels were known to be present in 950 of the 1km squares surveyed. They were actually seen in 848 (89%) of these squares during just two site visits between April and June (Newson and Noble, 2006). In the 2004, Living with Mammals survey, grey squirrels were seen at 75 % of the 790 sites surveyed (Mammal Trust UK, 2004).

4.1.3 Rationale behind the survey

For many species of mammal, a key objective is to understand changes in range rather than changes in actual numbers. This is particularly true for introduced species that are continuing to spread (Harris and Yalden, 2004). In the case of the grey squirrel, there is usually little need to obtained quantified population data as the main monitoring goal is to record range expansion. Inevitably, this is then asssociated with a subsequent contraction of the red squirrel's range (O'Teangana *et al.*, 2000) and damage to broadleaved trees (Lawton, 1999). Quantified population data would only be required, for example, when grey squirrels are present in or close to a red squirrel conservation area and a forest management plan or grey squirrels control programme is being planned or when conducting research into grey squirrel ecology.

For these reasons, assuming both species of squirrel were present in an area, the probability of detecting grey squirrels is much greater than the probability of detecting red squirrels. The distribution survey, therefore, focussed on grey squirrels and on those areas where range expansion was likely. Observations made about red squirrels presence/absence were noted.

4.1.4 Objective

To determine the range expansion of the grey squirrel in the Republic of Ireland since 1996.

4.2 Methods

4.2.1 Distribution survey

In conducting this survey it was decided to assess as far as possible the quality of the information supplied by the respondents. To this end, a questionnaire (Appendix 1) was designed which categorised the information supplied into firsthand information, secondhand information and anecdotal information. The questionnaire was not distributed to respondents. Rathar it was completed by the researcher during, and following, telephone conversations with the respondents. This allowed the researcher to question the respondent more fully regarding what the information supplied i.e. who saw the squirrel(s), was the squirrel(s) alive, trapped, shot, roadkill or were grey squirrel hairs obtained in hairtubes, was there one or more squirrels seen, was the squirrel(s) seen on one or more occasion, when and where was the sighting, when was the respondents first sighting of grey squirrels in that area, was there any control of grey squirrels in place, what was the respondents interest and knowledge in the area and how reliable did the respondent feel the information being supplied was?

Wherever possible information was gathered on grey squirrels that had been trapped, shot or seen as roadkill as it was felt that this information was more reliable than sighting data and would serve to authenticate sighting data in an area.

Sightings which were west of the Shannon and were not accompanied by physical evidence of grey squirrel presence or which extended the previously known range of the grey squirrel and were at least one $10km^2$ square removed from the next nearest sighting and were not accompanied by physical evidence of grey squirrel presence were regarded as unconfirmed sightings.

Prior to the telephone conversations with respondents, where a postal or email address was available, respondents were sent either a letter or email explaining what the survey was about and what information was needed. Respondents then had the option of phoning the researcher or awaiting a phone call from him. Letters or emails were sent to all NPWS District Conservation Officers, NPWS Conservation Rangers, Regional Game Council Officers, Forest Service Inspectors, Teagasc Farm Forestry Advisors, and to some Teagasc Research Centres and Agricultural Colleges, Golf Clubs, Birdwatch Ireland members, lecturers in third level institutions and consultant foresters in the relevant counties. These contact details were obtained from the NPWS, Ely Place Dublin 2, from the Forestry and Timber Yearbook 2006, Irish Timber Growers Association, 17 Castle Street, Dalkey, Co. Dublin and from internet the homepages of the various bodies. Postal addresses were not available for Coillte foresters who were contacted directly by phone. The website www.ireland.ie proved very useful for obtaining names, postal and email addresses and telephone numbers for Historic Buildings, Houses and Castles which often have some mature broadleaved woodland surrounding them capable of supporting grey squirrel populations and whose owners were all very interested and helpful in conducting this survey.

Only data which was firsthand data or secondhand data was used. i.e. the respondent had to see the grey squirrel personally or else receive this information from an identifiable individual.

A small number of respondents replied by email to state that they had no information, to confirm data already gathered, or gave comprehensive information by email, for example, Dr. Don Cotton supplied a spreadsheet of all his sightings of red and grey squirrels. In these cases these respondents were not contacted directly by telephone.

An emphasis was placed at all times on obtaining contacts and data from areas in which the grey squirrel was expanding its range or not recorded in

the previous survey by Reilly (1997). To this end the focus was on counties Wicklow, Wexford, south Kilkenny, south Carlow, Tipperary, north Waterford, north Cork, east Limerick and Donegal, Sligo and north Leitrim. There are many records from areas with few or no grey squirrel sightings. Also, every effort was made to talk directly with the individuals directly responsible for grey squirrel sightings e.g. greenkeepers in Golf Clubs to obtain firsthand information at all times.

4.2.2 Rate of spread of grey squirrels in Ireland

The one dimensional rate of spread since the previous survey ((Reilly (1997), completed in 1996) was measured by calculating the shortest distance from the centre of a previously occupied square to a newly occupied one (O'Teangana *et al.* 2000). These measurement were divided by 11, the number of years between the respective surveys, and the modal rate of spread in kilometres per year obtained.

The one dimensional rate of spread since 1911 was measured by calculating the maximum distance travelled by the grey squirrel in a northerly, north-easterly, south-easterly and southerly direction. In these directions the grey squirrel in Ireland has, or did have until 2007, further area to disperse into. These measurements were divided by 96, the number of years the grey squirrel has been in Ireland and the mean one dimensional rate of spread obtained.

Using the equation from Okubo *et al.*, (1989)

V (km per year) = $2 (AD)^{1/2}$,

where V is the speed of dispersal and A is the intrinsic rate of increase of the Grey Squirrel (estimated as 0.82 using a modified Leslie matrix from known reproductive/death rates), the two-dimensional diffusion coefficient for the grey squirrel since 1911 was estimated.

4.3 Results

4.3.1 Distribution survey

The telephone survey format worked very well. There was a concern that respondents may be irritated by the intrusion of a telephone call and for this reason letters or emails were sent first to inform the respondent about the survey. Respondents were all asked if the researcher had phoned at a bad time and if they would like him to call back at a later stage. Most respondents were willing and able to discuss the matter there and then. It was felt that some categories of individuals e.g. academics might prefer to complete a survey form in their own time and would find a telephone call intrusive. However, it was clear that the majority of individuals targeted in this survey e.g. NPWS Conservation Rangers, foresters, gun club members responded better to a personal telephone call and that a greater number of response and more information was obtained this way.

Many respondents gave contact details of colleagues or interested parties they felt would be able to help. Many respondents from gun clubs undertook to enquire from fellow members if they had seen or shot grey squirrels and provided contact details for these individuals at a later stage.

Table 4.1 gives a breakdown of respondents by county and by occupation. See appendix 2 for full details of respondents and distribution data.

The data gathered was compared with the data from the survey by Reilly (1997) and previous grey squirrel distribution studies in Ireland (Figures 4.3 and 4.4). Reilly (1997) did not have any records of grey squirrels present counties Waterford, Cork, Limerick, Clare, Galway, Roscommon, Sligo or Donegal.

Table 4.1 Breakdown of respondents by county and by occupation/category.

County	Landowner	Gun Club Officer/ Member	NPWS Ranger	Forester	Forest Service/ Teagasc	Academic	Golf Club	Other	NGO	Total
Carlow	3					1				4
Kilkenny	1		1		4	2		1		9
Cork		1	2	2	5		4			14
Limerick	2	1	2	1					3	9
Waterford	1				1			1		3
Sligo	2		2	3	2	1				10
Leitrim	2	4	1				2			9
Roscommon		1	2		2					5
Cavan								2		2
Donegal	4	2	7	10	1	1	1	2		28
Wicklow	1	3	4		1	2	19	2	1	33
Dublin							4			4
Tipperary	19	9	1	7	6	2	9	7	5	65
Wexford	4		3	1	9		3		5	25
Clare		3							2	5
Galway			4	1			3			8
										233



Figure 4.1 Map of Ireland showing rivers and mountains mentioned in the text.

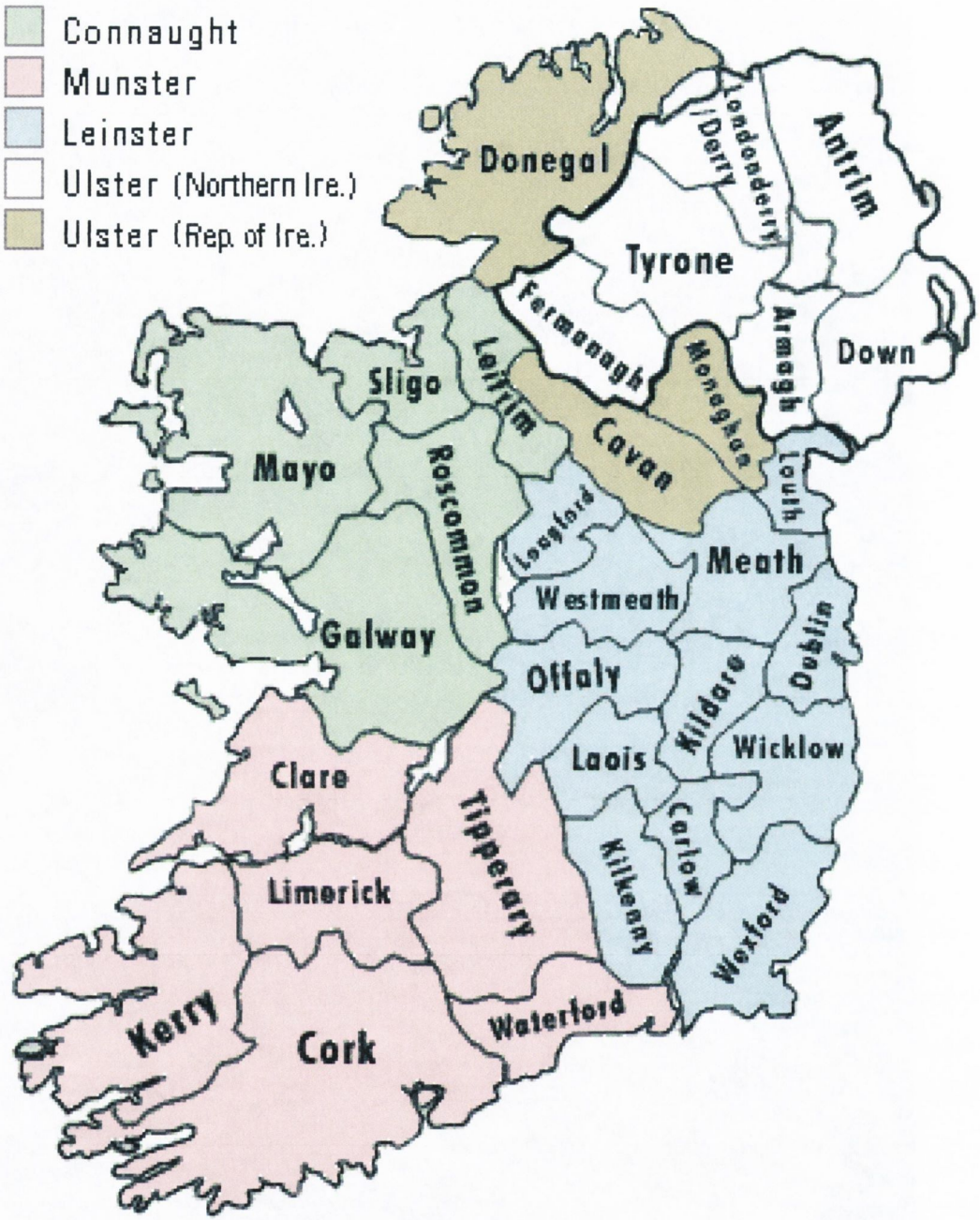


Figure 4.2 Map of Ireland showing counties, provinces and border with Northern Ireland.

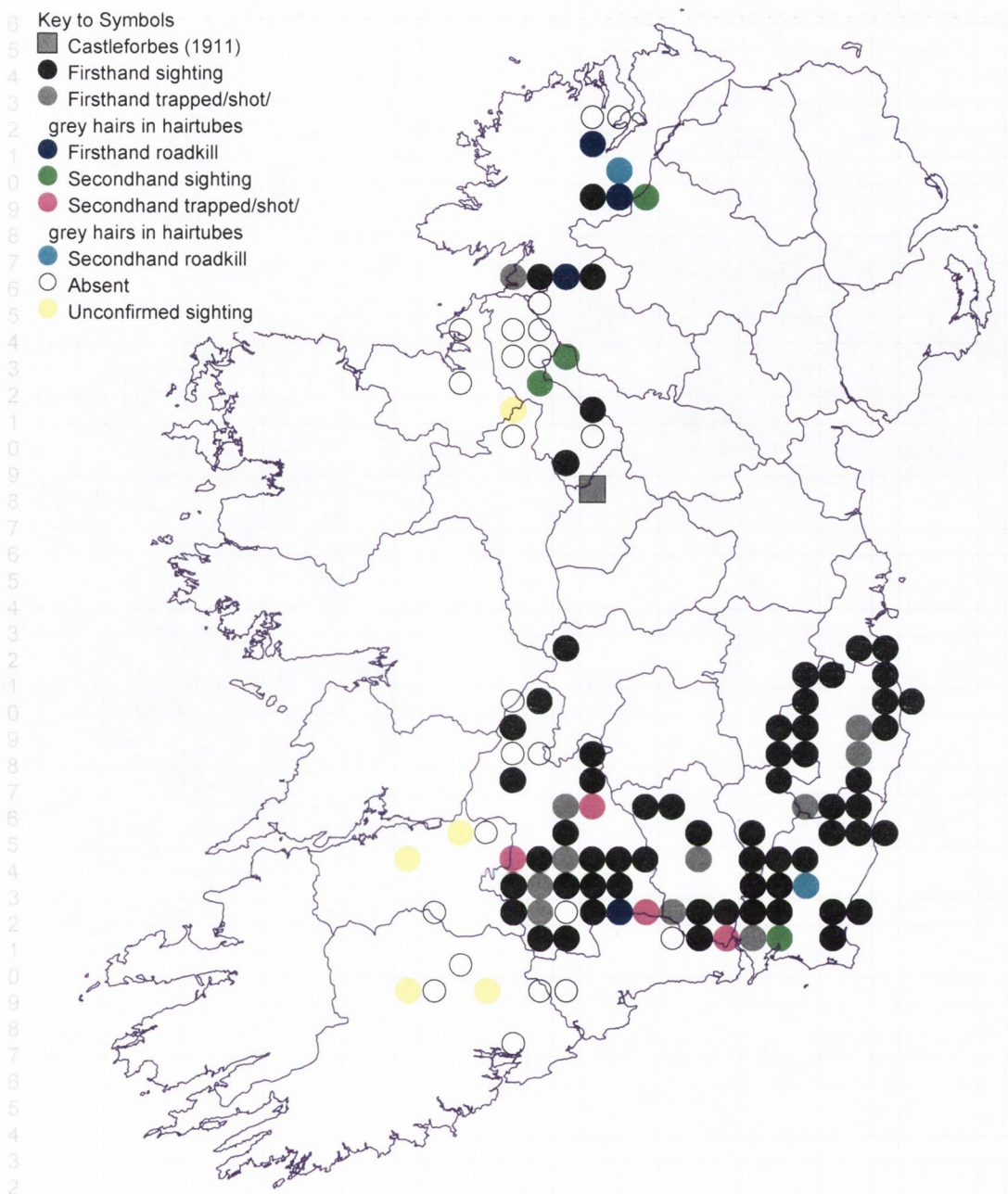


Figure 4.3. 10km² grid map showing all grey squirrel distribution data gathered during the survey. Unconfirmed sightings are sightings which are west of the Shannon and are not accompanied by physical evidence of grey squirrel presence or which extend the previously known range of the grey squirrel, are at least one 10km² square removed from the next nearest sighting and are not accompanied by physical evidence of grey squirrel presence.

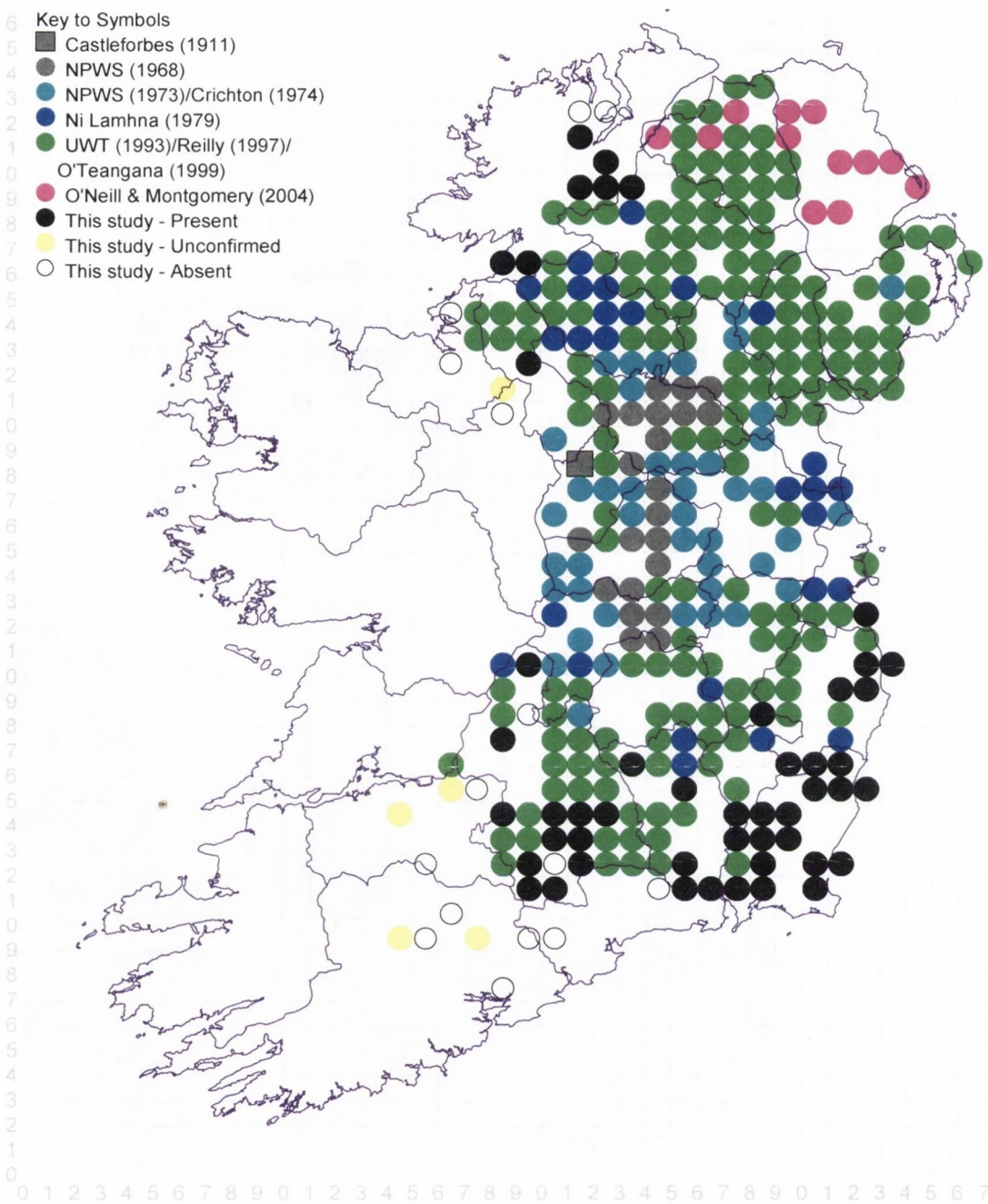


Figure 4.4. 10km² grid map detailing the range expansion of the grey squirrel since its introduction to Castleforbes in 1911. The map shows the new 10km² squares the grey squirrel was present in during each successive survey. Unconfirmed sightings are sightings which are west of the Shannon and are not accompanied by physical evidence of grey squirrel presence or which extend the previously known range of the grey squirrel, are at least one 10km² square removed from the next nearest sighting and are not accompanied by physical evidence of grey squirrel presence.

Wicklow

Reilly (1997) noted that that two records for the grey squirrel, one in Wicklow and one in Wexford were particularly isolated and that none of these areas had recorded a permanent population of the grey squirrel. This is no longer the case. Grey squirrels are common in the public parks and golf clubs of south Dublin and north Wicklow, for example Marley Park, Rathfarnham Golf Club, Old Conna Golf Club. They have spread to most, if not all, lowland areas surrounding the Wicklow mountains and are present in some upland areas, for example Kippure Estate and at Glendalough, where grey squirrel hairs were present in hair tubes. They are also spreading along river valleys, for example the Glenmalure valley where they are present at Ballinacor Estate, on the south side of the Avonbeg River, and further up the valley as far as the Glenmalure Hotel.

Wexford

Grey squirrels are also now widespread throughout Co. Wexford. In north Wexford and Wicklow several people independently reported that grey squirrel were remarkably abundant in 2006. In the JFK Arboretum in southwest Wexford, grey squirrels have been trapped by Dr. Chris Wilson, Conservation Ranger, the first in December 2005. There were 5 reports of grey squirrels in the Johnstown Castle Estate, a 400 ha estate in southwest Wexford which houses offices for the Forest Service, the Environmental Frotection Agency, Teagasc etc. Grey squirrels were also reported present on the east Wexford coast in Courttown Woods and in the Raven Woods, Curracloe. The local conservation ranger reported grey squirrel roadkill in the townland of Jamestown, Oilgate. The grey squirrel was only recorded as being absent from an area within 5-10km of the south Wexford coast.

Kilkenny

Reilly (1997) reports the grey squirrel as spreading along the River Nore but had few reports from south Kilkenny. Grey squirrels were reported as being present in most if not all the woodlands of the Rivers Nore, which runs through Co. Kilkenny, and of the River Barrow, which forms part of the

Carlow/Kilkenny boundary by the NPWS Conservation ranger. While undoubtedly true, this data was recorded as anecdotal data on the survey form and not subsequently used. 35 grey squirrels were trapped by the author in Mount Juilet, Thomastown, Co. Kilkenny. Consequently, the survey focussed on establishing their presence south of this location. On the Kilkenny/Waterford border, just north of the River Suir, grey squirrels were first recorded in Kildalton College, Pilltown in 1999. They have subsequently been studied here using hair tubes by Dr. Peter Turner of Waterford Institute of Technology. A number of foresters reported considerable damage caused by grey squirrels to young broadleaved plantations near Windgap, Mullinavat and to Kyleadohir Nature Reserve near Callan. The grey squirrel was recorded as being present in all the 10km^2 squares of south Kilkenny.

Carlow

The grey squirrel was recorded as being present in south Carlow, in woodland along the banks of the River Barrow, for example Clashganny Forest a broadleaved woodland of oak, hazel, beech and holly.

Tipperary

Reilly (1997) notes the grey squirrel as having reached the Glen of Aherlow within the previous 5 years but regarded it as then uncommon in south Tipperary. It is now abundant throughout much of south Tipperary where it is hunted keenly as a game species. Two grey squirrels were seen by Dr. Douglas Butler, a zoologist, while shooting in conifer plantations in the Knockmealdown mountains. This sighting would appear to be at the edge of its southerly range. Strangely, there are no reports of grey squirrels present in parts of west Tipperary, which is bordered by the River Shannon, for example Dromineer, either in this survey or in Reilly (1997).

Waterford

Reilly (1997) had no reports of grey squirrels from Co. Waterford. The River Suir appears to have acted as a boundary to the expansion of the grey squirrel from Co. Tipperary to into Co. Waterford. However, this is no longer

the case. There are three reports of grey squirrels in Co. Waterford, south of the River Suir near the bridge at Kilsheelan. One report is of a juvenile grey squirrel killer by a terrier, another of grey squirrels present in the area for at least three years. The final report was of "definite" grey squirrels in the same area i.e. on the edge of a 1000 hectare mixed conifer –broadleaved plantation.

Cork

There was a report of two grey squirrels seen in woodland outside Fermoy and of two grey squirrels in an estate outside Mallow, both on the River Blackwater. All other reports, for example from Doneraile Forest Park, Mallow, Charleville and Mitchelstown Golf Clubs, NPWS Conservation Rangers, foresters etc. indicated that there were no grey squirrel in north Cork. Reilly (1997) had no reports of grey squirrels from Co. Cork.

Limerick

In this survey, there was one report of a grey squirrel population in Adare Co. Limerick. This was contradicted by many reports from foresters, gun club members, Birdwatch Ireland members and NPWS Conservation rangers which stated that there were no grey squirrels in Co. Limerick. There was also one report of a single grey squirrel seen in Annacotty, near the Tipperary border. Reilly (1997) had no reports of grey squirrels from Co. Limerick.

Sligo

Neither this survey nor Reilly (1997) received any records of grey squirrels from Co. Sligo. In particular they were noted as being definitely absent from Lissadel House and from the woodland around Lough Gill.

Leitrim

Reilly (1997) obtained reports of grey squirrels from unnamed estates outside Manorhamilton. However, gunclub members from Manorhamilton,

Dromahair, Rossinver and Glenfarne were all adamant that grey squirrels were never present in their areas.

Donegal

The expansion of the grey squirrel in northwest Co. Donegal was perhaps the most surprising aspect of the survey. A grey squirrel was shot and photographed 5km northwest of Letterkenny in early 2006, the furthest point north and west in Ireland grey squirrels had reached, and a copy of the photograph obtained (Plate 4.1). On Nov 26th, 2006 a freshly killed carcass was found by Pat Vaughan, NPWS on the road at Doon Glebe, 5km west of Letterkenny. Further south there were sightings of grey squirrels around Raphoe, Ballybofey and Pettigo. David McDonagh, NPWS found grey squirrel roadkill 2km southwest of Pettigo on July 14th, 2005. Further southwest again, grey squirrels were shot just outside Ballyshannon on the Bundoran Rd. Grey squirrels appear to be rapidly colonising the lowland areas south and east of the Blue Stack and Derryveagh mountains. Reilly (1997) had no reports of grey squirrels from Co. Donegal.

Cavan

Only two 10km x 10km squares in the northwest tip of Cavan, on the border with Leitrim were surveyed. This area is dominated by the Cuilcagh mountains and there is relatively little afforestation. There were secondhand reports of grey squirrels around Blacklion and Dowra.

Clare

There were no reports of grey squirrels west of the Shannon in Co. Clare

Galway

There were no reports of grey squirrels west of the Shannon in Co. Galway.

Roscommon

There were two reports of grey squirrels west of the River Shannon in Co. Roscommon, contrary to the belief to date that grey squirrels have not

crossed the Shannon. Anthony Prins, a trapper with NPWS reported that he saw grey squirrels in Lough Kee Forest Park in 1979. Paddy McManus, a trapper with the Department of Agriculture and a taxidermist, reported there were grey squirrels in the woods on the shores of Lough Allen, Lough Kee and Lough Arrow 15-20 years ago, in woods outside Drumshambo, on O'Connors Island in Lough Arrow and in beechwood around Ballyfarnan. He also reported that a grey squirrel was shot on the parapet of the bridge in Geevagh and that he found a grey squirrels tail left by an animal in hazelwood between Kilmactranny and Lough Arrow 3-4 years ago. The accurary of these reports has not been confirmed with actual physical evidence of grey squirrel presence in the area.



Figure 4.5 A grey squirrel shot and photographed 5km northwest of Letterkenny (Grid reference C1216) in early 2006.

4.3.2 Rate of spread of grey squirrels in Ireland

A modal rate of spread of 0.90km/year was calculated since 1996 based on 49 records of grey squirrels expanding to previously unoccupied 10km squares. However, as only 11 years have passed between the collection of data by Reilly (1997) and this survey, and most range expansion involves the movement of grey squirrels to adjacent 10km squares, the modal rate of spread calculated is not very meaningful.

Grey squirrels have spread 130km in a northerly direction, 178km north-easterly, 171km southerly and 192km south-easterly since 1911. The mean rate of spread from 1911-2007 is 1.75km/year. The two-dimensional diffusion coefficient was calculated as 0.93km^2/year.

4.4 Discussion

Grey squirrel expansion to the north, south and east has continued unabated since the survey of Reilly (1997). In just over 10 years grey squirrels have become firmly established in Co. Wicklow and Co. Wexford in the east and in south Carlow and south Kilkenny. In the northwest grey squirrels are spreading rapidly into Co. Donegal and in Northern Ireland only the glens in northeast Antrim are free of grey squirrels (O'Neill and Montgomery, 2003). Previous hindrances to grey squirrel range expansion in the south such as the River Suir and the large, upland conifer plantations in the Knockmealdown mountains have been overcome. Only in the west does the River Shannon appear to have largely maintained an effective barrier to grey squirrel movement, although it has been outflanked by the grey squirrel moving through Cavan and Fermanagh into south Donegal.

Red squirrels populations were reported from all areas in which grey squirrels were absent. For example, in Co. Limerick, at the edge of the grey squirrel range on the Co. Tipperary/Co. Limerick border, red squirrels were reported in Glenstal Abbey, Murroe and in the (River) Clare Glens. They were also reported in Galtee Castle Wood, the Darragh Hills, Carrigkerry, Currachchase, Ballinruane, Creeves and Adare, all in county Limerick. In Co. Waterford they are present in large conifer plantations near both Kilsheelan and Portlaw and in broadleaved estates in Cappoquin and Lismore. There were also many report of red squirrels near Fermoy and Mallow in north Cork, and around Lough Gill in Co. Sligo, in north Leitrim and in Donegal, where they are said to be rapidly colonising conifer plantations in the Inishowen peninsula.

The situation with regard to red squirrels in areas colonised by grey squirrels in the past 15-20 years is less encouraging. In several broadleaved woodlands grey squirrels have replaced red squirrels, for example in Knoxes Wood, Thurles, Co. Tipperary, Castlecomer Demense, Co. Kilkenny, Huntington Castle and Gardens, Clonegal, Co. Carlow, Tulfarris Estate,

Blessington, Co. Wicklow and also in several golf clubs, for example Rathfarnham Golf Club, Co. Dublin and Beech Park Golf Club, Co. Dublin. Red and grey squirrels now coexist in many woodlands recently colonised by grey squirrels, for example, in the JFK Arboretum and Raven Wood, Co. Wexford. In Ballinacor Estate, a shooting estate in Co. Wicklow it was noted that the red squirrel had become scarce after only 6-7 years of co-existance with the grey squirrel. A more detailed survey, particularly of large conifer plantations, would be needed to accurately gauge the red squirrel's present distribution in Ireland.

Reports were received of significant local declines in some grey squirrel populations. It has been suggested, for example by Carey (2006), that predation by pine martens may be responsible. A report received from a landowner living in a 80 acre parkland estate of 'old beech' near Nenagh, Co. Tipperary (R8885) noted that the pine marten had 'done away with' all the grey squirrels in the estate. On 50 acre estate of 'mature beech' near Fenagh, Co. Leitrim (H1007) the landowner reported that grey squirrels had not been present for 9 years. He estimated the year of their arrival as '30 years ago' and noted that they had been abundant during his childhood. Reilly (1997) also received a report of a population crash which led to localised extinctions of grey squirrel populations in Longford and Cavan and from which they never fully recovered. Watt (1923) reported that at Castleforbes, the site of the original introduction of grey squirrels to Ireland, 'about four years ago over three hundred [grey squirrels] were killed in the course of the year'. While trapping there over a four-week period in 2005, in open, mature parkland in front of the Castleforbes castle, the author caught no grey squirrels. Estate workers reported that no grey squirrels had been seen on the estate in over 10 years and speculated that the pine marten was responsible for the decline in grey squirrel numbers. The author did catch one grey squirrel in a trap placed at the edge of broadleaved woodland with dense undergrowth at the rear of the castle, but did not investigate this area further. These are anecdotal reports from around the country of grey squirrel population declines which may, or may not, be linked to an increase in

population size and range in pine martens. For example, reports from north Leitrim also noted a marked increase in the numbers of pine martens but that grey squirrels were not, and never had been, present in the area.

Predation has not been known to have a significant impact on grey squirrel population size (Gurnell, 1990) and research into the diet of pine martens where grey squirrels are present would be interesting. The reason for the marked local declines in grey squirrel populations described above is unknown. A short-term decline in grey squirrel numbers could occur if there was a poor tree seed crop in several successive years (Gurnell, 1983). Disease, insect infestation and advanced stand age could contribute to a loss of tree vigour and a more long-term decline in tree seed production (Wear and Greis, 2002) and consequently grey squirrel population size. Disease epidemics in grey squirrels, such as coccidiosis, may also cause population crashes (Keymer, 1983; Gurnell, 1987). Whether the observed local population declines are merely normal, stochastic changes in population size or something more interesting remains to be seen.

Substantial populations of invasive non-native species occasionally collapse dramatically, for example cane toads in Australia (Freeland, 1986). Simberloff and Gibbons (2004) present detailed information of 17 population crashes, 10 of which have no strong evidence suggesting a cause, although disease is often invoked. Several other widely cited collapses of introduced species may simply be temporary lows during a more or less regular boom-and-bust cycle. In several instances, apparently spontaneous crashes were in fact probably caused by subsequently introduced competitors (Simberloff and Gibbons, 2004). For example, King (1990) attributes the decline in the New Zealand weasel population in the mid 1900's to predation by the larger stoat (*Mustela erminea*).

The grey squirrel survey indicates that the rate of grey squirrel range expansion continues at a steady pace. O'Teangana *et al.* (2000) calculated one-dimensional rates of spread of 0-13.4km/year, with a modal rate of 0.56

km/year and a mean rate of 1.94km/year since 1911. This survey calculated a modal rate of 0.90 km/year since 1996 and, using a different methodology, a mean rate of spread of 1.75km/year since 1911. Williamson and Brown (1986) calculated a one-dimensional rate of spread for grey squirrels of 7.66 km/year in East Anglia and Wauters *et al.* (1997) calculated a rate of 4.72 km/year in the Po plain in Italy. These differences between countries are most likely due to landscape differences, particularly in the amount of suitable woodland for grey squirrels to colonise and also in barriers to dispersal.

These rates of spread are calculated as though grey squirrels always move the minimum distance between two points. In reality they must take a more circuitous route, depending on barriers to dispersal, the availability of suitable habitat etc. Therefore, rates of spread do not reflect the actual dispersal capability of grey squirrels but provide only a minimum estimate of annual grey squirrel movement.

Given that a mean rate of spread of 1.75km/year is a minimum estimate of grey squirrel dispersal, this conflicts with data from Hungerford and Wilder (1941), Flyger (1960), Mosby (1969), Taylor *et al.* (1971), Fitzgibbon (1993), and Hale *et al.,* (2001b) who describe most grey squirrel dispersal through the matrix between woodlands as being less than about 1.5km. However, the mean rate of spread encompasses movement through both continuous woodland and the surrounding matrix. Grey squirrels spread will be more rapid through more densely wooded areas and along riverine corridors than through highly fragmented woodland with large areas of intervening matrix. Also the above data on inter-woodland movement may not capture occasional long distance dispersal events, which are the most important factor in range expansion (Shigesada and Kawasaki, 1997).

The two-dimensional diffusion coefficient for grey squirrels has been calculated as 0.93km^2/year in Ireland (see Section 4.3.2), 1.15 km^2/year in Ireland (O'Teangana *et al.* 2000), 7 km^2/year in Italy (Wauters *et al.* 1997)

and 17.9 km^2/year in East Anglia (Okubo *et al.* 1989) using the diffusion model in Okubo *et al.* (1989). Diffusion models have been used to calculate the historic rate of spread and predict future spread of introduced species, for example the muskrat (*Ondatra zibethica*) in Europe (Skellam, 1951). The models predict that the squared distance the species move increases linearly with time (Okubo, 1980). Three assumptions underlie this: a homogenous environment, random movement (dispersal) and contiguous movement (dispersal). For greys squirrels, landscapes are not homogenous, as mountains, rivers and the matrix between woodland will hinder dispersal; movement (dispersal) is not random but will favour wooded areas, for example, along riverine corridors and movement (dispersal) is not contiguous but dependent on population density and food supply (Gurnell, 1987). Non-contiguous movement has been shown by O'Teangana *et al.* (2000) who calculated one-dimensional rates of spread of between 0-13.4km/year, with a modal rate of 0.56km/year, in Ireland. The differences in two-dimensional diffusion coefficients between countries is most likely due to landscape differences, as stated previously, but the results should be treated with some care, as the assumptions of the diffusion models are not met for grey squirrels.

The grey squirrel is now likely to continue its range expansion into Donegal along river valleys such as the River Finn and throughout lowland areas around the coastline. As mentioned above, it has outflanked the River Shannon and is moving towards Co. Sligo via Donegal where it is about 25km from Lissadel Forest. From there, the woodland surrounding Lough Gill and ultimately south Sligo, Roscommon and east Galway is within reach. In Waterford old broadleaved estates in Lismore and Cappoquin are almost within reach as are the woodlands of the River Blackwater which runs throughout north and east Cork. There is ample woodland in Limerick and Kerry to permit range expansion to the west coast and, thereafter, south to the broadleaved woodlands of Killarney National Park.

The only imponderable concerning grey squirrel expansion in Ireland is how will the grey squirrel fare in the blanket bog, mountains and associated conifer plantations of Mayo and west Galway. Are there sufficient hedgerows, tree rows or wooded riverine corridors to permit range expansion in this region? This question may best be answered by modelling the spread of the grey squirrel in Ireland using the model developed by Rushton *at al.*, (1997). This model which simulates the distribution of the grey squirrel in the landscape has two main components. The first is a geographical information system which stores habitat and animal population information. This system undertakes data manipulation and abstraction and provides input for the second component. This consists of programs simulating the population dynamics of grey squirrels and their interactions and dispersal within the GIS-held landscape. Much work is underway to introduce red squirrels to squirrel-free conifer plantations in this part of Ireland (Lawton, 2006) in the hope that they will act as a refuge for red squirrels in Ireland. This model could be used to help predict what areas are most like to remain grey squirrel free for the longest time and conservation efforts for the red squirrel could then be focussed on suitable conifer plantations therein.

5. The prevalence of squirrel pox virus in the Irish grey squirrel population and its impact on red squirrel distribution.

5.1 Introduction

5.1.1 Disease in wildlife

The study of disease in wildlife is a relatively new scientific discipline when compared to the study of disease in human or domestic animals. In the past, the study of disease in wildlife tended to focus on infections that affect people (for example, rabies) or livestock (for example, bovine tuberculosis). However, during the past 10 years conservation biologists have become increasingly concerned about the threat of infectious diseases to populations of endangered species, as it has become clear that pathogens can occasionally cause sudden and unexpected local declines in abundance. Examples include the impact of avian malaria (*Plasmodium relictum*) on indigenous Hawaiian forest bird populations (Freed *et al.,* 2005), the near eradication of the black-footed ferret (*Mustela nigripes*) by canine distemper (Williams *et al.,* 1988) and the associated impact of sylvatic plague (*Yersinia pestis*) on prairie dogs (*Cynomys spp.*) which make up 90% of the black footed ferret diet, and the effects of rabies and canine distemper on Ethiopian wolves (Laurenson *et al.,* 1998)

Pathogens may be a direct cause of population extinction. For example, canine distemper virus reduced the last known wild population of black footed ferrets from about 130 to 18, leading to a decision to capture these last surviving individuals for a captive breeding programme (Thorne and Williams, 1988). Alternatively, it has been suggested pathogens may suppress the size or resilience of their host population, increasing the probability of extinction due to other factors, as in the

case of grey wolves (*Canis lupus*) in Minnesota and canine parvovirus (Mech and Goyal, 1995).

Generalist pathogens as opposed to host specific pathogens present the most serious extinction risks to endangered species. Pathogens maintained in relatively common species can cause local extinction in populations of threatened species when they spill over into threatened hosts. Rabies and canine distemper virus are maintained in populations of domestic dogs in Africa and both viruses have threatened populations of Ethiopian wolves (Laurenson *et al.,* 1998) and African wild dogs (Alexander and Appel, 1994). Lethal pathogens with short infectious periods, which are not maintained in reservoir hosts, are predicted to persist only in large, spatially structured host populations with high turnover. Such pathogens cannot persist in the small isolated populations typical of many threatened species (Lyles and Dobson, 1993).

For endangered species management the key issue is to identify the risk of infection entering the host population and the probability that the infection will result in host extinction. Population viability analysis has been used to show that infections can significantly increase the risk of extinction, for example with canine distemper and rabies in the Ethiopian wolf (Mace and Sillero-Zubiri, 1997). In contrast, Gurnell *et al.,* (2006) studied the squirrel pox virus (SQPV) disease threat to red squirrels in the largest, designated red squirrel refuge site in England, Kielder Forest (50,000 ha), using modelling techniques. Assuming that grey squirrels do not settle within Kielder Forest because of the unfavourable nature of the spruce habitat, Gurnell *et al.,* (2006) predicted that SQPV disease would burn out at the edges of the forest, although many red squirrels would die.

When disease in a reservoir population threatens an endangered species, it may be possible to devise management strategies that minimise

infectious contacts between host populations. Red squirrels are being introduced into isolated conifer woodlands in the west of Ireland in the hope that grey squirrels, and hence squirrel pox virus, will be unable to reach these woodlands (Lawton, 2006). It has also been proposed to prevent grey squirrels from entering the Inisowen Peninsula in Co. Donegal by maintaining the 20km x 20km strip connecting the Inisowen Peninsula to the remainder of Co. Donegal as a grey squirrel free buffer zone (E. Johnston, pers. comm.).

Where it is impossible to prevent contact between threatened hosts and reservoir hosts, reducing the population size of the reservoir host might reduce the risk of disease transmission. In the case of grey squirrels, a reduction in population size could be achieved by culling or by using an immunocontraceptive. Alternatively, management could focus on the eradication of the infection by vaccination of the reservoir host. However, given the high birth/death rates and rapid turnover that characterise grey squirrel populations, and the fact that no immunocontraceptive or vaccine is available; none of these options are feasible in practice.

Vaccination of threatened hosts is the most certain and direct method of protecting individuals from disease and has been attempted for a variety of host pathogen systems. Oral rabies vaccines and bait delivery systems have been successful in reducing the incidence of rabies in several populations including red foxes in Europe (Pastoret and Brochier, 1998). However, direct vaccination may be impractical. Administering any vaccine to large populations of red squirrels would lead to logistic difficulties and require the development of an oral vaccine. In the case of red squirrels, no safe, effective vaccine, either injectable or oral, is available.

5.1.2 Disease in squirrels

Keymer (1983) subjected 40 red squirrels and 30 grey squirrels to full postmortum examination and reported the results, together with other reports of disease affecting freeliving squirrels in Britain. Coccidiosis (*Eimeria sciurorum* infection) was common in red squirrels and both species frequently harboured other *Eimeria* spp. parasites in the intestinal tract. Other protozoa that occurred included haemogregarines (probably *Hepatozoon* sp.) and *Toxoplasma.* Helminths were relatively uncommon, but *Enterobius* spp. were found in the gut of reds. Both species were commonly infested with fleas, mainly *Monopsyllus sciurorum* in red squirrels and *Orchopeas howardi* in grey squirrels. Lice, especially *Neohaematopinus sciuri,* and ticks (*Ixodes ricinus*) also occurred in both species. Keymer (1983) concluded that there appeared to be a higher incidence of disease in red squirrels than in grey squirrels.

5.1.3 Squirrel pox virus (SQPV)

The Poxviridae family is divided into two subfamilies, Chordopoxvirinae, the vertebrate poxviruses and Entomopoxvirinae, the insect poxviruses (Smith and Kotwa, 2002). Squirrel pox virus (SQPV) represents a previously unrecognised genus of the Chordopoxviridae (Thomas *et al.,* 2003). The extinction of local populations of red squirrels in the UK has been attributed to SQPV (Sainsbury *et al.,* 2000; Tompkins *et al.,* 2003). The disease is characterised by ulcerated and haemorrhagic scabs affecting the skin around the eyes, nose and lips which later spread to ventral thorax, inguinal area and feet (Thomas *et al.,* 2003). Experimental infection with a mixture of isolates from various outbreaks of scab disease confirmed that SQPV is the agent responsible for the disease observed in red squirrels. In contrast, infection of grey squirrels failed to induce disease although all developed antibodies to the virus (Tompkins *et al.,* 2002). Further confirmation that this virus could induce SQPV disease was also obtained by Thomas *et al.,* (2003), who

infected two captive bred red squirrels with virus isolated directly from scab material.

A serological survey of red and grey squirrels from the UK revealed that 61% (136/223) of apparently healthy grey squirrels had antibody to SQPV, whereas only 2.86% (4/140) of red squirrels tested were seropositive. 3 out of 4 of the seropositive red squirrels were found dead or dying with symptoms of pox virus disease and poxvirus particles were identified by electron microscopy in these red squirrels. Only one red squirrel had antibodies to SQPV without having confirmed SQPV associated disease, which suggests that the disease was in the incubation stage in this squirrel or it was a solitary example of a red squirrel which had survived the infection and seroconverted. A high seroprevelance of antibodies to SQPV and the low prevelance of the disease in grey squirrels suggest that grey squirrels may act as reservoir for SQPV, which may then be transmitted to red squirrels causing lethal disease (Sainsbury *et al.*, 2000). SQPV is not present Italy or Scotland. However, the virus is likely to move through the expanding grey squirrel range in northern England and infect Scottish grey squirrels where they already come into contact in the Solway area (Gurnell *et al.*, 2006).

In 1999, a SQPV seroprevalence survey was carried out in Northern Ireland by testing serum from 163 grey squirrels in 17 forests in 4 counties. 20% were seropositive (McGhie, 2000). This was followed up by a survey of 181 grey squirrels from 16 forests in all 6 counties of Northern Ireland. 33% were seropositive and SQPV was found to be prevelant in all 6 counties (McKay *et al.*, 2004). Disease associated with SQPV in red squirrels been confirmed by electron microscopy from at least four regions of the U.K. (Cumbria, Lancashire, Northumberland and Thetford Chase), (Sainsbury *et al.*, 2000) but has not yet been reported in red squirrels in Ireland.

5.1.4 Pathogen mediated competition and decline in red squirrel distribution

Pathogen mediated competition or apparent competition occurs where one species can be a superior competitor simply by harbouring and transmitting a pathogen to a more vulnerable species, Squirrel poxvirus (SQPV) is an example of pathogen-mediated competition between an invasive species, the grey squirrel, and a native species, the Eurasian red squirrel. SQPV causes disease with high mortality in red squirrels but appears non-pathogenic in grey squirrels. Not all populations of introduced grey squirrels carry the virus, notably those in Scotland and Italy, and the rate of red squirrel replacement by grey squirrels is 17-25 times faster in those areas where grey squirrels carry the virus (Rushton *et al.,* 2006).

Rushton *et al.,* (2000) developed a stochastic, individual-based model for simulating the dynamics of pox virus in sympatric red and grey squirrel populations. The disease model was integrated with a spatially explicit population dynamics model that simulated red and grey squirrel populations in real landscapes. The model was used to investigate the dynamics of red and grey squirrels in Norfolk between 1966 and 1980. The model predicted that pox virus would cause a more rapid local replacement of red squirrels by grey squirrels.

Tompkins *et al.,* (2003) modified the two-host shared microparasite model of Bowers and Turner (1997 in Tompkins *et al.,* (2003)) to make it specifically applicable to the squirrel/virus system and tested whether the model could replicate the dynamics of the expansion of grey squirrels and decline of red squirrels that occurred in Norfolk from 1960 to 1982, In the spatial model the rate of ecological replacement incorporating both interspecific competition and poxvirus was very close to that seen in the actual Norfolk data. Without the virus, the natural rates of

competition alone could not explain the red squirrel decline and the grey squirrel expansion that was observed.

Rushton *et al.,* (2006) subsequently analysed the incidence of SQPV disease and changes in distribution of red and grey squirrels in Cumbria, from 1993 to 2003 and compared these data to the predictions of the Rushton *et al.,* (2000) model. A comparison of rates of decline in red squirrel populations in areas where SQPV was present, Cumbria and Norfolk, and in areas where SQPV was absent, Scotland and Italy showed that declines were 17–25 times higher in regions where SQPV was present. The model predicted spatial overlap of 3–4 years between the species before red squirrels were replaced and that was observed in the field. A grey squirrel population control of > 60% effective kill was needed to stop the decline in red squirrel populations in Cumbria.

Gurnell *et al.,* (2006) developed strategies to manage the SQPV disease threat to red squirrels by reference to the largest, designated red squirrel refuge site in England, Kielder Forest (50,000 ha). Using modelling techniques, they identified four main corridors by which grey squirrels will reach Kielder Forest. There would be high, repeated, local mortality amongst red squirrels on the periphery of the forest. This predicted pattern of disease was contingent on two important assumptions. First that the progression from infection with SQPV to death in red squirrels was very rapid, and there is evidence that this is less than three weeks (Tompkins *et al.,* 2002). Second, that grey squirrels do not penetrate deeply into Kielder Forest and become residents because of the unfavourable nature of the spruce habitat. They concluded that the conservation of red squirrels will depend on minimising contact between red and grey squirrel populations, and advocated monitoring grey squirrels in corridors within buffer zones around refuge areas, and removing them when detected.

5.1.5 Hypothesis

It was hypothesised that (1) SPV would be present in grey squirrel populations throughout Ireland and (2) the rate of decline in red squirrel distribution in Ireland would be similar to Norfolk and Cumbria and not Italy and Scotland.

5.1.6 Objectives

To determine the prevalence of squirrel pox virus in five population in Ireland.

To calculate the rate of decline in red squirrel distribution in Ireland and compare it to Norfolk and Cumbria, where SPV is present and to Italy and Scotland, where SPV is absent.

5.2 Methods

5.2.1 Testing serum for squirrel pox virus

205 grey squirrels were collected from 5 populations in Ireland; Shane's Castle, Castle Leslie, Beau Parc, Clongowes Wood and Brittas Castle (Figure 5.1).

In Shane's Castle and Brittas Castle grey squirrels were shot and stored in at freezer at –20 C. In Castle Leslie, Beau Parc and Clongowes Wood grey squirrels were euthanised as described in Section 2.2 and stored in a freezer at –20 C.

11 red squirrel serum samples were obtained for testing from 4 areas, Killarney, Co.Kerry, Adare, Co.Limerick, Curtlestown, Co. Wicklow and Killiney, Co. Dublin (Figure 5.1). These samples were taken from red squirrels found dead in woodlands or as roadkill.

Serum samples were then collected as follows. Squirrels were removed from the freezer and allowed to thaw at room temperature for 12-18 hours. A squirrel was pinned to a dissecting tray ventral side up. A scalpel was used to make an incision in the squirrel's skin, starting underneath the jawbone and running down the centre of the thorax, along the sternum, to the bottom of the rib cage. A small incision was made through the skin at the bottom of the rib cage into the abdomen. A scissors was placed in this opening and the rib cage cut in half following the line of the first incision. The rib cages were prised apart with the scissors, the thoracic cavity exposed and membranes and ligament surrounding the heart cut away. The heart was punctured with a sterile, large bore 16G needle attached to a sterile 5ml syringe. 0.5-2ml of blood was drawn into the syringe and transferred into a sterile 25ml centrifuge tube. The scalpel blade and scissors were the wiped clean and flamed in alcohol to prevent cross contamination. The blood samples were

centrifuged at 2500 rpm for 15 minutes. 0.1-0.2ml of supernatant was drawn off and stored in a screwcap eppendorf tube at –20 C. These serum samples were sent to Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian, EH26 OPZ, U.K. (Tel 00441314455111 Email: info@moredun.ac.uk) for testing for squirrel pox virus.

Sainsbury *et al.,* (2000) developed a direct enzyme-linked immunosorbent assay (ELISA) for squirrel pox virus which was performed on the serum samples. Squirrel sera were tested for antibody to the red squirrel parapoxvirus using a standard ELISA in 96 well microplates. Duplicate wells were coated overnight with a 1/300 dilution of an NP-40 extract of parapoxvirus infected cells and duplicate neighbouring wells were coated with similarly extracted uninfected cells. Test sera, diluted 1/50 were added to the four wells and incubated for 2 hours at 37 °C. After washing, all wells received a 1/1000 dilution of protein G conjugated to horse radish peroxidase and plates were incubated for a further 2 hours before further extensive washing. The substrate orthophenylenediomine was added for approximately 4 minutes after which the reaction was stopped with 2.5M H_2SO_4. The optical density was read at 492 nm. Corrected optical densities (ODs) were calculated by subtracting the mean of the ODs from the two control wells from the mean of the ODs from the infected wells. Based on the results obtained from an initial 45 grey and 9 red squirrel sera when developing the test, the cut-off point for a positive result was an OD of 0.2.

5.2.2 Calculating the rate of decline in red squirrel distribution in Ireland

The rate of decline in red squirrel distribution in Ireland was then investigated in two ways using data by O'Reilly (1997), O' Teangana (1999) and Ni Lamhna (1979). Firstly, the red squirrel distribution data

in Ni Lamhna (1979) was compared with the red and grey squirrel distribution data in O'Reilly (1997)/O'Teangana (1999). Where grey squirrels were recorded as present and red squirrels absent in a 10km square in O'Reilly (1997)/O'Teangana (1999), and red squirrels had previously been recorded as present in that square in Ni Lamhna (1979), then it was assumed that the grey squirrels had replaced red squirrels from that square. The Ni Lamhna (1979) records were collected from 1950-78. It was assumed that any red squirrel records collected post 1950 also reflected red squirrel presence in 1950. Therefore the Ni Lamhna (1979) distribution data was considered to reflect red squirrel distribution in 1950. The rate of decline in red squirrel distribution was therefore calculated from 1950-1995.

Using the O'Reilly (1997)/O'Teangana (1999) data, the total number of 10km squares occupied by grey squirrels but not by red squirrels was calculated. Taking the year of grey squirrel introduction as 1911 and the end of fieldwork on the O'Reilly (1997)/O'Teangana (1999) survey as 1995 the rate of decline in red squirrel distribution in km^2/year was calculated. This assumes that any area occupied by grey squirrels would otherwise be occupied by red squirrels but for the presence of grey squirrels.

5.3 Results

5.3.1 Prevalence of squirrel pox virus in Ireland

SQPV was found to be present in four out of five grey squirrel populations tested in Ireland, (Shane's Castle, Co. Antrim, Castle Leslie, Co. Monaghan, Beau Parc, Co. Meath and Clongowes Wood, Co. Kildare), with seroprevalence ranging from 27-60%. SQPV was absent only from the 23 grey squirrels tested in the most southerly population in Brittas Castle, Co. Tipperary. All red squirrels tested were negative for SQPV (Figure 5.1; Table 5.1a).

7 juveniles/subadults (i.e. less than 10-12 months old) were infected with SQPV, the remaining 62 individuals infected were adults (Table 5.1b). There was a significant association between age class and infection with SQPV (Chi squared test, Chi-Sq = 16.746, DF = 1, P-Value <0.001). There was no association between squirrel sex and infection (see Table 5.1b) (Chi squared test, Chi-Sq = 0.934, DF = 1, P-Value = 0.334).

The location of each squirrel with SQPV captured in Castle Leslie, Beau Parc and Clongowes Wood was plotted to investigate if the locations of capture of infected squirrels tended to be clustered together. This would suggest that infected squirrels shared overlapping or adjacent home ranges. The locations of capture of infected squirrels were not clustered but were distributed throughout each woodland.



Figure 5.1 Map of Ireland showing sites where grey squirrel and red squirrel serum samples were collected for testing for Squirrel Pox Virus (SQPV). The figures indicate the number of squirrels which were positive for SQPV and total number tested at that site.

Table 5.1a. Results of tests for Squirrel Pox Virus (SQPV) on 205 grey squirrels from 5 populations in Ireland

Location	Grid reference	Number tested	Number positive for SQPV	Percentage positive for SQPV
Shanes Castle	J1389	22	7	32
Castle Leslie	H7241	60	18	30
Beau Parc	N9472	52	31	60
Clongowes Wood	N8729	48	13	27
Brittas Castle	S1261	23	0	0

Table 5.1b. Results of tests for Squirrel Pox Virus (SQPV) on 182 grey squirrels from the 4 populations testing positive (Shanes Castle, Castle Leslie, Beau Parc and Brittas Castle) by sex and age class.

SPV status	Male		Female	
	Adult	Juvenile	Adult	Juvenile
Negative	30	19	40	24
Positive	34	2	28	5

Table 5.2 Results of tests for Squirrel Pox Virus (SQPV) on 11 red squirrels in Ireland

Location	Grid reference	Number tested	Number positive for SQPV	Percentage positive for SQPV
Killarney	V98	7	0	0
Curtlestown	O160180	2	0	0
Adare	R455450	1	0	0
Killiney	O263260	1	0	0

5.3.2 Rate of decline in red squirrel distribution in Ireland

Comparing the red squirrel distribution data in O'Reilly (1997)/O'Teangana (1999) with Ni Lamhna (1979), reveals that red squirrels were absent from 28 10km squares in 1995 in which they were present in 1950. This is a decline in red squirrel distribution of 2,800 km^2 or 62km^2/year.

In O'Reilly (1997)/O'Teangana (1999) the total number of 10km squares occupied by grey squirrels but not by red squirrels was 68, equaling 6,800 km^2. This represents a decline of 81km^2/year in red squirrel distribution since 1911. The reasons behind the difference in these two figures for decline in red squirrel distribution in km^2/year will be discussed below.

5.4 Discussion

Similar to the decline in red squirrels, over the past 20 years there have been declines in many wildlife populations attributed to pathogens. For example, populations of 14 species of Australian rainforest frogs have either declined or become locally extinct. Disappearances have progressed from south to north, commencing just north of Brisbane in 1985 (Laurance *et al.*, 1996; Campbell, 1999) and have been attributed to a novel fungal pathogen *Batrachochytrium dendrobates* (Berger *et al.*, 1998; Skerratt *et al.*, 2007).

Rapid decline in red squirrel populations is the result of apparent competition mediated by SQPV. Apparent competition can be difficult to demonstrate conclusively as it is difficult to distinguish from resource competition. It has been demonstrated in the laboratory, in the absence of resource competition, with the ichneumonid parasitic wasp *Venturia canescens* and two of its moth hosts *Plodia interpunctella* and *Ephestia kuehniella* (Bonsall and Hassell, 1998). Among introduced species, transmission of the caecal nematode *Heterakis gallinarum* from introduced pheasants *Phasianus colchicus* to native wild grey partridges *Perdix perdix* has also been implicated in the decline of partridge populations in the U.K. (Tompkins *et al.*, 2001). Likewise transmission of rabies from domestic dogs to both Ethiopian wolves and wild dogs is threatening populations of these species (Alexander and Appel, 1994; Laurenson *et al.*, 1998). In contrast, experimental studies found no evidence that the cestode parasite *Cylindrotaenia* sp. was implicated in the competitive replacement of the native gecko *Lepidodactylus lugubris* by the invader gecko *Hemidactylus frenatus*, as originally suspected, leaving resource competition as the most likely explanation (Hanley *et al.*, 1998).

Skerratt *et al.*, (2007) outlined three pieces of evidence needed to establish that a spreading pathogen is causing population decline.

Firstly, the pathogen must be highly pathogenic, virulent and transmissible. Tompkins *et al.*, (2002) and Thomas *et al.*, (2003) confirmed that a pathogen, SQPV, caused a fatal skin disease by infecting red squirrels with isolates from various outbreaks of scab disease. The lesions documented were the same as those in squirrels recovered dead from the wild. The incubation period of SQPV is short 5-8 days (Tompkins *et al.*, 2002) or <15 days (Carroll *et al.*, 2009) as is the course of the disease - an average of 10 days (Carroll *et al.*, 2009). The disease is transmissible. Red squirrels from Kielder Forest which were seronegative for SQPV, but translocated to Thetford Chase, later contracted SQPV and died (Carroll *et al.*, 2009). The exact mode of transmission of SQPV is unknown although it appears direct, and not vector borne (Sainsbury *et al.*, 2008; Bruemmer *et al.*, 2010).

Second, the population declines and the pathogen must show temporal-spatial patterns of spread. Also the pathogen has at least one source from which it has emerged (Skerratt *et al.*, 2007). Sainsbury *et al.*, (2008) mapped the expansion of SQPV disease in the north of England. SQPV disease only occurred in those areas also inhabited by seropositive grey squirrels. As the geographic range of the grey squirrel expanded, SQPV disease occurred in these new grey squirrel habitats and red squirrel populations declined. SQPV has emerged from the introduced American grey squirrel and a role for the grey squirrel as a reservoir host for SQPV is supported by the above data.

Finally, the pathogen must be present during declines and mass mortality events and appears to be causing the mortality based on its epidemiology and pathogenesis. There are reports of widespread epidemics of skin disease in red squirrels in English counties associated with a decline in red squirrel distribution since 1930 (Middleton 1930; Shorten 1954; Tompkins *et al.*, 2002) and SQPV has been isolated from skin lesions characteristic of this disease (Thomas *et al.*, 2003).

In Ireland seroprevalence rates for SQPV in grey squirrel populations ranged from 27% - 60%. In England and Wales seroprevalence rates ranged from 42% to 100% (Sainsbury *et al.,* 2000), but individuals in the southern range of the grey squirrel in Ireland, in Scotland and in parts of northern England were not infected. In this study, in the four Irish populations in which SQPV was present the overall seroprevalence (38%) was similar to that detected in Northern Ireland (33%, McKay, 2000) but lower than that in the UK (61%, Sainsbury *et al.,* 2000)).

Further testing will be required to determine if SQPV is, as yet, largely absent from the southerly range of the grey squirrel in Ireland or if the Brittas Castle population is an isolated SQPV-free area or population. As the Irish grey squirrel population began with a single introduction of 12 individuals in Castleforbes, Co. Longford and spread to the north, east and south, it may be possible that a lineage which colonized areas of the south is, by chance, SQPV-free. Such a pattern of occurrence of SQPV in Ireland may arise from the historical pattern of introduction of SQPV. It is possible that only a few of the original introduced grey squirrels carried the disease into the country. This could readily occur because the virus does not have a high prevalence in grey squirrels in its native homeland nor is it present in grey squirrels throughout its natural range (Gurnell *et al.,* 2006). However, even if SQPV is absent from areas in the grey squirrels southern range, this is unlikely to remain so indefinitely, given the prevalence of SQPV in the remainder of the country.

A comprehensive survey of SPV prevalence in red squirrels in interface areas was not possible in this study. The 11 red squirrels tested for SPV were originally collected for a population genetic study by Dr. Laura Finnegan. Only 3 were from interface areas, 2 from Curtlestown Co. Wicklow and 1 from Killiney, Co.Dublin. All red squirrels tested were negative for SQPV antibodies.

There have been no reports of red squirrels showing clinical signs of SQPV disease in Ireland. Whether this is because there have been no outbreaks of SQPV disease among red squirrels in Ireland or whether it is due to a lack of awareness of the symptoms of the disease among wildlife workers in unknown. Detection of the disease depends on corpses or sick animals being found and many red squirrels may die undetected (Rushton *et al.,* 2006). Prior to Reilly (1997) there were no research publications on red squirrels in Ireland other than distribution surveys. Consequently interest in red squirrels and awareness of pox virus is relatively new among wildlife workers in Ireland.

Rushton *et al.,* (2006) calculated rates of decline in red squirrel distribution in different regions of England, Scotland and Italy. In Cumbria and Norfolk, where SQPV was present, observed rates of red squirrel loss were 76km^2/year and 92 km^2/year. In Scotland and Italy, where SQPV was absent, observed rates of red squirrel loss were 4.4 km^2/year and 3.6 km^2/year. Therefore in Cumbria and Norfolk where SQPV was present, the decline in distribution of red squirrels was some 17–25 times faster than in Scotland and Italy where SQPV was absent. In Ireland the rate of decline of red squirrels from 1950-1995 was 62 km^2/year, and from 1911-1995 81 km^2/year. Therefore, the decline in Ireland, where SQPV is prevalent is comparable with Cumbria and Norfolk. In conclusion, the similarity between the rates of decline in red squirrel distribution in Ireland, calculated by either of the above methods, and the rates of decline in Cumbria and Norfolk provides an additional piece of evidence for the critical role of SQPV in the replacement of red squirrels by grey squirrels.

There is obvious potential for bias in the Irish data. For example, the distribution data in the studies by Ni Lamhna (1979), O'Reilly (1997) and O'Teangana (1999) and were not derived from systematic surveys of red and grey squirrel populations in Ireland. All studies relied on records of sightings from members of the public. The survey design and effort

involved differed between the studies and the raw data for both species may have exhibited bias. In particular for Ni Lamhna (1979) the assumption that where red squirrels were recorded post 1950 they were also present in 1950 will not always be true. An increase in the planting, and subsequent maturing, of conifer plantations throughout the 1900's would have created new habitats for the red squirrel which would not have been present in 1950. Where this assumption is not true it has the effect of underestimating the rate of decline. Also, the red squirrel distribution data was collected over the period 1950-1978. It is possible that some red squirrel populations may have been present but unrecorded in 1950 (or thereafter) and later been replaced by grey squirrels, thus remaining unrecorded forever. This would also lead to an underestimatation in the rate of the red squirrel decline between 1950-95. The assumption that where grey squirrels are present, red squirrels would be present but for the grey squirrels is reasonable as red squirrels can exist in all squirrel habitat types, broadleaved, mixed or conifer woodlands, parks, golf courses etc., in the absence of the grey squirrels.

Despite this potential for bias, there is a strong similarity in the rate of decline in red squirrel distribution in Cumbria, Norfolk and Ireland and this does not bode well for the future of the red squirrel in Ireland.

6. Discussion

This study examines the vagility of an introduced species, the grey squirrel, with an emphasis on dispersal movements and gene flow, through studies of population genetic structure (chapter 2), control (chapter 3), and range expansion (chapter 4). Finally, it deals with the impact of this vagility and the presence of a lethal pathogen, squirrel pox virus, on the rate of decline in red squirrel distribution in Ireland (Chapter 5).

6.1 Genetic diversity

The Irish grey squirrel populations displayed moderate genetic diversity generally comparable to sciurid and other mammal populations worldwide (Hale *et al.,* 2001a; Todd, 2000; Trizio *et al.,* 2005; Shibata *et al.,* 2003; Gunn *et al.,* 2005; Dewoody and Avise, 2000; Hampton *et al.*, 2004; Redeker *et al.*, 2005).

However, it difficult to determine underlining genetic diversity in a population, or to compare genetic diversity data between studies, using microsatellite data as microsatellite loci are not chosen randomly. Researchers tend only to work with the most polymorphic loci available and rarely report data for monomorphic loci or for loci with little polymorphism. Observed and expected heterozygosities reported in the literature will generally be considerably greater than those that would be obtained from a random sample of microsatellites. For example, Ogden *et al.,* (2005), reported a mean H_E 0.24 for the red squirrel population on Anglesea, lower than that reported in any of the studies above. However, this figure was obtained from an analysis of 3 monomorphic and 3 polymorphic loci in the red squirrel population. In contrast, Peakall *et al.*, (2006) used 11 microsatellite loci which were hypervariable, with the number of alleles varying from 11-25 per locus, when studying the

Australian bush rat. Interpretation of microsatellite genetic diversity data and comparison between studies therefore, requires caution.

Also, there was insufficient genetic diversity in the microsatellies used to answer questions concerning fine scale genetic structure, relatedness and breeding success (see Huck *et al.*, 2008). Hale *et al.,* (2001a) isolated 31 microsatellites from grey squirrel tissue samples obtained in the north of England. The 10 most polymorphic of these were analysed at the beginning of this study and 6 were found to have 3 or more alleles. Of these 6, one was found not to be in Hardy Weinberg equilibrium. The 5 remaining microsatellites used had insufficient polymorphism to detect bottlenecks (Piry *et al.,* 1999), to detect sex biased natal dispersal (Peakall and Smouse, 2006) and to identify parent/offspring combinations using software such as CERVUS (Marshall *et al.*, 1998). Additional molecular markers and/or a greater number of microsatellites with greater polymorphism, as for Peakall *et al.*, (2006), would be required to answer these questions.

6.2 Interpopulation or breeding dispersal

Although the grey squirrel is a vagile animal, it is a habitat specialist requiring woodland habitat, and data from this study suggest that inter-population dispersal between neighbouring woodlands is somewhat restricted. Genetic differentiation between populations was significant, even at a local level. Direct measures of dispersal during the removal experiment found only one movement of a grey squirrel between woodlands. Genetic data indicated that survivors of the cull in 2003, and not immigrants, were responsible for the population recovery observed. Restricted dispersal has also been found in other introduced (Taylor *et al.*, 1971; Fitzgibbon, 1993) and native grey squirrel populations (Flyger, 1960; Mosby, 1969).

Direct measures of dispersal in other small mammal species may also be

low. Hale *et al.*, (2001b) found that the maximum dispersal distance of red squirrels over unfavourable ground was probably less than or equal to 1.5km. 49 adult fox squirrels were radiotagged in 12 woodland sites in Indiana. No movements between woodlands were observed over 17 months (Sheperd and Swihart, 1995). Common voles were studied in six natural populations at distances of 0.4–2.5 km in three different seasons (fall, spring, summer) corresponding to different life-history stages. Field observations provided no direct evidence for movements of individuals between populations (Schweizer *et al.*, 2007). Telfer *et al.*, (2003) noted that between 1997 and 1998, 29 of 823 water voles trapped were caught in more than one population. The median natal dispersal distance for individuals that reached breeding status was 180m for both females and males. As with grey squirrels (Taylor *et al.*, 1971; Fitzgibbon, 1993), the negative exponential function fitted the distributions of breeding dispersal distances well (Telfer *et al.*, 2003).

There are limitations associated with direct capture-mark-recapture measures of dispersal. If there is considerable heterogeneity in capture probability, the resulting dispersal estimates will only be representative of a portion of the population. Practical restrictions on the size of the study site also limit the detection of long distance dispersal (Latch and Rhodes, 2006; Ogutu *et al.*, 2006). Genetic measures of dispersal, involving direct parentage assignment or indirect methods, reveal somewhat higher rates of dispersal than capture-mark-recapture estimates, but similar distribution of dispersal distances. While field observations of common voles provided no direct evidence for movements of individuals between populations, assignment analyses identified a proportion of individuals as being immigrants to the population where they were captured (Schweizer *et al.*, 2007). Low rates of dispersal have also been documented using capture-mark-recapture in juvenile kangaroo rats (*Dipodomys spectabilis*) but parentage analysis has shown that dispersal may occur prior to first capture (Waser *et al.*, 2006). In racoons (*Procyon lotor*) parentage analysis indicated that

dispersal distance distribution is highly positively skewed with 85% of raccoons, both male and female moving less than 3km. The tail of this distribution indicated a small proportion (<4%) moved more than 20km (Cullingham *et al.*, 2008).

Restricted dispersal is expected where the intervening matrix, is hostile, for example a busy urban environment (Tamura and Hayashi, 2007) but can also occur where the matrix appears benign, in this case pasture with small fields and a high density of hedgerows. Similar low dispersal rates and fine scale genetic structure have been documented for other small mammal species, for example the red squirrel, *Sciurus vulgaris*, (Trizio *et al.,* 2005), the common vole, *Microtus arvalis* (Schweizer *et al.*, 2007), the bank vole *Clethrionomys glareolus* (Aars *et al.*, 1998), the white-footed mouse, *Peromyscus leucous* (Mossman and Waser, 2001), the hairy footed gerbil, *Gerbillurus paeda,* (Meyer *et al.*, 2008), the bush rat *Rattus fuscipes* (Peakall *et al.*, 2006) and the sand dune tuco-tuco, *Ctenomys australis* (Mora *et al.*, 2010). The banner tailed kangaroo rat has been studied as a model system for mammalian dispersal for more than 25 years. Extensive field data suggest that dispersal between populations is low and more than 80% of dispersing individuals remain within 100m of their birthplace (Busch *et al.*, 2005). Likewise, no evidence of dispersal between population fragments was detected for the bush rat *Rattus fuscipes*, despite inter-fragment distances that were substantially shorter than distances moved in continuous habitat (Bentley, 2008).

If dispersal between woodlands is restricted, it would be expected that 'isolation by distance' would be present. There was evidence of isolation by distance emerging but it appears that a physical barrier to dispersal, the River Boyne, was disrupting this pattern. Major rivers have also been noted as a barrier to gene flow in Eastern Chipmunks (*Tamias striatus*) in Canada (Chambers and Garant, 2010). For grey squirrels in Ireland, lower levels of gene flow between habitat fragments, would appear to be

responsible for the greater genetic differentiation seen in comparison with red squirrels in continuous, conifer forest in the Alpine Alps (see Trizio *et al.*, 2005).

The relatively slow expansion of the grey squirrel in Ireland over the last 100 years also adds support to the finding of restricted dispersal between woodlands. The mean one-dimensional rate of spread since 1911 is 1.75km/year (this study) or 1.94km/year (O'Teangana *et al.*, 2000). Movement will be more rapid through continuous woodland than the surrounding matrix. Also self-sustaining populations of grey squirrels are not yet established west of the Shannon.

6.3 Population recovery

Removal methods have been used to study dispersal and recolonisation in small mammals for several decades. Such studies usually assume that trapping removes all resident animals from the study area and recolonisation, (which is usually rapid) occurs by immigration from adjacent populations (Stickel, 1946; van Vleck, 1968; Dobson, 1981; Gaines *et al.*, 1979; Joule and Cameron, 1975; Middleton and Merriam 1981). The recolonisation of woodland by grey squirrels following control is assumed to occur through immigration (Taylor, 1977; Rowe, 1980; Lawton and Rochford, 2007). However, Stenseth and Lidicher (1992) caution that these assumptions may not be true as efforts to remove all animals from a site may not be successful. This was found to be the case in this study. The Clongowes Wood populations of 2003-2005 partitioned together 86 percent of the time following 1000 bootstrap replicates of the allele frequencies data and the application of the UPGMA algorithm of PHYLIP (Felsenstein, 2004). This suggests that the Clongowes Wood populations of 2004 and 2005 are derived largely from survivors of the cull in 2003 and not from immigrants from the K Club, Mountarmstrong or elsewhere.

The methodology used in a removal experiment may influence the conclusions reached. For example, removal effort can vary greatly between studies and species. Lindenmayer *et al.*, (2005) studying bush rats, *Rattus fuscipes*, placed traps approximately 10m apart (100/ha). Trapping continued until no new animals were captured for at least 4 successive nights and for a minimum of 5 nights in each patch, but frequently the number of nights exceeded 10 and in one case 20. When trapping grey squirrels, Lawton and Gurnell (2007) set 1.08-1.75 traps/ha in 12 ha sites for 5 days. This study set 2.5-3.3 traps/ha in a 12 ha site for a total of 37 days. For small rodents, a very considerable effort is required to remove all the individuals from a site. If this effort is insufficient, resident individuals are likely to remain on the study site.

Removal experiments study different forms of movement. In some studies individuals were removed from small plots embedded in continuous habitat (for example Stickel, 1946; Gaines *et al.*, 1979; van Vleck, 1968) and recolonisation is possible through a combination of exploratory movements, natal dispersal and/or breeding dispersal. In this study and others, populations and sites are discrete (Middleton and Merriam, 1981) and longer distance movements are required for recolonisation. Therefore, different removal experiments may have different outcomes. Dispersal is more likely to contribute to the recolonisation of sites embedded in continuous habitat than more isolated sites.

Individuals in habitat surrounding the depopulated study site may be trapped and marked, to identify immigrants, (Stickel, 1946; van Vleck, 1968) or unmarked (Lawton and Rochford, 2007). It is difficult to mark all neighbouring individuals and to remove all resident individuals, so genetic techniques may be necessary to aid identification of colonising individuals (Lindenmayer *et al.*, 2005). Depending on the methodology used and data gathered, different conclusions may be reached. For example, Middleton and Merriam (1981) removed all white footed mice,

Peromyscus leucopus, from a small 2 ha wood and monitored recolonisation from three neighbouring tagged populations. Recolonisation was rapid, but no mice came from other woods. It was assumed the immigrants were untagged mice from farm buildings 300m away. Had genetic data been available it would have indicated if immigrants or residual individuals were responsible for the recolonisation observed.

A clear limitation of the removal experiment in this study is that the sample size was 1. Clearly, there are landscapes and study areas where dispersal might play an important role in population recovery following control. For example, if the woodland in which the control was carried out was adjacent to, or part of, a large broadleaved wood with a large grey squirrel population, with good connectivity to the study area, then the proportion of immigrants in the post control population would certainly be greater (see Gurnell and Steele, 2002). Similarly, differences in food availability between years will affect the level of dispersal, survival and fecundity in these years (Gurnell, 1987). When food supplies are good, dispersal is low, survival is high, fecundity is high and few young or adults disappear from the population; when food supplies are poor dispersal is high, survival is low, fecundity is low and many young and some adults disappear (Gurnell, 1983), There are also very occasional reports of very large numbers of grey squirrels dispersing in the autumn. Flyger (1969) is a well-documented example. These large-scale movements probably occur as a result of very large populations (resulting from good seed crops the previous year), with very poor autumn seed supplies; so the squirrels move in search of food (Gurnell, 1987). Therefore, the ratio of local recruits to immigrants in a grey squirrel population after control will depend on the efficacy of control, the distance to the nearest woodlands, the grey squirrel population size in these woodlands, connectivity between woodlands, whether or not grey squirrel control is practised in these woodlands and the effects of natural food availability on dispersal, survival and fecundity.

Lindenmayer *et al.*, (2005) suggest a number of demographic criteria for assessing if residual animals or immigrants are primarily responsible for population recovery following control. If residual animals are responsible then (i) there will be no relationship between rate of recovery and distance from source populations, (ii) recovery of populations by residual individuals will be quicker relative to the recovery by immigration (iii) the proportion of juveniles and males in recovering populations will be unchanged (this assumes control is unbiased and dispersal is juvenile and male biased). In a study of grey squirrel recovery following control by Lawton and Rochford (2007), the more isolated population (Ardmulchan) recovered more quickly; recovery was extremely rapid (in 3 out of 4 springtime culls the population recovered within ten weeks); post cull populations tended to consist of unmarked adults; post cull populations had a greater percentage of males but population sizes were low and this may not have been statistically significant. Based on the above criteria this would suggest that residual animals were responsible for the population recovery observed.

In a removal experiment similar to this study, Peakall and Lindenmayer (2006) investigated the mechanism of population recovery in the Australian bush rat, *Rattus fuscipes*, over 24 months using genetic monitoring, following the removal of animals by intense trapping. The outcomes of genetic analysis at 11 microsatellite loci and mtDNA indicated that population recovery was achieved predominately by residual animals: recovery populations were genetically more similar to their respective pre-treatment population than nearest neighbours; assignment tests detected few immigrants and there was no influx of new immigrant alleles and haplotypes. This finding was consistent with emerging evidence for restricted gene flow in bush rats. Naulty and Hayden (2008) removed 99 grey squirrels from a 5 ha site in the Phoenix Park over 12 months and found that there was little subsequent movement of grey squirrels into this site. In a related study of agile antechinus *Antechinus agilis* and bush rat *Rattus fuscipes* population

recovery following wildfire, residual individuals and not immigrants were found to be the starting point (Banks *et al.*, 2011). These data agree with the findings of this study that residual animals, and not immigrants, are primarily responsible for population recovery following control.

6.4 Sex Biased Dispersal

The costs and benefits of dispersal are expected to affect the sexes differently, depending on a species life history strategy. This may lead to a sex biased dispersal pattern. Male–biased dispersal is more common in mammals and female biased dispersal more common in birds (Greenwood, 1980) but many exceptions to this general rule exist (Lawson Handley *et al.*, 2007). The single population spatial autocorrelation analysis did not detect any genetic structure (i.e. any evidence of sex biased natal dispersal) in the Beau Parc population at any distance class for males, females or the entire population. In contrast, all the indices estimated in this study suggest that in grey squirrels breeding (inter-population) dispersal rates are higher among males than females. Significant differences between the sexes were observed in the mean assignment index and in the F_{IS}, whereas differences in the assignment index variance and F_{ST} were not significant. This finding is in agreement with field data (Taylor *et al.*, 1971; Mosby, 1969; Gull 1977 in Gurnell, 1987; Don 1981, in Gurnell, 1987) which suggest a male bias in breeding dispersal. Field data also exist which indicates there is a male bias in natal dispersal in introduced (Gurnell *et al.*, 2001) and native (Koprowski, 1996) populations. The possible reasons for failing to detect this in the genetic studies have been discussed.

Several evolutionary models exist to explain female philopatry. These are local resource competition, local mate competition, cooperative behaviour amongst kin and inbreeding avoidance.

Local resource competition

Familiarity with the natal area is crucial for the sex responsible for territory acquisition and defence (Greenwood, 1980). Home range areas of adult male grey squirrels are large (2-15 hectares, Don 1983), vary in size depending on population density and food supply (Don 1983) and overlap considerably with other males and females (Gurnell, 1987). Home ranges of females tend to be smaller and overlap with other females to a lesser extent (Gurnell, 1987; 1990). However, following breeding female grey squirrels tend to occupy small core areas well separated from other females (Gurnell, 1987), described as intra-sexual territoriality by Wauters *et al.,* (1994) (as observed for red squirrels). Havera and Nixon (1978, in Gurnell, 1987) found that breeding female fox squirrels in Illinois had den sites which tended to be uniformly distributed with at least 50m between them. Gull (1977, in Gurnell 1987) found a similar spacing pattern in female grey squirrels and noted that intensively used areas of forest were not shared between members of different family groups. Kenward (1985) gives a mean home range size of 1.6ha for lactating female grey squirrels, 3.5 ha for females which failed to wean litters and 4.7 ha for females which did not breed. When breeding/lactating, familiarity with the natal area would be beneficial in selecting an appropriate home range and core areas in which to obtain sufficient food resources, avoid predators and wean juveniles and would favour philopatry in females.

Local mate competition

A proximate cause of male biased dispersal may be forceful eviction as a result of aggression from adult males, in response to the increasing maturity of male adolescents. Such intra-sex eviction would point to a competition for mates as an ultimate cause (Lawson Handley and Perrin, 2007). In grey squirrels, females come into heat on one day only and male grey squirrels follow the female (the mating chase) waiting for an

opportunity to mate with her. The dominant male in the chase takes up a position closest to the female and chases away subordinate males who attempt to approach the female (Gurnell, 1987). Thus, eviction of male adolescents prior to the breeding season would also serve to reduce mate competition and would also promote male biased dispersal.

Cooperative behaviour amongst kin

The benefits of kin cooperation may provide further competitive advantages to residents and thereby promote philopatry. This effect would only be evident in species with good kin-recognition abilities (Lawson Handley and Perrin, 2007), as has been shown for many species of rodents, for example, Belding's ground squirrels (*Spermophilus beldingi*) (see Mateo, 2003 for a review).

Koprowski (1996) noted that adult male and female squirrels interacted less than expected, suggesting that the social systems of the sexes were separate. Amicable behaviors were directed only toward closely related females ($r = 0.5$) within kin groups; aggression was directed primarily at unrelated females. Cooperation should help to defend resources by preventing unrelated competitors from joining the group. Communal nesting occurred in all seasons, but was most frequent in winter. Unrelated adult males commonly nested together. In females, communal nesting was almost exclusive between related females. Of 52 nesting groups that included more than one adult female, related females ($r > 0.25$) nested exclusively in 82.7% of cases (Koprowski, 1996). Kinship also appeared to influence recruitment patterns of female gray squirrels. Of the 18 adult female gray squirrels that were recruited into the population (i.e., became resident for >1.0 year), only two females were not related to resident females ($r < 0.25$) (Koprowski, 1996).

Female grey squirrels therefore, demonstrate a pattern that appears common in the social ground squirrels, for example Belding's ground

squirrel; close relatives receive more amicable behavior than distant relatives, which are treated in a manner similar to unrelated females. Sharing space and nests with descendant kin appears to be a strategy to maximize direct fitness and also promotes female philopatry.

Inbreeding avoidance

Inbreeding avoidance is also speculated to be a primary cause of sex-biased dispersal in birds and mammals (Greenwood, 1980). Dispersal lowers the risk of mating with relatives and hence inbreeding depression. In polygynous species, such as grey squirrels, females pay a higher cost by inbreeding since they have higher reproductive investment than males, and hence are expected to disperse more than males (Waser *et al.,* 1986). This prediction is reversed if mate choice is allowed. For inbreeding load exceeding a certain threshold value (set by relatedness), females should prefer immigrant males, and hence boost male dispersal (Lehmann and Perrin, 2003). Thus, kin recognition, as demonstrated in grey squirrels (Koprowski, 1996), could increase the potential for cooperation (sharing space/nests with kin) in the sex in which resource acquisition (a high quality core area for breeding) is crucial, promoting philopatry. It could also avoid the potential for close inbreeding and indirectly select for dispersal in the sex that less crucially depends on social bonds (Lehmann and Perrin, 2003).

6.5 Disease

The rates of decline of the red squirrel in Cumbria (76km^2/year) and Norfolk (92 km^2/year) where squirrel pox virus (SPV) is present are much greater compared to Scotland (4.4 km^2/year) and (Italy 3.6 km^2/year) where SPV is absent (Rushton *et al.*, 2006). Data from this study confirm this pattern for Ireland, where SQPV is present, and the rate of decline is high (1950-1995: 62 km^2/year, 1911-1995: 81 km^2/year).

The vagility of an invading species carrying a disease or pathogen will be critical in determining the rate of spread of the pathogen. Bruemmer *et al.*, (2010) found that SQPV prevalence was male biased, increased with weight and varied seasonally. As male squirrels and adult squirrels have larger home-ranges they have an increased probability of encountering the virus. Seroprevalence rose from autumn to spring coinciding with increased movements of squirrels during the autumn dispersal period. Seroprevalence did not coincide with the time of year when one would expect increased parasite burdens pointing to a direct disease transmission route, which may involve environmental contamination (Bruemmer *et al.*, 2010). The seropositive grey squirrel population in northern England has begun to expand into Scotland and cases of SQPV have been detected there (Sainsbury *et al.*, 2008) with disease occurring in red squirrels approximately 3 years after the coexistence of red and grey squirrels (Rushton *et al.*, 2006). In Ireland, grey squirrels continue to spread into the west and south. The steady range expansion of seropositive grey squirrels makes the future of the red squirrel in Ireland and Britain very uncertain.

6.6 The grey squirrels as an introduced species in Ireland

The Irish grey squirrel population has lost relatively little genetic diversity relative to other sciurid populations worldwide. Loss of genetic diversity is a process that occurs between generations, even in cases of a single colonising event. Where the founding population consists of more than a few individuals, and providing the population rapidly increases in size, relatively little genetic diversity is lost (Austerlitz *et al.*, 1997). As with grey squirrels in Ireland, the introduction of the marsh frog, *Rana ridibunda*, into England from Hungary involved only 12 individuals; yet due to rapid population growth, the established non-native population exhibited a level of genetic diversity similar to that of the source population (Zeisset and Beebee, 2003). Based on a review of the relevant literature, Wares *et al.*, (2005) concluded that introduced populations

often retain as much as 80% of the genetic variation of the source population, or even more.

Restricted dispersal and sex biased dispersal are features of the Irish grey squirrel population and other introduced (Taylor *et al.*, 1971; Fitzgibbon, 1993; Gurnell *et al.*, 2001) and native grey squirrel populations (Flyger, 1960; Mosby, 1969; Koprowski, 1996).

Range expansion by grey squirrels in Ireland has been relatively slow (1.75 km/year: this study) in comparison with Italy (4.72 km/year: Wauters *et al.*, 1997) and the United Kingdom (7.66 km/year: Williamson and Brown (1986). In comparison, the muskrat inhabited the European continent within 50 years of its initial introduction (Hengeveld, 1989) with observed rates of spread between 1-25 km/year and 51-230 km^2/year (Andow *et al.*, 1990). Range expansion varies in other introduced species; in the cabbage butterfly it was 15-170km/year (Andow *et al.*, 1990), in the cereal leaf beetle 27-90km/year (Andow *et al.*, 1990) and in the gypsy moth 3-20km/year (Liebhold *et al.*, 1992). Similar to the Irish grey squirrel, range expansion northward in the sea otter was slow: 1.4km/year and 13.5 km^2/year (Lubina and Levin, 1988). Range expansion in introduced species will vary hugely depending on species, life history factors, habitat preferences, habitat available, interactions with other species, climate etc. For example, grey squirrels were introduced to South Africa in about 1900 but have not expanded their range. They are confined to the Cape Peninsula and areas of the Western Cape, associated with oak trees and pine plantations, as they are unable to utilise the fynbos vegetation of the region.

6.7 Future research

Much theory relating to biological invasions has been developed from studies of plants and sessile/sedentary animals (Pysek *et al.*, 2008). It is

not yet clear how well these ideas apply to more mobile animals, and what role behaviour plays in the invasion process of these animals. Thus more invasion studies of mobile animals are needed to discover the generality of much of the current invasion theory.

It is difficult to draw broad conclusions on the management of an invasive species from a single piece of research. Two of the most notable examples of successful eradication involving mammal species are the eradication of the North American muskrat and the coypu from Great Britain (Gosling and Baker, 1989), and the recent eradication of pigs from Santa Cruz Island, California (Morrison *et al.*, 2007). Each of these efforts was meticulously organized and involved comprehensively implemented trapping and monitoring plans. Management and control of non-native species will benefit from further research conducted on-site, within the actual ecosystem of interest and which is carefully planned, executed and documented.

As conventional control by shooting and/or trapping is only likely to be useful in relatively small, isolated island habitats, research into alternative methods of control may be required. Mammalian predators have often been introduced to try to control other pest mammal species such as rabbits and rats but this approach that often has resulted in undesirable impacts on native wildlife (Loope *et al.*, 1988). Currently underway are studies to evaluate the effectiveness of introducing particular pathogens and parasites to control species such as the brown tree snake (*Boiga irregularis*) and cane toads (Hoddle, 2004). The development of an immunocontraceptives in oral form continues to be investigated for brushtail possums (*Trichosurus vulpecula*) in New Zealand using either bacterial ghosts (Duckworth *et al.*, 2007) and transgenic plants (Polkinghorne *et al.*, 2005). These studies may yet point the way for new areas of research into grey squirrel control.

A clear imperative in squirrel research is to prevent the extinction of the red squirrel. As squirrel pox virus has been identified as the most likely cause of the rapid decline in red squirrel abundance and distribution in Ireland, England and Wales future research must focus on understanding the epidemiology of this virus and on the possibility of developing a vaccine. Further basic research is needed to gain a better understanding of SQPV. In Castle Leslie, Beau Parc and Clongowes Wood the locations of capture of squirrels infected with SQPV were not clustered suggesting that in these study sites infected squirrels did not tend to share overlapping or adjacent home ranges. On a much larger scale, in south and central Cumbria, the number of tetrads in which red and grey squirrels with SQPV were found was much less that the number of records suggesting that the occurrence of SQPV in both species at a landscape scale was clumped (Rushton *et al.,* 2006). The route of transmission, the persistence of the virus in the environment, the encounter rate between healthy and infected squirrels, the probability of an infection occurring following such an encounter and other factors which might explain the pattern of SQPV occurrence are all unknown and must be investigated. Routes of transmission to be investigated include by air, saliva, via scent marks or by ectoparasites (fleas, lice, ticks and mites), which may transfer from squirrel to squirrel in the drey. The relatively low prevelance of SQPV among juveniles/subadults, in comparison with the adult population, would suggest that vertical transmission is not a major factor in the spread of SQPV. The apparent survival of some red squirrels following SQPV infection raises the possibility that a vaccine could raise a sufficient immune response prevent infection and control SQPV in some populations.

6.8 Conclusion

Intriguing ecological insights may be gained when an invasive species interacts with a novel environment. For example, the impact of apparent

competition on red squirrels, mediated through a novel pathogen introduced by grey squirrels, was realised only recently. The focus on invasive species, and their economic and ecological impacts, has led to the idea that biological invasions are an ecologically distinct phenomenon and that invasion biology is a unique subdiscipline in ecology.

However the processes studied in invasion biology of colonization, establishment, competition, turnover, persistence and spread are common to all branches of ecology, in particular population ecology, community ecology and biogeography. Vermeij (2005) argues that the differences between the biotic interchanges of the past and the human assisted invasions of today are exaggerated. Davis (2009) suggests that the study of invasions should be practiced more as part of mainstream ecology as, from an ecological perspective, there is little about biological invasions that make them so unique that a specialized subdiscipline is required to study them.

Generalisations are more readily found between species of similar taxa, be they native or invasive, than species of different taxa, which share only the characteristic of being invasive. The possibility of finding large-scale generalisations, unique to the field of invasion biology, which encompasses such a myriad of species and ecosystems, seems unlikely. In studying invasive species, an increased emphasis should be placed on how findings enhance understanding in ecology generally, rather than focusing solely on the field of invasion biology.

7. References

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