Island syndrome in rodents; a comparative study on island forms of the bank vole, Myodes glareolus

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Submitted to the University of London for the degree of Doctor of Philosophy September 2009

Abstract

Islands are extremely variable habitats, differing in shape, size, degree of isolation, geography and climate. They are often described as 'natural laboratories' and have proven beneficial for testing theories on evolution and adaptation. Rodents on islands are often characterised by differences in demography, morphology and behaviour compared to adjacent mainland populations. One of the most notable and extensively reported differences is in body size. Several adaptive theories have been suggested to explain these phenomena, which have been termed 'island syndrome', yet few have been empirically tested.

The bank vole (*Myodes glareolus*) is a good model for studying the evolution of island syndrome, being present throughout the British mainland as well as on 13 small offshore islands. Voles on four of these islands exhibit the gigantism characteristic of island syndrome. The aim of this study was to compare insular and mainland populations of voles to determine whether island syndrome is truly an adaptive response to life in insular habitats, or whether it is driven by more random processes such as founder effects and genetic drift.

In this thesis, I present data on body size, demography and skull morphology along with phylogenetic analyses based on mitochondrial DNA sequences from island and mainland populations of bank voles around the UK. Whilst I was able to demonstrate insular changes in body size, I was unable to demonstrate any demographic differences consistent with the predictions of island syndrome. Phylogenetic analyses revealed that body size differentiation on islands was not related to phylogeographic history. There was little evidence for a single unifying theory explaining the existence of island syndrome, thus I conclude that this biological pattern is probably caused by multiple environmental and genetic factors.

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Acknowledgements

First and foremost I would like to thank my supervisors, Professor John Gurnell and Dr. Chris Faulkes, to whom I will forever be indebted. Both have consistently gone above and beyond the call of their supervisorial duties. As well as his expertise in everything to do with small mammals, I want to thank John for some of the most hilarious field trips I could ever hope to remember (getting stuck in amidst wobbly grass tussocks on Skomer springs to mind), and for his endless patience and good humour particularly when answering my phone calls at ungodly hours. Chris' assistance with the genetic aspects of this project was invaluable, as was his quirky sense of humour ("beaver beaver!"), especially during stressful periods. Although unrelated to this project I would also to thank like Chris for allowing me to accompany him on a mole-rat catching expedition to South Africa, despite the various injuries sustained! I sincerely hope that you both already know how deeply grateful I am without reading this.

Although not officially one of my supervisors, Dr. Steve Le Comber has selflessly contributed a significant amount of time, effort and academic support to this project. In addition he has been a true friend and will no doubt remain so. I must also thank Steve for the copious amounts of fruit, lunches and pints he has supplied over the years. I will pay you (some) back when I'm no longer an impoverished student, honest!

The thesis would not have been possible without the funding provided by NERC, Countryside Council for Wales and the States of Jersey. For access to field sites I would like to thank the following organisations: Countryside Council for Wales, Welsh Wildlife Trust, Field Studies Council, Forestry Commission, Tilhill Economic Forestry Limited, Royal Society for the Protection of Bird and Peoples Trust for Endangered Species. I would also like to thank the following people for helping me to obtain the relevant permissions: Chris Milligan, Greg and Lisa Morgan, Liz Halliwell, Stuart Maidment, Kevin Sutton, Kenneth Knott and Donald Macleod. For assistance on Skomer I particularly thank Juan Brown. For access to museum collections I thank Paula Jenkins and Louise Thomsett (Natural History Museum, London) and Andrew Kitchener (Royal Museum of Scotland, Edinburgh).

For their help and support on academic aspects of this project I would like to thank Dr. Steve Rossiter, Prof. David Polly, Dr. Tim Healing, Dr. Charles Mein, Alex Curtotti and Prof. Andrew Leitch. I would also like to thank Andrew and his family for the kind loan of their cottage during my stays in Pembrokeshire. Louise Magris was an absolute star during my visits to Jersey. Not only did she give up her time (and garage!) in the interests of vole science, she helped organise field sites and funding, and she and her husband Douglas also welcomed me into their home. Many thanks to you both. For making my stays on Skomer some of the most memorable parts of this project I thank, Richard Brown, James Darke, Dave Boyle (also for providing the photo on the title page of this thesis), Juan Brown, Jane Matthews and the researchers and volunteers who are too numerous to mention. I am also enormously grateful to my many field assistants for their hard work and helping to make field work so enjoyable. In no particular order these include: Janie Steele, Alison Triggs, Rachel Toresen, Dr. Fern Slingsby and Nicky Bull. I also thank Dr. Peter Lurz for attempting to catch voles in Kielder in my absence.

Finally, I must thank my family and friends who have graciously put up with me throughout the many moments of pure insanity. There are too many to list, but you know who you are. Nevertheless, Alison Triggs, Julie Warne, Dr. Leeann Reaney, Dr. Peter Staves and Dr. Golo Maurer deserve special mention. Most of all I thank *you* Rachel Toresen (thought you weren't getting special mention, didn't you!) and my boyfriend Simon Percival, without whom I would simply not have got through the last few months of this PhD. Thanks!

Chapter 1: General introduction

1.1. What is an island?

The term 'island' can be used to describe any landmass surrounded by a body of water. However, in studies of island biogeography the term is usually applied to small oceanic islands that differ in biota to adjacent continental landmasses. Thus, whilst Australia and Great Britain are technically islands, many biogeographic studies have considered these as mainland habitats (e.g. Keogh *et* al. 2005, Kier *et al.* 2009, Lomolino 2005, Meiri *et al.* 2008). In the context of this study, the term 'island' is used to describe small offshore landmasses of no more than 1200 km^{2a}.

1.2. Islands as evolutionary laboratories

"Islands are an enormously important source of information and an unparalled testing ground for various scientific theories" (Mayr 1967). Islands are extremely variable in their physical characteristics with each island differing for example in shape, size, degree of isolation, geography and climate. Colonisation opportunities and extinction risks vary concurrently with these physical characteristics, with features such as island area and distance from the mainland having a particularly strong effect. Consequently, islands often encompass unique biotic assemblages and thus have been described as a series of 'natural experiments' that are ideal for studying evolutionary mechanisms (Whittaker 1998).

[a] An arbitrary value derived from the area of Anglesey, the largest offshore landmass in the UK that contains bank voles.

Seawater provides a significant barrier to the dispersal of most terrestrial species, thus species richness is predicted to decrease with increasing isolation from adjacent landmasses (MacArthur and Wilson 1967). Smaller landmasses are generally able to support fewer species than larger areas, primarily because maximum population size is related to island area (Marquet and Taper 1998) and because extinction risk increases greatly with decreased population size (Frankham 1998, 2005). The complex relationship between colonisation and extinction processes operating on islands often results in the occurrence of a depauperate flora and fauna with respect to mainland communities. Therefore newly colonising species may find themselves in the absence of a normal suite of competitors and predators and in vastly different habitat to that from which they came. Thus, the selective pressure on islands may be significantly different to those experienced by mainland communities and this can drive rapid evolutionary changes through adaptive radiation, as classically exemplified by Darwin's finches of the Galapagos Islands (Grant and Grant 2003). However, this is not the only evolutionary mechanism through which variation on islands can occur. Colonisation events typically involve small numbers of individuals thus a genetic bias inherent in the founding population can significantly affect the phenotype of future generations, the so-called 'founder effect'. In addition, the effects of genetic drift are greatly enhanced in small populations (Frankham 1998), thus the chance fixation of random mutations may drive further differentiation between island and mainland forms. Fortunately, despite the great variability amongst island biota, some consistent patterns can still be observed. This combination of variability and uniformity means that islands form natural 'laboratories' that can help us to understand the driving forces of evolution.

1.3. The importance of islands as biodiverse habitats

The properties of isolated landmasses, as previously discussed, have led to the evolution of a multitude of endemic forms occurring on islands both above and below the species level. As such, islands contribute a significant amount to total global biodiversity (Heaney 1986, Sax and Gaines 2008). A recent study by Kier *et al.* (2009) produced estimates of global 'endemism richness' (an index incorporating both endemism and species richness) for vascular plants and vertebrates. These authors demonstrated that endemism richness of plants on oceanic islands was 9.5 times that of the mainland, whilst vertebrate endemic richness was 8.1 times greater on islands.

However, island populations are susceptible to increased risk of extinction, either through naturally occurring disturbances or through human impacts such as the introduction of alien species, disease and habitat destruction (Whittaker 1998). A case in point is that 20% of the world's avian species and subspecies are endemic to islands, but these island endemics contribute 90% of avian species that are known to have become extinct since 1600 (Cox and Moore 2000). Furthermore, Kier *et al* (2009) predicted current threats to biodiversity through human impact to be significantly greater on islands than mainland landmasses and thus concluded that islands should be "a high priority in global biodiversity conservation this century".

1.4. Island syndrome and the Island rule

Island forms are often distinct from their mainland counterparts in terms of morphology, demography and behaviour. These phenomena and hypotheses that attempt to explain them are discussed in turn below.

1.4.1. The Island Rule

1.4.1.1. Evidence for the existence of the island rule

Early research on insular forms focused greatly on the morphological peculiarities of island populations with respect to mainland forms. One of the main patterns to emerge from these studies was a tendency amongst island races to exhibit a change in body size in comparison to mainland conspecifics. This trend was first described in mammals by Foster (1963, 1964) who noted that insular rodents had the propensity to increase in body size on islands whilst ungulates and carnivores often decreased in body size. The theory was later extended by Van Valen (1973) and others (Damuth 1993, Heaney 1978, Lomolino 1985) to describe a tendency towards dwarfism on islands for large animals and a tendency towards insular gigantism by small mammals. Thus, this phenomenon became one of the so-called ecogeographical rules; the 'island rule'.

Since inception, support for the existence of the island rule has been reported in a number of different vertebrate taxa. Some of the most recent examples include; birds (Blondel 2000, Clegg and Owens 2002, Robinson-Wolrath and Owens 2003, Scott *et al.* 2003), reptiles (Boback and Guyer 2003, Pafilis *et al.* 2009, Wikelski 2005) amphibians, (Lampert *et al.* 2007, Wu *et al.* 2006), ungulates (Palombo *et al.* 2008, Simard *et al.* 2008, Van Vuren and Bakker 2009), primates (Broham and Cardillo 2007, Welch 2009), rodents (reviewed by Adler and Levins 1994), elephants (Palombo 2007) and vertebrates in general (Lomolino 2005). However, the evidence is equivocal in several of these taxa. In particular, large-scale meta-analyses seem to provide little support for the generality of the island rule in carnivores (Meiri *et al.* 2004, Meiri *et al.* 2005), sand lizards (Meiri 2007) and mammals in general (Meiri *et al.* 2006, Meiri *et al.* 2008). However, some of these discrepancies may be attributable to the different measures of body size employed in these studies, the sizes of

islands included in the analyses and the statistical tests used (Lomolino 2005, Meiri *et al.* 2006, Price and Phillimore 2007, Welch 2009). Welch (2009) was able to demonstrate that conformity or non-conformity to the island rule in primates was dependant both on the choice of body size measurement and on the null hypothesis being tested. Welch showed that when body mass and skull length were used, primates conform to the island rule (this is contrary to the findings of Meiri *et al*. 2008) but that head-body length only yielded support when the 'wrong' null hypothesis was used. Nevertheless, upon revision of their earlier analyses (i.e. Meiri *et al.* 2004, Meiri *et al*. 2005), Meiri *et al*. (2008) found evidence for the existence of the island rule in some clades of mammals when phylogeny was controlled for. These authors concluded that there was a significant trend towards insular dwarfism for artiodactyls, carnivores and heteromyid rodents and a highly significant trend towards insular gigantism in murid rodents[b].

1.4.1.2. Hypotheses explaining dwarfism and gigantism

Explanations for the occurrence of differential size evolution on islands have been even more equivocal. The main hypotheses are outlined below. However, it should be noted that several of these theories are interlinked, as is evident from the empirical evidence.

Cope's rule states that population lineages tend to increase body size over time. This is based on the idea that larger animals may be metabolically more efficient, better able to avoid predators (in some cases), better able to predate upon other species, more able to resist periods of low food availability, experience greater longevity, experience increased intelligence with increased brain size, and that increased body size usually confers an advantage through interspecific and intraspecific competition thereby positively influencing

[b] Four species from the genus *Myodes*, family Cricetidae, were included as murids in the analysis performed by these authors (under the former name *Clethrionomys*).

survival and fecundity (Hone and Benton 2005). However, these authors point out the disadvantages of increased body size include, higher nutritional requirements, increased development time which gives rise to slower rates of evolution and thus a reduced ability to survive environmental perturbations and, in the long-term, lower fecundity because larger species tend to invest more in fewer offspring (a k-selection strategy rather than an r-selection strategy).

Damuth (1993) disagreed with the validity of Cope's rule for mammals. He argued that metabolic efficiency leads to an 'optimal body size' of around 1 kg for mammals and that given the absence of other selective pressures and phylogenetic constraints mammals will evolve towards this size. He used this theory to explain that gigantism and dwarfism in insular populations could be the result of optimisation of body size in the absence of predators and competitors.

Whether or not Cope's rule operates is largely immaterial for the purpose of this study. However the selective pressures involved in body size evolution and the pros and cons of evolving to larger size may indeed differ in relation to island and mainland populations. For example, slower rates of evolution and a reduced ability to survive environmental perturbations would obviously be a distinct disadvantage on islands where populations are generally small, particularly given the inability to disperse to other habitats if environmental perturbations should arise. Thus, this could select for a decrease in body size in larger mammals. In spite of this, to my knowledge there have been no studies that suggest a decrease in gestation time occurring in any island forms relative to mainland forms thus this is unlikely to account for insular dwarfism. Conversely, factors such as nutritional requirements (linked to resource availability), predation pressure and

interspecific competition have all been implicated in insular evolution of body size and these form the three main hypotheses that have been put forward to explain gigantism and dwarfism on islands.

1.4.1.3. Reduced predation pressure on islands

This hypothesis is based on the assumption that predator avoidance is a significant selective pressure on body size evolution of animals on the mainland. The evolution of large body size may confer an advantage to larger animals because this may reduce the opportunity for predators to attack. For example, the size of buffalo and elephants is a major deterrent to lions and only the young of these species tend to be vulnerable to predation. Increased body size will also be favoured if an animal's predator avoidance mechanism is running away or fighting (Palkovacs 2003). Conversely, in smaller animals decreased size may confer a significant advantage because smaller species tend to adopt a 'hiding' defence and commonly escape by retreating into refuges too small for predators to follow (Heaney 1978, Lawlor 1982). The number of predators on islands is commonly reduced compared to mainland populations (Adler and Levins 1994, Lomolino 2005, White and Searle 2007) due to incomplete colonisation and increased extinction risks. Therefore a release from predation pressure could allow animals on islands to evolve to a more optimal body size (Damuth 1993). The main evidence for this hypothesis seems to be the absence of certain predators on islands where species exhibit the island rule (e.g. weasels *Mustela nivalis* in the case of small mammals; Adler and Levins 1994). However the absence of these predators on islands where species do *not* exhibit the island rule must then surely count against this hypothesis. More direct evidence that refutes this hypothesis as a universal explanation comes from the marine iguanas of the Galapagos Islands, where predators are not size-specific but insular gigantism still occurs (Wikelski 2005).

1.4.1.4. Competitive release/niche expansion hypothesis

The number of species on islands tends to decrease with diminishing island size (MacArthur and Wilson 1967). In an absence of competitors on (small) islands, extant fauna may be enable to occupy habitat and utilise food resources which would otherwise be unavailable to them (Lomolino 2005). Palkovacs (2003) states that for this hypothesis to be workable, an increase or decrease in body size must somehow correspond to an increase in the range of food items available to a given species (i.e. larger species can eat larger items as well as smaller items). He argues that this is more likely to result in modification of feeding behaviour or the morphology of feeding structures, as is evident in Darwin's finches of the Galapagos Islands (Grant 1986), than a simple increase in body size. Niche-expansion leading to increased body size seems to be evident in the house mice (*Mus musculus*) of Gough Island, South Atlantic. Since introduction in 1810, the mice have doubled in size and have become predators of seabird chicks including the extremely large Tristan albatross (*Diomedea dabbenena*) (Cuthbert and Hilton 2004, Rowerowe and Crafford 1992). Palombo (2007) also felt the niche expansion hypothesis best described the patterns of insular dwarfism occurring in the extinct proboscideans of the Mediterranean islands (Masseti 2009).

Nevertheless, there is an alternative explanation as to how decreased interspecific competition could select for increased body size. Decreased competition and increased resource availability through niche-expansion is likely to increase the growth of individuals in the short-term. However, an increase in available resources is also likely to result in increased densities (see Section 1.4.5), particularly if the influence of predation is negligible and dispersal is limited, thus increased intraspecific competition may occur. Increased intraspecific competition may then select for increased body size in the long-term (Adler

and Levins, 1994). Increased resource availability (which was at least partially attributable to fewer competitors) leading to increased densities and an associated increase in intraspecific competition was proposed as the explanation for insular gigantism in Chinese rice frogs (*Rana limnocharis*) (Wu *et al.* 2006). Obviously this hypothesis fails to adequately explain the incidence of insular dwarfism and has significant overlap with the resource availability hypothesis.

1.4.1.5. Food resource availability

I have already discussed how increases in resource availability could lead to gigantism in smaller animals, thus this section will mainly refer to how food resource availability is likely to influence dwarfism in larger mammals. The increased nutritional requirements inherent with larger body size are likely to have a substantial effect on populations living on small islands where resources are finite and dispersal to other areas is restricted. Whilst increased intraspecific competition could theoretically select for increased body size, there is a further constraint on these species, which will not impact so greatly on smaller animals; body size affects the number of animals that can live in a given area (Marquet and Taper 1998). Thus if large animals were to increase in size on islands, population size would have to decrease. However, because the risk of extinction increases with decreased population size (Frankham 1998), populations that evolve in this direction may be less likely to persist. Conversely, the evolution of smaller body size would allow higher densities of animals to occur by increasing the carrying capacity of the environment, thereby increasing the chance of population survival. Furthermore, large animals with a delayed maturation rate and low fecundity would also be at a disadvantage in a seasonally resource limited environment.

The most conclusive evidence in support of this 'resource limitation' hypothesis leading to dwarfism in larger animals has to be the white-tailed deer (*Odocoileus virginianus*) of Anticosti Island, Quebec. Deer were introduced to this island in 1896 and have since experienced a 50% reduction in body size with respect to the ancestral population (Simard *et al.* 2008). These authors were able to demonstrate that high levels of herbivory had caused a change in the vegetative fauna and a decrease in the nutritional levels of vegetation consumed by the deer and that vegetation quality was significantly related to body mass. Further evidence for the impact of resource availability on body size comes from Keogh *et al*. (2005). These authors demonstrated that gigantism and dwarfism in insular Australian tiger snakes (*Notechis* spp.) was directly related to the size of prey items available on the respective islands.

It is clear from the empirical evidence that none of these theories provide an all-encompassing explanation for the occurrence of the island rule. Indeed, most studies have implicated a combination of factors as the cause of insular body size divergence. For example, Meiri (2008b) concluded that the evolution of differential body size in sand lizards is driven by a combination of predator release, dietary specification and thermal requirements. In small mammals there are several other selective pressures that may affect body size evolution on islands. However, these are discussed in detail in Chapter 5.

1.4.2. Island syndrome

Whilst much less well studied than dwarfism and gigantism, there are several other patterns that have become apparent in island populations. These include a tendency to display demographic differences (e.g. increased population size, increased survival, increased stability) and behavioural differences (e.g. reproductive behaviour, aggression) in

comparison to mainland populations. Adler and Levins (1994) termed these differences collectively, the 'island syndrome'.

1.4.2.1. Increased densities

It has been noted that in several taxa, insular populations can often reach extraordinarily high densities (e.g. frogs - Wu *et al.* 1998; birds - Grant 1966, Blondel *et al.* 1988, Wright 1980; reptiles – Rodda *et al.* 2001, Rodda and Dean-Bradley 2002; deer - Simard *et al.* 2008; rodents - Jewel 1966, Sullivan 1977, Gliwicz 1984). In essence there are two main types of hypotheses that attempt to explain the occurrence of excess density in island populations. However it is worth noting at this point that within both types of explanation, an abundance of available food resources is (unsurprisingly) implicit. Furthermore both theories tend to encompass the idea of impoverished insular fauna leading to decreased competition and/or predation.

(a) Density compensation hypotheses

The idea of a 'carrying capacity' of a given environment is well entrenched in ecological theory (Begon *et al.* 2006). This term describes the size of a population that can be sustained in a particular habitat at a particular time given the available resources. Since islands often have a subset of fauna in comparison to the mainland, it follows that, in the absence of competitors, a single species may have access to additional food and space resources which would otherwise be unavailable in a comparable mainland site (the niche-expansion hypothesis). Thus, one may expect an increase in the density of this species relative to its mainland densities. However, this poses the question of whether density in insular species simply increases to fill the absence of other competitive guild members, or whether the carrying capacity of islands is actually higher than in an equivalent mainland

site (Wright 1980). Thus, if the first scenario is true, one would expect total densities and total biomass of species within the same competitive guild to be roughly equal between mainland and island sites; the density compensation hypothesis. Whereas if the latter scenario were true, one would expect total densities (or more accurately, total biomass) on islands to greatly exceed that of a comparable mainland site; the excess density compensation hypothesis (MacArthur *et al*. 1972).

In insular populations of birds, excess density compensation and density compensation have been shown to occur and both are thought to result from some form of niche-expansion in the absence of competitors (MacArthur *et al*. 1972). Increasing levels of faunal impoverishment have also been implicated in excess density compensation occurring in insular lizards (Rodda and Dean-Bradley 2002). This demonstrates that, under some conditions, the carrying capacity of islands can exceed that of typical mainland sites. However, these meta-analyses have largely relied on the absence of competitors to infer the occurrence of niche-expansion. Conversely, through controlled introduction experiments of competitive rodent species, Crowell (1983) was able to demonstrate that increased population densities in insular populations of *Myodes gapperi*, *Peromyscus maniculatus* and *Microtus pennsylvanicus* were not a result of niche-expansion and competitive release, but were attributable to the effects of restricted dispersal (the 'fence effect') in combination with predator release.

(b) The 'fence effect'

In confined populations, such as those on small islands, opportunities for dispersal are likely to be rare. Thus in the absence of predation pressure, populations of animals may build up to very high densities because there is no marginal habitat for them to move into

(i.e. no 'dispersal sink'). Krebs *et al*. (1969) named this the fence effect. Increased density due to dispersal restrictions has been demonstrated in artificially confined populations of small mammals (see Adler and Levins 1994, Gliwicz 1980, and references therein) and fenced populations. However the consistency and validity of these experiments has been questioned (Ostfeld 1994). Nevertheless there is some support for this hypothesis in wild populations. For example studies of feral house mice on Great Gull Island, New York, showed populations do not reach elevated densities because areas of unfavourable habitat appear to act as dispersal sink (Anderson 1970). Furthermore artificially confined populations appear to show normal demographic patterns when provided with a dispersal-sink (Tamarin *et al*. 1984).

1.4.3. Consequences of increased density and behavioural adaptations

Implicit in the Krebs *et al*. (1969) model of the fence effect, is the idea that in the absence of a dispersal sink, populations will build up to extremely high levels, exploit all the available resources and subsequently crash. This tenet has proven to be particularly incorrect for some small mammal populations, where relatively high densities on islands have been said to be more stable than in mainland populations (e.g. *Microtus californicus* on Brooks Island, California - Lidicker 1973; *Microtus breweri* on Muskeget Island, Massachusetts - Tamarin 1977). Gliwicz (1980) proposed that this apparent stability in confined populations may be attributable to the ability of animals to respond to changing levels of resources in the environment. She argued that in insular populations where dispersal of animals is restricted and the effects of predation and competition are ameliorated, population size may remain relatively stable if individuals adjust their reproductive allocation according to resource availability. Indeed, some island populations do apparently show modified reproductive behaviour in comparison to mainland

conspecifics in the form of shortened breeding seasons (e.g. Jewel 1966, Tamarin 1977, Sullivan 1977), delayed maturity (e.g. Jewel 1966, Tamarin 1977) and decreased litter/clutch size (Cody 1971 in MacArthur *et al*. 1972, Simard *et al*. 2008). Increased densities may lead to increased tolerance of conspecifics by selecting for decreased aggressiveness (Adler and Levins 1994, McNab 2002, Knell 2009). Whilst less aggressive island forms do exist (Garten 1976, Halpin 1981, Gray and Hurst 1998), there are no studies that have attempted to quantify this trait in direct relation to population density.

1.5. The bank vole: general biology, island and mainland forms

British bank voles (*Myodes glareolus*; Pallas 1811[c]) are a good model species for studying 'island syndrome' because this species occurs throughout the mainland and also on the 13 offshore islands of Ulva, Mull, Raasay, Scalpay, Arran, Bute, Handa, Skomer, Ramsey, Anglesey, Hayling, Wight and Jersey (Shore & Hare 2008). Four of these populations reportedly show morphological differences with respect to the British mainland voles (*M. g. britannicus*) and currently hold (questionable) subspecific status; Jersey, *M. g. caesarius*, Skomer, *M.g. skomerensis*, Mull, *M. g. alstoni* and Raasay, *M. g. Erica* (Corbet 1963).

Shore and Hare (2008) have recently reviewed the literature on bank voles (see references therein). The bank vole belongs to the rodent family Cricetidae, although it is sometimes included in the Muridae or the subfamily Microtinae. Its range extends from the north of the Arctic Circle to the Pyrenees and Italy and from western Ireland to central Siberia, although in the eastern part of the range its distribution is not continuous. Bank voles in Britain commonly inhabit mixed or deciduous woodland but can occur in coniferous forest, hedgerows, grasslands, fenland and road margins. However the presence of this species is

[c]The genus name for this species in most previous literature is listed as *Clethrionomys*. The change to *Myodes* occurred *sensu* Carlton *et al.* 2003.

almost always dependent on thick ground cover. Weights of adult voles vary with latitude, altitude and geology but typically adult voles on mainland Britain weigh between 17 g and 27 g. Population cycles in Britain are annual, generally following a pattern of spring decline followed by autumn peaks in densities. However, multiannual cycles of between 3-5 years occur in northern European populations. Bank voles are mostly herbivorous and a large proportion of their diet comes from green leaves (40-50%) but fleshy fruits, seeds and dead leaves are also important. Roots, grasses, moss, flowers, invertebrates and animal matter may also be consumed although these form a less significant part of the diet. Bank voles are active both day and night with peak activity occurring at dawn and dusk. The maximum lifespan in wild populations is 18-21 months although captive animals can live up to 40 months. Breeding season on the mainland ranges from March-April to September-October although this varies with latitude. Breeding can occur over winter if food supplies, such as seeds, are abundant. Mean litter size in Britain is 3.5-4.1, multiple litters can be produced in a season and multiple-sired litters occur. Ovulation is induced. Mean gestation length is 19.6 days. Young voles are fully weaned by around 18 days and weigh around 9-10 g. Females born early in the season can breed the same year, but those born later in the season will usually not mature until the following breeding season and overwinter as sub-adults. Field voles are competitively dominant over bank voles and their presence may effect female survival, territory size and maturation of young females. There is some evidence to suggest that wood mice may have an effect on bank vole numbers and activity patterns but neither species seems behaviourally dominant over the other.

1.6. Aims of this thesis

Whilst considerable amount of work has been carried out on island populations of *Apodemus*, *Mus* and *Microtus* (e.g. Berry 1964, Berry *et al.* 1991, Berry *et al.* 1992, Delany 1970, Reynolds and Gorman 1999), few detailed studies have been carried out on island populations of red-backed voles (*Myodes* spp.). In addition, such studies have tended to focus on a particular island population and their closest mainland relatives, rather than incorporating studies across a whole range of islands. By adopting a comparative approach and integrating phylogeographic evidence with morphological, demographic and ecological data, this study seeks to test hypotheses about the causes of island syndrome, using bank voles as a model species.

1.7. Thesis layout

Chapter 2 sets the scene by describing the different islands and field sites that were visited and studied between 2005 and 2008. Chapter 3 considers the phylogeography of the island forms, and this is followed by Chapter 4 that looks in detail at the ecology of the voles on the different study islands. Thereafter there are two chapters dealing with morphometrics. Chapter 5 looks at body size, a key component of island syndrome, and Chapter 6 considers whether island and mainland forms differ in skull morphometry and whether there is any evidence of developmental instability in island populations. Chapters 3 to 6 each have their own introduction and discussion. The final chapter, Chapter 7, provides a synthesis and overview of the main findings and discusses what research should be carried out in light of these findings. The specific aims of the data chapters (Chapters 3-6) are discussed in more detail below.

1.7.1. Chapter 3

In Chapter Three I investigate the phylogeographic origins of six insular populations of bank voles surrounding the British coastline, as inferred by sequence analysis of the mitochondrial cytochrome *b* and control region loci. I specifically test the following hypotheses: (i) extant bank vole lineages colonised all offshore islands via human-mediated transportation rather than 'naturally' (with the exception of the Isle of Wight), (ii) colonisation of all offshore islands occurred after the Last Glacial Maximum, and (iii) clinal size variation and phylogeographic history are responsible for the observed size differentiation in the four insular subspecies.

1.7.2. Chapter 4

In Chapter Four I investigate demographic parameters of four island populations and one mainland population of bank voles to see whether populations conform to patterns predicted by the 'island syndrome' hypothesis. Specifically, I ask whether there is evidence of differences between insular and mainland populations in the following: (i) density, (ii) rodent biomass, (iii) length of breeding season, (iv) survival, (v) population stability, (vi) catchability of individuals, and (vii) trap-revealed movement.

1.7.3. Chapter 5

In this chapter I investigate body size variation in terms of length, weight and condition between four mainland and six insular populations of bank voles. As well as discussing the most appropriate measurements for body size comparisons, I particularly address the following hypotheses: (i) island subspecies are larger than mainland conspecifics (ii) insular gigantism in voles occurs as a result of selective pressures rather than random genetic effects. By combining data from this study with data from the published literature

across the species range I question whether the following selective pressures are responsible for the increased body size of insular subspecies of bank voles: (i) reduced interspecific competition, (ii) increased intraspecific competition, (iii) reduced predation pressure, (iv) Bergmann's Rule, and (v) climatic variables (e.g. temperature, rainfall, altitude). I also test the hypothesis that reverse sexual size dimorphism (females larger than males) is not apparent in island vole populations.

1.7.4. Chapter 6

In Chapter Six I employ geometric morphometric analyses to investigate cranial variation between island and mainland populations of British bank voles. Specifically I test the following hypotheses: (i) island vole subspecies are morphologically distinguishable in features other than body size, (ii) skull morphology does not support the subspecies classification of voles from Mull and Raasay, (iii) sex and age influence vole skull morphology, (iv) there is regional variation in skull morphology, and (v) island vole populations without designated subspecies have skull morphology that is indistinct from mainland vole populations. Using the landmark coordinates from the geometric morphometric analysis, I also examine levels of fluctuating asymmetry within vole populations to see whether there is greater evidence of developmental instability within island populations.

Chapter 2: Field sites

2.1. Introduction

Several field sites were visited during the course of this study (Fig. 2.1). Sites were selected on the basis that they fulfilled one of the following criteria; (i) island with a named subspecies of bank vole, (ii) easily accessible island with bank voles reportedly similar to mainland voles, (iii) mainland area in close proximity to one of the island sites, containing extensive habitat in line with the preference of bank voles (e.g. deciduous woodland). This chapter provides details of locations and brief site descriptions for the different sites. Particular attention is paid to the five main sites visited for the purposes of the demographic studies: the islands of Skomer, Raasay, Mull and Jersey, and the mainland 'control' site at Orielton in Pembrokeshire, Wales. Details of trapping grids and times of trapping for these main sites are provided in Chapter 3. Information, including times of trapping, for seven additional field work sites used for the collection of body size and genetic data are given in this chapter. Climate and bank vole predators and rodent competitors, with particular respect to British islands, are also considered here. This information will be referred to at several points later in the thesis.

Figure 2.1: Locations of field sites employed during the course of this study

2.2. Site descriptions

2.2.1 Skomer Island

2.2.1.1. Island geography and history

Skomer Island is a small island of 292 ha that lies ~1 km off the coast of south west Wales (Pembrokeshire), to the south of St. Brides Bay (51°44'N, 5°17'W). Between Skomer and the mainland lies the much smaller island of Middleholm. Both islands are effectively isolated from the mainland by fast flowing sounds that commonly exceed 4 knots (Healing, 1984).

Grimes (1950) estimated that Skomer has been inhabited by man since the first century BC. Remains of an Iron Age farming settlement, which probably housed up to 200 people, are evident across the island. The farmhouse complex, currently situated in the island's

centre, was built around 1840 and was thought to replace a similar sized building dating from around 1700. Historically, agriculture has been important on Skomer and the field systems present throughout the central parts of the island have been used to grow cereals, hay and graze livestock. All farming ceased on the island in 1950 and the island is now a protected wildlife reserve, owned by the Countryside Council for Wales (CCW) and leased by the Wildlife Trust of South and West Wales. Nowadays the island is inhabited from February to October by a warden and assistant warden, and an ephemeral population of volunteers, research staff and overnight visitors.

2.2.1.2. Island flora and fauna and locations of trapping grids

Skomer has a depauperate mammalian fauna when compared to mainland Britain, consisting of bank voles, wood mice (*Apodemus sylvaticus*), common shrews (*Sorex araneus*) and rabbits (*Oryctolagus cuniculus*). Pygmy shrews (*Sorex minutus*) have historically been recorded on the island but their current status is unclear (J. Brown, pers. comm.). Of these species, rabbits are likely to have the largest impact on the distribution of voles because their extensive grazing and burrowing significantly alters the suitability of the habitat.

Skomer Island is an important nesting site for many species of seabird and the island hosts over 40% of the world's breeding population of Manx shearwater (*Puffinus puffinus*). As a burrow nesting species, shearwaters undoubtedly create habitat for the voles but they may also have compete for tunnels during the breeding season. Puffins (*Fratercula artica*) are also present on the island but the burrows of nesting colonies are restricted to two coastal sites covering a much smaller area and thus probably have less impact on the voles. Other nesting colonies that may affect the distribution of voles by excluding them from certain

areas include herring gulls, *Larus argentatus* and lesser black backed gulls, *Larus fuscus* (Healing *et al.* 1983).

The vegetation on Skomer is impoverished with respect to the mainland fauna. Large areas of the central parts of the island are covered by covered by bracken (*Pteridium aqulilnum*). Healing *et al.* (1983) described nine different vegetation categories across the island and their habitat use by voles (Table 2.1). Figure 2.2 shows the approximate location of the three trapping grids employed for this study with respect to vegetation types. Grid T made use of a grid previously established by Healing (1984) in an area of known high density of voles. This was to enable direct comparisons between current data and existing long-term population data on the voles. Grid E1 covered an area of patchy bracken and rabbit lawns, with a small area of bramble/mixed cover. Grid E2 was located in an area of continuous bracken/sorrel, with some bracken/grass habitat (Table 2.1, Fig. 2.2).

Table 2.1: Vegetation categories on Skomer Island, according to a 1981 survey, with the dominant plant species in each category. Density of voles is according to a 1981 survey. Table adapted from Healing *et al*. 1983.

Vegetation Categories	Vole density	Flora
Bracken/sorrel	High	Tall bracken (*Pteridium aquilinum*), dense bluebell (*Endymion non-scriptus*), sorrels (*Rumex* spp.)
Bracken/grass	Medium	Medium to tall extensive bracken, some bluebell, Yorkshire fog (*Holcus lanatus*)
Patchy bracken	Low	Mainly yorkshire fog, patches of bracken
Heather	Medium	Heather (*Calluna vulgaris*), heaths (*Erica* spp.)
Yorkshire fog	Low	Mainly yorkshire fog, some *Poa* spp., *Festuca* spp., clumps of ragwort (*Senecio jacobea*)
Clifftop/rocky outcrop	Low	Sea campion (*Silene maritima*), mixed grasses (mainly *Festuca* spp.), thrift (*Armeria maritima*), lichens, bare rock
Rabbit lawn	Low	Mixed grasses: well grazed, short
Moor grass/rushes	Medium	Tussocks of purple moor grass (*Molinia caerulea*), clumps of rushes (*Juncus* spp.)
Bramble/mixed cover	High	Clumps of bramble (*Rubus* spp.) Umbellliferae and thistles (*Cirsium* spp.)

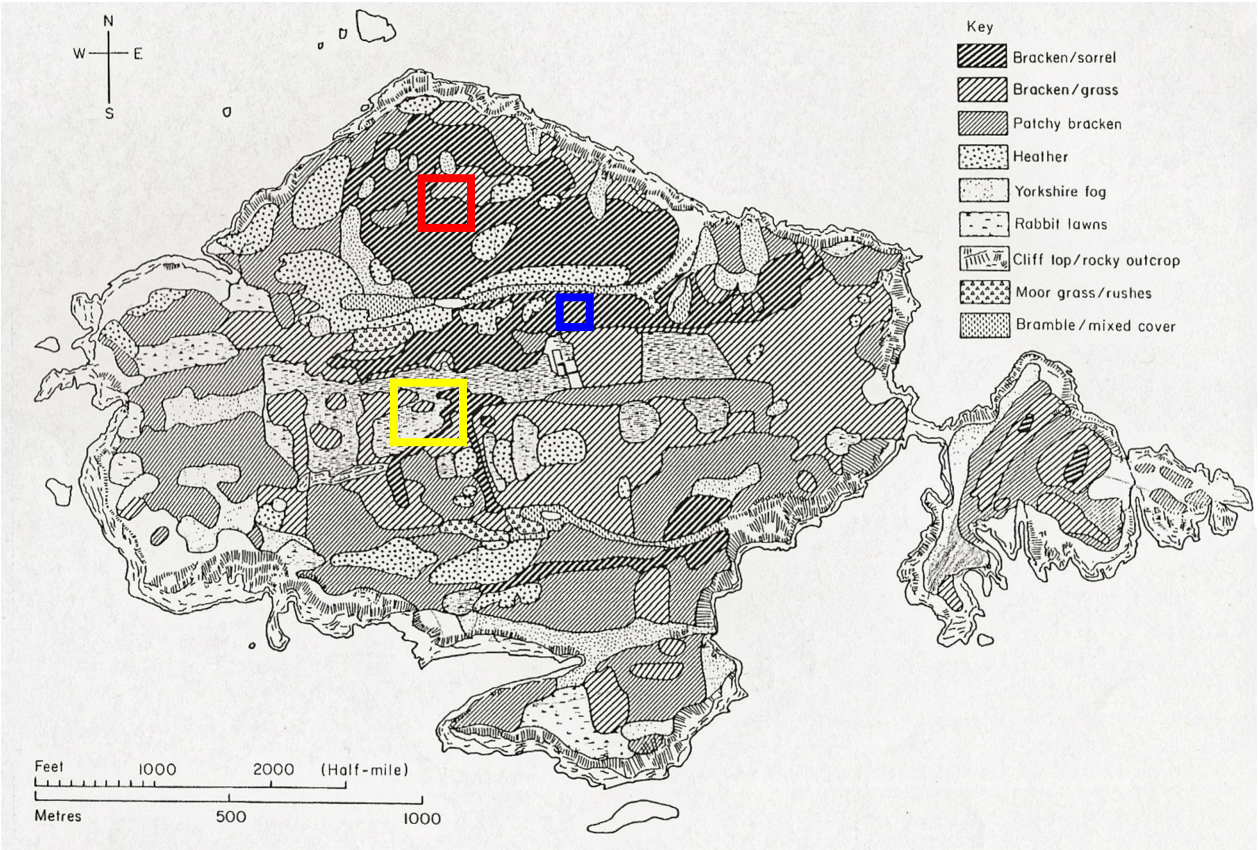


Figure 2.2: Location of Skomer trapping grids overlaid on a vegetation map of the island taken from Healing *et al*. (1983). Habitat categories are as described in Table 2.1. Grid T is shown in blue, Grid E1 in yellow and Grid E2 in red.

2.2.2. Jersey

2.2.2.1. Island geography and history

Jersey is an 11,630 ha island belonging to Channel Islands archipelago situated in the English Channel. The island lies around 22 km from the Contenin peninsula of the Normandy region of France (Berry 2009). The Channel Islands include five inhabited islands along with a number of much smaller uninhabited islets. Listed in order of decreasing size these inhabited islands are; Jersey, Guernsey, Alderney, Sark and Herm. Interestingly, bank voles are absent from the entire archipelago with the exception of Jersey. The Channel Islands are considered British Crown dependencies and are not technically part of the United Kingdom because they are politically separate. Both Jersey and Guernsey have their own legislative assemblies, administrative and financial systems and courts of law. Thus, Jersey is largely autonomous because the other inhabited islands fall under the jurisdiction of the States of Guernsey.

Archaeological evidence suggests the landmass now known as Jersey was first occupied by mammoth hunters during the Palaeolithic 250,000 years ago (Berry 2009). However, subsequent evidence suggests a break in human habitation between 50,000 and *c.*6000 years BP. There is significant evidence of agricultural activity since reoccupancy, and the bones of domestic animals are present in fossil deposits from around 5500 BP (Berry 2009). The current human population on Jersey is estimated to be 90,800 (States of Jersey website 2009).

2.2.2.2. Field site

Previous island-wide studies of small mammals on Jersey indicated that voles reach particularly high densities in both grassland and heathland habitats (Magris 2000). Thus,

field sites were selected in heathland areas because there was less likelihood of disturbance from members of the general public. Initially a grid (J1) was established in the northwest of the island in the heathland area of Les Landes (approximately 49°14’52.64 N, 2°14’52.60 W). However, following low capture success on Grid J1 in autumn 2005, an alternative grid (J2) was established on Portlet Common (Fig. 2.4). This area of heathland is in the southwest of the island (49°10’28.48 N, 2°11’07.52 W) and was chosen because a high density of voles had previously been recorded there (Magris 2000). The dominant vegetation types on both grids were heather (*Erica* spp. and *Calluna vulgaris*) and gorse (*Ulex europaeus*). However Grid J2 also contained patches of bramble (*Rubus fruticosus* agg.) and bracken with various grass species (Fig. 2.4).

Figure 2.3: Vegetation on trapping grid, Jersey (site 2).

2.2.3. Mull

2.2.3.1. Island geography and history

The Isle of Mull (87,794 ha) lies approximately 1.8 km off the western coast of Scotland and belongs the island chain known as the Inner Hebrides. Mull is the second largest of the islands in the Inner Hebrides and the current human population is approximately 2696 (Berry 2009). Mull appears to have a long history of pre-historic human habitation and has probably been occupied almost continuously since the Neolithic (*c.*8500 BP). There is archaeological evidence of Mesolithic hunter-gatherers, Bronze Age settlements and Iron Age settlements occurring on the island.

2.2.3.2. Field site

The field site was located in the Garmony area of Mull on Forestry Commission land (approximately 56°29’30.89 N, 5°46’35.64 W). The trapping grid was situated adjacent to a conifer plantation and a small area of deciduous woodland (Fig. 2.4). The vegetation consisted of large areas of grassland, punctuated by patches of bramble, other scrub vegetation (e.g. gorse) and some tree cover (mainly silver birch *Betula pendula*, but with coniferous cover along the edge). However parts of the grid were very wet and as such the dominant vegetation in these parts was either moss (*Sphagnum* spp.) or rush (*Juncus* spp.).

Figure 2.4: Vegetation on trapping grid, Mull.

2.2.4. Raasay

2.2.4.1. Island geography and history

The Isle of Raasay is an Inner Hebridean Island measuring 6,405 ha. Raasay is situated in between the mainland (approximately 6.5 km offshore) and neighbouring Skye. The narrowest part of the Channel between the two islands measures approximately 1.1 km, but interestingly bank voles are absent from Skye. Historically, Raasay is similar to many of the other islands in the Inner Hebrides in that there appears to be evidence of human settlers from the Neolithic period (6000-5000 BP) onwards. The current human population is only 194 (Berry 2009).

2.2.4.2. Field site

The field site was chosen on the basis that it was the only area of broadleaved woodland on Raasay. The site was located on private land, approximately halfway between the north

and south of the island on the west side (57°23’53.59 N, 6°03’58.86 W). Unfortunately the chosen area was insufficient to accommodate a standard grid of size 65 m x 65 m. Therefore two smaller grids were established, Grid One (Fig 2.5) was in a partially wooded area of silver birch and willow (*Salix* sp.) at the top of a hill and Grid Two (Fig. 2.6) was situated in a similar wooded area at a slightly lower altitude. The dominant ground vegetation on both grids consisted of grass and moss but Grid One contained patches of bracken whereas Grid Two contained patches of bog myrtle (*Myrica gale*) and mixed heather species.

Figure 2.5: Vegetation on trapping grid, Raasay (Grid 1).

Figure 2.6: Vegetation on trapping grid, Raasay (Grid 2).

2.2.5. Orielton

Since Skomer Island was the subject of more detailed population studies than the other three islands, a mainland site was established in nearby Pembrokeshire, south west Wales, to enable direct demographic comparisons. This site was situated in the grounds of Orielton, a Field Studies Council (FSC) centre, because it is one of the only extensive broadleaved woodland areas located in Pembrokeshire. The site is situated near the village of Hundleton (51°39'24.70 N, 5°57'17.00 W), approximately 22 km distance from Skomer Island. The trapping grid was established in Limebridge Wood, in an area with relatively dense ground vegetation, corresponding to the habitat preference of mainland bank voles (Fig. 2.7). The dominant tree species on this site were sycamore (*Acer pseudoplatanus*) and ash (*Fraxinus excelsior*) and these were interspersed with beech (*Fagus sylvatica*), oak (*Quercus* spp.) and hazel (*Corylus avellana*). The ground vegetation consisted largely of bramble and various species of fern (mainly; broad buckler

Dropteris dilatata, hart's tongue *Phyllitis scolopendrium* and soft shield *Polystichum setiferum*).

Figure 2.7: Vegetation on trapping grid, Orielton.

2.2.6. Ramsey Island

In addition to the five main field sites, Ramsey Island was visited to collect body size and genetic data. Since Ramsey is only ~ 12 km from Skomer Island, off the west coast of Wales, some details of the island are given below.

Ramsey is a 265 ha island situated to the north of St. Brides Bay, in Pembrokeshire, south west Wales (51°51'36 N, 5°20'27 W). The island lays approximately 0.75 km off the coast of St. David's Head. Ramsey is owned by the Royal Society for the Protection of Birds (RSPB) and is managed as a nature reserve. The warden and his wife currently inhabit the island. Along with neighbouring Skomer, the island has a long history of agricultural use. However, unlike Skomer, Ramsey still houses livestock (approximately

200 sheep along with a small number of ponies and red deer). Brown rats (*Rattus norvegicus*) were accidently introduced to Ramsey in the 1800's and remained on the island until a successful poisoning programme led to their eradication in 2000.

Voles were live trapped on Ramsey for the purposes of this study during April 2006, June 2007 and August 2007.

2.2.7. Other sites

Six other sites were visited in order to collect genetic samples and body size data. Since population data were not gathered from these sites, traps were deployed in irregular lines rather than a grid system and were placed in areas deemed to be most in line with bank vole habitat preferences. Relevant information about these sites is provided in Table 2.2.

2.3 Climate data

Climate data from the nearest weather station to each of the field sites was gathered from the Met Office website (www.metoffice.gov.uk/climate). Data from weather stations for the nearest mainland station and nearest island station were gathered for Hebridean Islands. Data were averaged for the time periods stated in the column 'Years' (Tables 2.3 to 2.6) Temperature and rainfall data for the five main study sites are illustrated in Fig. 2.8.

Table 2.2: Details of six additional field sites visited during the study to collect vole body size and genetic data.

Site	Habitat	Grid Coords	Time of trapping	Additional Notes
Isle of Wight	Briddlesford Woods, mixed deciduous woodland	50°42'N, 1°13'W	March 2006	31,800 ha island in English Channel, 1.3 km (min) from mainland
France	Mixed deciduous woodland and hedgerows	41°32'N, 1°45'W	October 2006	Site near the village of Dol-de-Bretange, ~70 km from Jersey
Surrey, England	Mixed deciduous, scrubby woodland	51°11'N, 0°51 W	February 2006, March 2008	Alice Holt Forest
Bedfordshire, England	Garden, field hedgerows	51°59' N, 0°37' W	-	Cat kills
Wooltack Point, Pembrokeshire	Gorse/scrub patches in rough grassland	51°44'N, 5°14'W	March 2006	Headland on mainland opposite Middleholm and then Skomer
Morvern, Scotland	Mixed deciduous, scrubby hedgerows	56°24'N, 5°25 W	April 2008	On Scottish mainland ~18 km from Mull field site

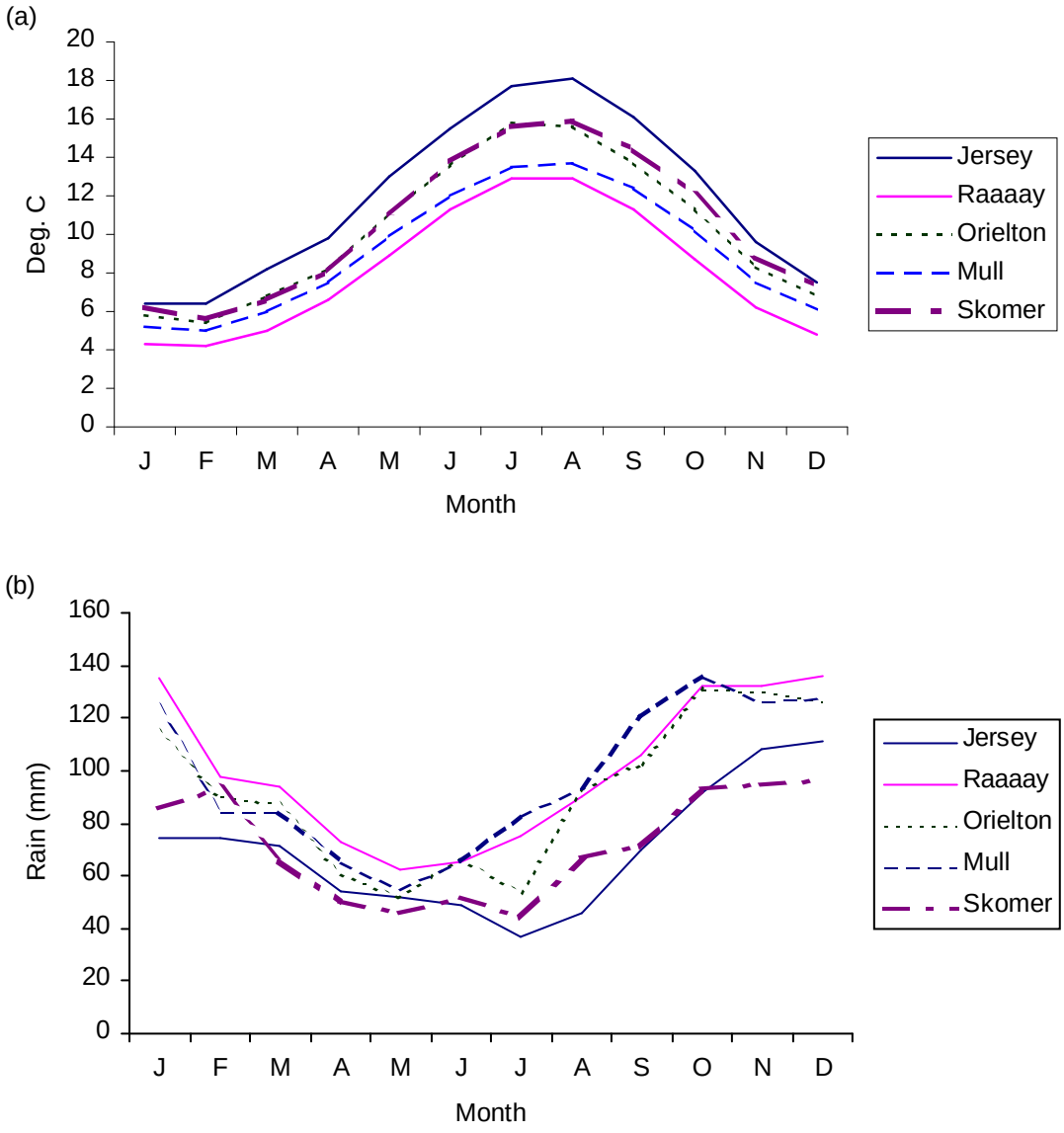


Fig. 2.8: Long-term average monthly (a) temperature and (b) rainfall records for the five main study sites. Raasay data from Isle of Lewis, Mull data from Isle of Tiree (Table 2.2).

Table 2.3: Mean monthly temperatures (°C). Data are averaged from minimum-maximum temperature data for each month.

Site	Weather station location	J	F	M	A	M	J	J	A	S	O	N	D	Mean	Years
Jersey	Jersey, Channel Islands	6.4	6.4	8.2	9.8	13.0	15.5	17.7	18.1	16.1	13.3	9.6	7.5	11.8	1971-2000
Raasay	Kinlochewe, Ross-Shire	3.7	4.0	5.5	7.2	10.2	12.4	14.4	14.1	11.7	9.0	5.8	4.4	8.6	1971-2000
Raasay	Stornaway, Isle of Lewis	4.3	4.2	5.0	6.6	8.9	11.3	12.9	12.9	11.3	8.7	6.2	4.8	8.1	1873-2007
Ramsey	Aberporth, Dyfed	5.1	5.0	6.3	7.8	10.6	13.0	15.1	15.2	13.4	10.8	7.8	6.1	9.7	1971-2000
Orielton	Tenby, Pembrokeshire	5.8	5.4	6.8	8.2	11.1	13.6	15.8	15.6	13.7	11.3	8.3	6.8	10.2	1971-2000
Mull	Dunstaffnage, Argyllshire	4.6	4.7	5.8	7.6	10.6	12.5	14.3	14.3	12.4	10.0	6.9	5.5	9.1	1971-2000
Mull	Isle of Tiree, Argyllshire	5.2	5.0	6.0	7.5	9.9	12.0	13.5	13.7	12.4	10.2	7.5	6.1	9.4	1931-2001
Alice Holt	Wisley, Surrey	5.2	5.1	6.3	7.9	10.8	13.1	15.0	14.9	13.0	10.6	7.6	6.1	9.7	1971-2000
Morvern	Paisley, Renfrewshire	4.0	4.3	6.0	8.4	11.4	14.1	15.5	15.3	12.9	9.9	6.3	4.5	9.5	1959-2008
Skomer	Dale, Pembrokeshire	6.2	5.6	6.6	8.1	11.0	13.8	15.6	15.9	14.4	12.1	8.8	7.3	10.5	1961-1990
Wight	Everton, Hampshire	5.3	5.1	6.9	8.6	11.8	14.4	16.7	16.7	14.5	11.6	8.1	6.3	10.5	1971-2000

Table 2.4: Mean rainfall (mm).

Site	Weather station location	J	F	M	A	M	J	J	A	S	O	N	D	Mean	Years
Jersey	Jersey, Channel Islands	74	74	71	54	52	49	37	46	70	92	108	111	837	1971-2000
Raasay	Kinlochewe, Ross-Shire	284	213	227	115	94	104	99	129	205	235	288	287	2278	1971-2000
Raasay	Stornaway, Isle of Lewis	135	98	94	73	62	65	75	90	106	132	132	136	1199	1873-2007
Ramsey	Aberporth, Dyfed	89	63	64	51	48	61	50	68	76	104	98	99	870	1971-2000
Orielton	Tenby, Pembrokeshire	115	90	87	61	52	67	53	93	102	131	130	126	1107	1971-2000
Mull	Dunstaffnage, Argyllshire	192	140	153	80	67	83	102	119	163	187	182	192	1661	1971-2000
Alice Holt	Wisley, Surrey	63	41	48	48	51	52	40	49	61	71	60	65	647	1971-2000
Mull	Isle of Tiree, Argyllshire	125	84	84	65	55	65	83	93	120	136	126	128	1164	1931-2001
Morvern	Paisley, Renfrewshire	133	94	99	64	68	66	70	89	111	131	125	130	1180	1959-2008
Skomer	Dale, Pembrokeshire	86	93	66	50	46	52	44	67	71	93	95	96	859	1961-1990
Wight	Everton, Hampshire	81	59	60	48	46	52	38	50	67	88	84	91	764	1971-2000

Table 2.5: Mean hours of sunshine.

Site	Weather station location	J	F	M	A	M	J	J	A	S	O	N	D	Total	Years
Jersey	Jersey, Channel Islands	66	88	134	191	239	247	257	246	182	124	80	59	1912	1971-2000
Raasay	Kinlochewe, Ross-Shire	18	46	64	101	147	125	113	109	82	52	24	14	894	1971-2000
Raasay	Stornaway, Isle of Lewis	34	63	105	147	192	167	128	132	107	77	44	26	1223	1929-2007
Ramsey	Aberporth, Dyfed	55	74	110	169	214	191	199	185	141	100	63	46	1545	1971-2000
Orielton	Tenby, Pembrokeshire	58	75	116	185	218	206	219	201	149	106	72	50	1654	1971-2000
Mull	Dunstaffnage, Argyllshire	34	60	86	146	190	175	143	142	98	76	46	31	1224	1971-2000
Alice Holt	Wisley, Surrey	52	71	108	152	194	188	204	201	143	113	67	43	1535	1971-2000
Mull	Isle of Tiree, Argyllshire	39	68	110	169	226	199	161	163	123	81	48	30	1417	1931-2001
Morvern	Paisley, Renfrewshire	36	63	95	135	177	166	154	146	111	82	51	32	1247	1959-2008
Skomer	Dale, Pembrokeshire	60	79	131	190	229	215	224	207	154	105	73	52	1718	1961-1990
Wight	Everton, Hampshire	62	81	122	182	223	212	232	223	160	120	81	53	1751	1971-2000

Table 2.6: Mean days of air frost.

Site	Weather station location	J	F	M	A	M	J	J	A	S	O	N	D	Total	Years
Jersey	Jersey, Channel Islands	12.0	10.5	8.2	5.7	2.3	0.1	0	0	0.3	2.2	7.5	10.8	59.6	1971-2000
Raasay	Kinlochewe, Ross-Shire	7.2	7.0	6.5	2.6	0.6	0	0	0	0	0.9	3.5	6.2	34.3	1971-2000
Raasay	Stornaway, Isle of Lewis	5.6	5.6	2.1	0.6	0	0	0	0	0	0	1.0	3.4	18.3	1873-2007
Ramsey	Aberporth, Dyfed	3.2	3.1	0.6	0.3	0	0	0	0	0	0	0.4	1.9	9.5	1971-2000
Orielton	Tenby, Pembrokeshire	7.6	5.9	3.9	2.0	0.5	0	0	0	0	0.3	3.4	5.7	29.4	1971-2000
Mull	Dunstaffnage, Argyllshire	9.5	9.6	6.3	3.9	0.9	0	0	0	0	2.2	6.6	8.4	47.4	1971-2000
Alice Holt	Wisley, Surrey	3.7	4.2	2.7	0.9	0	0	0	0	0	0	0.8	2.4	14.8	1971-2000
Mull	Isle of Tiree, Argyllshire	9.4	8.2	4.6	1.5	0.1	0	0	0	0	0.7	4.9	8.2	37.6	1931-2001
Morvern	Paisley, Renfrewshire	3.8	3.9	1.2	0.2	0.0	0	0	0	0	0	0.3	2.0	11.4	1959-2008
Skomer	Dale, Pembrokeshire	8.3	7.2	4.4	2.0	0.1	0	0	0	0	0.3	3.8	6.3	32.5	1961-1990
Wight	Everton, Hampshire	8.3	7.2	4.4	2.0	0.1	0	0	0	0	0.3	3.8	6.3	32.5	1971-2000

2.4. Predators and competitors

Many of the theories concerning 'island syndrome' invoke the absence of predators and/or competitors on islands as a causal explanation (Chapter 1). Table 2.7 lists British islands that have bank voles, with known predators and rodent competitors. Information on mammalian predators and competitors was gathered from *Mammals of the British Isles: Handbook 4th Edition* (Harris and Yalden 2008). Data on avian predators was largely gathered from regional wildlife based websites. Data on both mammals and avian predators were supplemented with personal records and information from local people. Few records were available for Handa and Hayling islands. Many avian species are recorded on Hayling as probably present (L) on the basis these species are recorded on the adjacent mainland and nearby Isle of Wight.

Table 2.7: Presence/absence of bank vole predators and rodent competitors on British islands. A=absent, Y = present, L = probably present,? = unknown, V=vagrant, P=old records, possibly extinct, R=recent introduction, O=occasional records.

Name	Species	Jersey	Skomer	Raasay	Mull	Bute	Arran	Scalpay	Wight	Anglesey	Ramsey	Ulva	Handa	Hayling
Mammalian predators														
Weasel	*Mustela nivalis*	N	N	P	N	Y	N	R	Y	Y	V	N	N	Y
Stoat	*Mustela erminea*	P	N	Y	Y	Y	N	R	Y	Y	N	N	O	Y
Fox	*Vulpes vulpes*	N	N	N	N	Y	N	N	Y	Y	N	N	N	?
Avian predators														
Buzzard	*Buteo buteo*	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	?	Y
Kestrel	*Falco tinnunculus*	Y	Y	Y	Y	Y	Y	?	Y	Y	Y	Y	?	Y
Barn owl	*Tyto alba*	Y	Y	N	O	Y	Y	?	Y	Y	Y	O	?	L
Tawny owl	*Strix aluco*	O	N	Y	Y	Y	Y	?	Y	Y	N	Y	?	L
Short-eared owl	*Asio flammeus*	O	Y	N	Y	Y	Y	?	Y	Y	Y	Y	?	L
Long-eared owl	*Asio otus*	Y	N	N	Y	Y	Y	?	Y	Y	N	Y	?	L
Little owl	*Athen noctua*	O	Y	?	N	Y	?	?	Y	Y	Y	N	?	L
Rodent competitors														
Field vole	*Microtus agrestis*	N	N	N	Y	Y	Y	Y	Y	Y	N	?	?	Y
Wood mouse	*Apodemus sylvaticus*	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	?	?	Y

Chapter 3: Phylogeography of bank voles inferred from mitochondrial DNA sequence analysis

3.1. Introduction

When studying variation between island and mainland forms, it is important to first take colonisation history into account. Although most hypotheses that seek to explain 'island syndrome' focus on post-colonisation adaptive mechanisms (Chapter 1), founder effects often play a significant role in the phenotypic variation observed on islands (Berry 1992). Thus, information on the origin of founding populations, as well as the timing of colonisation may provide valuable insights into the evolutionary mechanisms behind insular variation.

There are several mechanisms through which terrestrial mammals can colonise offshore islands from adjacent landmasses. Colonisation could occur through the use of land or ice bridges, through swimming or rafting on debris across small channels or through human-mediated introduction (Whittaker 1998). Alternatively, colonisation may have occurred before islands broke away from the mainland and extant island populations may be relicts.

At the turn of the last century it was hypothesised that the microtine rodents present on the Orkney, Hebridean, Channel and Welsh islands were relicts of pre-glacial lineages that had been displaced throughout mainland Britain by post-glacial colonisers from Europe (Hinton 1910). This was largely based on the morphological peculiarities observed in these island races and their similarity to Scandinavian, Pyrenean and Alpine populations, also at the time thought to be relicts. However, an

eloquent rebuttal of this hypothesis was put forward by Corbet (1961) who argued that the geological evidence did not support the 'relict theory' and that the random distribution of field voles (*Microtus agrestis*), bank voles (*Myodes glareoulus*) and wood mice (*Apodemus sylvaticus*) on offshore islands pointed more towards human-mediated introduction than natural colonisation processes. Corbet (1961) reasoned that if islands were colonised by natural processes one would expect species occurrence on islands to follow the same pattern as their currently observed distribution ranges and that interspecific competition would play a significant part in preventing subsequent colonisation attempts. Thus, field voles should be most prolific on British islands followed by bank voles, followed by wood mice, when in fact wood mice have the widest insular distribution and bank voles have the most restricted distribution. Indeed Corbet's theory has proven correct for several insular populations of rodents. Genetic analysis has shown that the Orkney vole (*Microtus arvalis orcadensis*) probably arrived with Neolithic people from southwestern Europe (Haynes *et al*. 2003). Whilst the wood mice (*Apodemus sylvaticus*) of St. Kilda, and the house mice (*Mus musculus*) of the Faroe Archipelago probably arrived with the Vikings (Berry 1986, 1992).

With geological evidence suggesting that the ice sheets of the Last Glacial Maximum (LGM) extended as far south as Norfolk and south Wales, the rest of Britain would have been tundra, thus it is unlikely that most British mammal populations would have been unable to survive the height of the LGM *in situ* (Yalden 1999). The first fossil records of bank voles after this period have been dated by pollen analysis to the Bolling Interstadial 12,500-12,000 years before present (BP) and the Allerod Interstadial 11,700-11,000 years BP (Campbell 1977 in Yalden 1982). Carbon dated

deposits also place bank voles in mainland Britain during the Younger Dryas, 10,590 BP (Bramwell 1977 in Yalden 1982). However, the climatic deterioration of the Younger Dryas (11,000-10,000 BP), causing a return to arctic conditions, make it extremely unlikely that any British bank vole lineages survived this period and thus it is likely that the carbon dating estimate was less than precise (Yalden 1982). It is also possible that these fossils represent the more cold-tolerant species of *M. rufocanus*, now absent from the British fauna, which may not be easily distinguishable from *M. glareolus* when using skeletal traits. Nevertheless, subsequent rapid warming of the climate appears to have enabled quick recolonisation of the British Isles and, in all probability, a full complement of native mammals were present on the mainland by 9,500 BP, before the flooding of the English Channel (Yalden, 1999).

In terms of the insular populations of interest in this study, the postglacial history of these islands regarding their separation from the mainland is somewhat unclear. However, considering the current depth of the sea floor between Raasay and the mainland is between 50 m and over 130 m (source Google Earth), it is likely that this island very quickly became isolated following the ice melt. Thus, overland colonisation is a highly improbable route for bank voles onto Raasay. The extant faunal assemblages, in particular the lack of field voles, also indicate that rising sea levels during the postglacial phase probably prevented land bridge colonisation by mammals onto the islands of Skomer and Ramsey. The origin of populations on Mull is less certain as its proximity to the mainland and the presence of field voles on this island indicates that ‘natural’ colonisation may have occurred.

The Channel Islands are well below the latitude to which the ice sheets extended during the Last Glacial Maximum (LGM; 20,000 years ago) and thus are thought to be the most likely of the British islands to retain some of their preglacial biota. However, only the most cold-tolerant species are likely to have survived *in situ* and this is unlikely to include bank voles. Berry (2009) estimates that following the LGM, Guernsey became an isolated island *c*.9200 BP and Jersey detached from mainland France *c*.5800 BP. Other authors (Yalden, 1982, 1999, 2008) have argued that whilst the current depth of the sea floor between Jersey and France may indicate land bridge connection during this period, the impoverished and random distribution of mammalian fauna of these islands suggests most extant mammalian species probably did not arrive by 'natural' means. For instance, bank voles are only found on Jersey whilst common voles (*Microtus arvalis*) are only present on Guernsey and field voles are absent from all of the Channel Islands. This is not the pattern one would expect if these animals had utilised land bridge connections from mainland France. In addition the presence of white toothed shrews (*Crocidura* spp) on several islands, particularly *C. suavolens* which is not present on the directly adjacent mainland, perhaps also points towards human-mediated introduction of at least some of the Channel Island small mammal fauna (Yalden, 1999, Berry 2009). Conversely, on the Isle of Wight the presence of most mammalian fauna typical of the British mainland indicates vole populations here possibly became established before the island was isolated from the mainland *c*.7500 years ago (Berry 2009).

Recently, phylogeographic studies of mitochondrial DNA (mtDNA) have proven pivotal in understanding the origins of small mammal populations in Britain and Ireland. Stuart *et al*. (2007) were able to show that bank voles were most likely

introduced to Ireland from Germany in the 1920's, probably arriving with materials transported for the construction of the Shannon Hydroelectric Scheme. Similarly, extant Irish pygmy shrew populations are thought to have been the result of human-mediated introduction from southwest Europe (Mascheretti *et al.* 2003, McDevitt *et al*. 2009). Conversely, Martínková *et al*. (2007) were able to show that Irish stoats were in all probability the result of a natural colonisation process during the LGM, coming across ice bridges from mainland Britain. White and Searle (2008) employed mtDNA sequences in combination with polymorphic nuclear loci (microsatellites) to show that common shrews (*Sorex araneus*) most likely colonised the Hebridean islands via ice bridges from the nearby mainland.

So far, only a limited amount of work has been carried out on bank vole phylogeography in the UK (Deffontaine *et al.* 2005). Analysis of the mitochondrial cytochrome *b* (cyt *b*) gene, from specimens from across the species' entire range, revealed that bank voles present within mainland Britain are part of a western phylogroup that colonised large parts of north western and central Europe following rapid population expansion from central European refugia after the last glaciations. Whilst it is hypothesised that insular races on the smaller islands surrounding the UK probably colonised from nearby mainland Britain (even with human-mediated introductions), and Channel Island voles from nearby mainland France, the exact origins of these populations are as yet undetermined. This study seeks to establish the phylogenetic origins of island races of bank voles around the UK. In particular I seek to test the following hypotheses:

1. Voles colonised all offshore islands via human-mediated transportation rather than 'naturally' (with the exception of the Isle of Wight).
2. Colonisation of all offshore islands occurred after the height of the Last Glacial Maximum.
3. Clinal size variation (see Corbet, 1964) and phylogeographic history are not responsible for the observed size differentiation in the four insular subspecies.

3.2. Materials and Methods

3.2.1. Sampling

Bank voles were collected from six island and five mainland sites around the UK as well as from one location in mainland France between 2005 and 2007 (Chapter 2). Specimens were captured using Longworth live traps. A 2 mm ear biopsy tissue sample was taken from each individual and stored in 70% ethanol before the animal was released. Island samples were collected from at least three different localities (with the exception of the Isle of Wight) in an attempt to ensure that a representative range of potential haplotypes were included in the analyses.

3.2.2. DNA extraction, amplification and sequencing

Deffontaine *et al.* (2005) analysed cytochrome *b* (cyt *b*) sequence data for 64 specimens from the western phylogroup that is thought to have colonised large parts of central Europe including Britain. They detected 36 individual haplotypes from 22 locations across 12 countries. Previous studies that examined genetic variation in the mitochondrial control region (d-loop) of *Myodes* found only 30 variable nucleotides within the entire locus from 54 *M. glareolus* samples taken from six countries across

the species range (Matson and Baker 2001). Thus, for the purposes of this study it was decided to sequence both the cyt *b* and d-loop genes in order to maximise the likelihood of obtaining enough variation to determine the origins of the island populations in question.

The genetic polymorphism of partial genetic sequences from the mitochondrial cyt *b* gene (993/1143 bp) and control region (d-loop) locus (870/946 bp) were analysed for 69 animals. Genomic DNA was extracted from the tissue samples using DNeasy Tissue Kits (QIAGEN). Partial segments of the cyt *b* gene and d-loop gene were amplified by the polymerase chain reaction (PCR: 1 initial denaturation cycle at 94°C for 3 mins, then 35 cycles of alternating denaturation at 94 °C for 30 secs, annealing at 50 °C for 45 secs and extension at 72 °C for 45 secs, followed by one final extension cycle at 72 °C for 10 mins). The cyt *b* gene was amplified using various combinations of primers 1-4 and the d-loop locus was amplified using the primers 5-6 (Table 3.1). PCR products were then purified using the QIAquick PCR Purification Kit (QIAGEN). Sequencing was carried out in both directions using combinations of primers to generate partially overlapping strands that produced unambiguous results. An Applied Biosystems (ABI) 3700 automated DNA sequencer was used to separate fragments produced using the Dynamic ET Terminator Sequencing Kit (Amersham, UK).

Table 3.1: Primers used for amplification of the bank vole mitochondrial genome.

No.	Name	Sequence	Amplified region
1	L14724	5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′	Cytochrome *b*
2	EHCB-R	5’-TGAGTAGGTCAGCTACTAGG-3’	Cytochrome *b*
3	EHCB-F	5’-GGACGAGGCATATACTATGG-3’	Cytochrome *b*
4	CB-R	5'-TTARTCTAGGTCYAKRATGTYGTTTTC-3'	Cytochrome *b*
5	DL2OP-F	5’-ACATTCTATTTAAACTACTTCT-3’	d-loop
6	HN00651	5’-TAACTGCAGAAGGCTAGGACCAAACCT-3’	d-loop

3.2.3. Analysis of mitochondrial DNA sequences

Sequences were aligned manually using BioEdit Sequence Alignment Editor Version 7.0.5.2 (Hall, 1999). Cyt *b* and d-loop data were initially explored independently and then as a concatenated sequence data set. Cyt *b* data only were used for molecular clock analyses (Section 3.2.5). Phylogenetic relationships between haplotypes were ascertained using PAUP* version 4.0b8 (Swofford 2001). Maximum parsimony analysis was performed using the heuristic search option with all characters given an equal weighting and with gaps treated as missing data. The maximum number of trees was restricted to 100 and the robustness of trees was evaluated by bootstrap resampling with a setting of 100 replications. MODELTEST 3.7 (Posada and Crandall 1998) was used to determine the most suitable evolutionary model for the data and the parameters were used to perform maximum-likelihood analysis in PAUP*. Because computing time can become problematic with increasing sample sizes, common haplotypes were submitted as a single representative sequence in these analyses. Sequences containing both cyt *b* and d-loop data for other *Myodes* species were not available from the published literature, therefore it was not possible to root trees with a closely related species as an outgroup.

The genetic structure of populations was investigated using analysis of molecular variance (AMOVA) implemented in the programme ARLEQUIN version 3.1 (Excoffier *et al*. 2005). This analysis uses pairwise distances to examine sequence variation within predefined groups, and the F-statistic analogues produced describe variation at three hierarchical levels; between groups (Φ_{CT}), between populations within groups (Φ_{SC}) and within populations (Φ_{ST}). In this instance the analysis was used to describe variation between geographically delimited groups consisting of; (i) Welsh islands and mainland Wales (ii) Scottish islands and mainland Scotland (iii) Isle of Wight and southern England (iv) Channel Islands and mainland France. The significance of these parameters was estimated by 10,000 permutations of the distance matrix.

3.2.4. Network analyses

Traditional tree building methods, such as maximum likelihood and maximum parsimony, are extremely useful for reconstructing between-species phylogenies yet they are often inadequate for dealing with intraspecific divergence. This is because population data tend to violate several of the basic assumptions behind these models (Posada and Crandall 2001). Genetic distance will be much greater between individuals of different species than between conspecifics because long periods of reproductive isolation combined with mutation lead to multiple fixations of different alleles, resulting in a hierarchical genetic relationship. Conversely, intraspecific genetic relationships are often non hierarchical because of sexual reproduction and the occurrence of recent mutations, which often lead to low levels of sequence variation, the persistence of ancestral haplotypes in the population, as well as multiple

decendants of a single ancestral haplotype. Thus, in these instances, genealogies are best represented by multifurcating trees, commonly produced using network analysis techniques, rather than the bifurcating trees produced by traditional tree building methods (Huson and Bryant 2006).

There are several network methods available for analysis of mtDNA sequence data (reviewed by Posada and Crandall 2001). The median-joining (MJ) network method was chosen to analyse the data from this study because it is an extremely fast, user-friendly method that can easily handle large data sets. In addition, in a critical evaluation of several of the most commonly used network tree-building methods, Cassens *et al*. (2003) found the MJ network produced the most parsimonious tree for cyt *b* data in dolphin populations. Furthermore, previous phylogeographic studies of bank voles have also employed the MJ network method to analyse mtDNA (Deffontaine *et al*. 2005, Stuart *et al*. 2007) making findings between studies much easier to compare than if an alternative method had been employed.

MJ networks were constructed for the cyt *b* and d-loop data combined using the programme NETWORK version 4.2.0.1 (available at www.fluxus-engineering.com; Bandelt *et al.* 1999). Unlike the maximum parsimony and maximum likelihood analyses, the MJ method is better able to handle sequence data containing haplotypes common to more than one animal and thus the complete set of sequence data were analysed. In an attempt to further resolve relationships within the resulting network, an additional 47 previously published bank vole cyt *b* sequences were downloaded from the GenBank database (Deffontaine *et al*. 2005, Kotlik *et al*. 2006) and compared to cyt *b* sequence data from this study (see Appendix Table 3.1 for

GenBank Accession numbers). To ensure minimal amounts of missing data were entered into the network analysis, cyt *b* sequences were restricted to 917 bp and 968 bp for comparisons with Deffontaine *et al*. (2005) and Kotlik *et al*. (2006) sequences respectively. This was an important step because the MJ analysis becomes increasingly unreliable as the amount of missing data increases.

3.2.5. Molecular clocks

Following methods described in Deffontaine *et al*. (2005), the percentage of genetic divergence was used to calculate an approximate divergence time between the two major phylogroups. A matrix of percentage distances between non-identical cyt *b* sequences was generated in PAUP* and the corrected distances (according to the K_3P model suggested by MODELTEST) were used to calculate mean genetic percentage divergences within and between phylogroups. The following formula was used to correct for ancestral mtDNA polymorphism:

$$P_{net} = P_{AB} - 0.5\ (P_A + P_B)$$

where P_{net} is the net genetic distance between phylogroups, P_{AB} is the mean genetic distance in pairwise comparisons of individuals from group A versus group B and P_A and P_B are mean genetic distances among individuals within each phylogroup.

The molecular clock rate of 3.6% K_3P distance per My (95% CI: 3.45-3.75%), calculated by Deffontaine *et al*. (2005), was applied to the data to estimate the divergence time between the two major phylogroups. These authors used relative-rate tests to confirm that bank vole cyt *b* sequences evolve in a clock-like manner and thus this analysis was not repeated.

3.3. Results

3.3.1. Haplotype diversity

74 polymorphic nucleotide sites were identified from the entire sequence of 1863 bp, resulting in 42 unique haplotypes among 69 individuals (Table 3.2). Of these variable sites, 50 were parsimony informative. The cyt *b* gene was slightly more variable than the d-loop locus with 4.12% and 3.56% of variable nucleotide sites within the amplified sequences respectively. The average transitions/transversions ratio was 8.26 and the base composition was A=0.30, C=0.275, G=0.13, T=0.29. Although several haplotypes were common amongst individuals within the sampled populations, no haplotypes were shared between populations when the entire sequence data were considered (Table 3.2). However, when only cyt *b* sequence data were analysed, some populations were found to share haplotypes; RM4 was common to four animals from Ramsey and all six animals sampled on Skomer Island, and haplotype SU3 also was found in two Morvern animals (Fig. 3.5, Table 3.2). When the cyt *b* sequence was restricted to 917 bp for comparison with published data, haplotype SU2 from Surrey was shared by two animals from Raasay and the BELG2 haplotype was sampled in both Surrey and France and present in Belgium from the Deffontaine *et al*. (2005) study (Fig. 3.5).

3.3.2. Phylogenetic analysis

The tree recovered from the maximum parsimony analysis (rooted using a default outgroup) along with bootstrap support for each node is shown in Fig 3.1. Unsurprisingly, the most strongly supported nodes tended to be those grouping

samples from the same populations. Although support for many of the other basal nodes was low (bootstrap values <50%) there was 100% support for the phylogroup containing the Welsh, Jersey, French and Bedfordshire samples, along with one haplotype sampled in Surrey (depicted in blue Fig. 3.1). However, relationships within this group were far less certain.

Maximum likelihood analyses for the combined cyt *b* and d-loop data were performed using the HKY85 + I + G model of sequence evolution suggested by the hierarchical likelihood ratio tests in MODELTEST version 3.7, with a proportion of invariable sites of I = 0.855, a gamma distribution shape parameter of $\alpha = 0.886$ and without a molecular clock enforced. The single most parsimonious tree (rooted using a default outgroup) did not differ significantly in appearance from the maximum parsimony tree (Fig 3.1, Appendix Fig. 3.1). When the tree was unrooted, the analysis uncovered two distinct groups and produced a tree that was very similar in appearance to that produced by the median joining (MJ) network analysis (Fig. 3.2).

Table 3.2: Geographical locations and sample abbreviations for bank vole haplotypes sampled during this study. Frequency of haplotypes for cyt *b* and d-loop concatenated data and cyt *b* data only are given separately.

Country	Site	Abbreviations	No. of animals with haplotype	
			Cyt *b* and d-loop	Cyt *b* only
Wales	Skomer	S1	4	
		S3	1	
		S5	1	
	Ramsey	RM1	1	1
		RM2	2	2
		RM4	4	10 (6 Skomer)
	Mainland Pembrokeshire	WP1	1	2
		WP2	1	
		P1	1	4
		P2	1	1
		P3	1	1
		P4	3	
Channel Islands	Jersey	J1	3	3
		J3	1	1
		J4	1	1
		J5	1	1
France	Dol-de-Bretangne	F1	2	2
		F4	3	3
		F3	1	1
England	Bedfordshire	B1	2	2
		B2	2	2
		B3	1	1
		B5	1	1
	Surrey	SU1	1	1
		SU2	1	1
		SU3	1	3 (2 Morvern)
		SU4	1	1
		SU5	1	1
		SU6	1	1
	Isle of Wight	IW1	3	1
		IW2	1	3
		IW3	1	2
		IW4	1	
Scotland	Morvern (mainland)	MV1	2	
		MV3	1	3
		MV6	3	1
	Mull	M1	3	5
		M2	2	
		M6	1	1
	Raasay	R1	3	4
		R3	2	2
		R5	1	

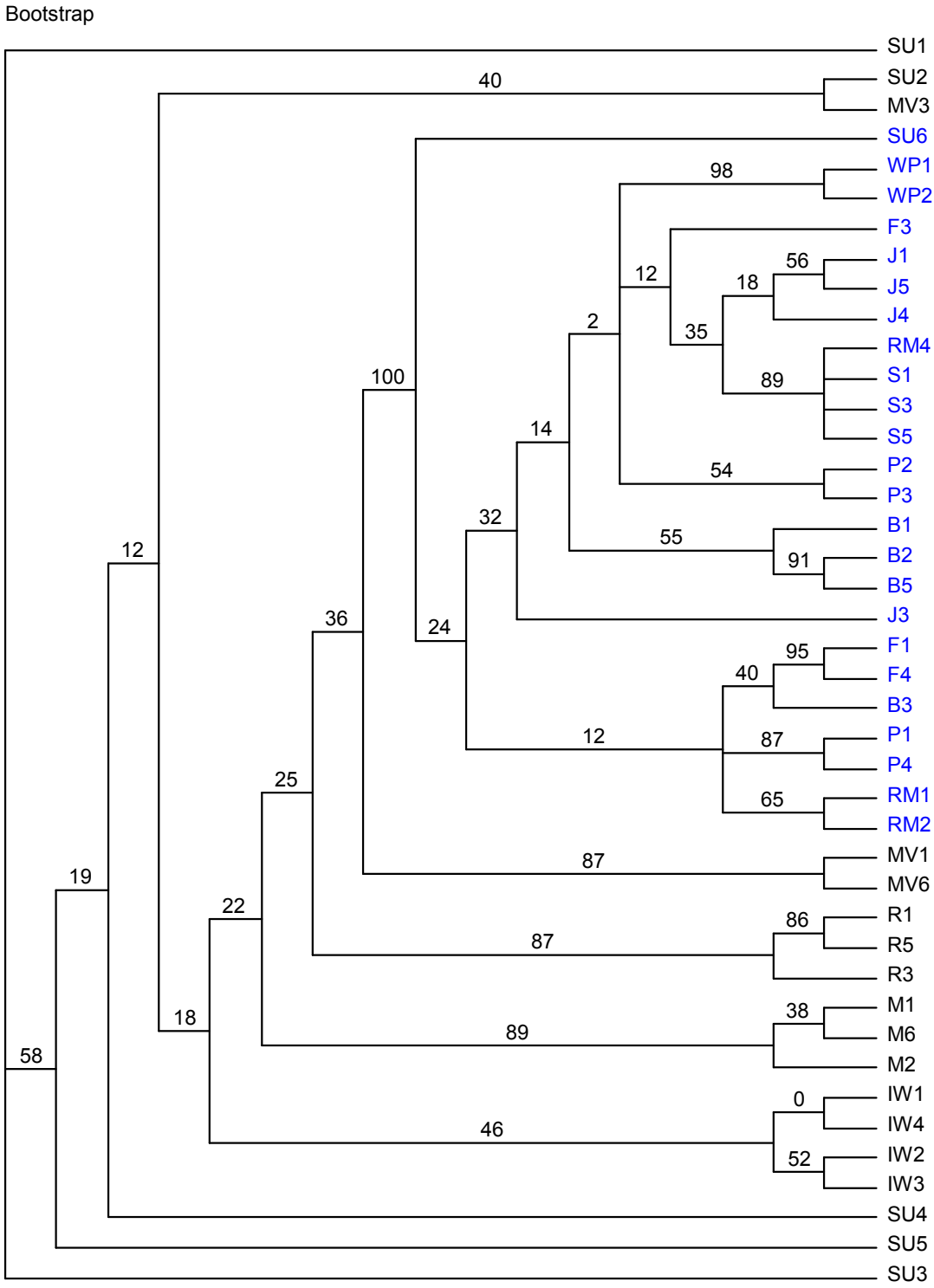


Figure 3.1: Maximum parsimony tree showing the phylogenetic relationships between 42 bank vole mtDNA haplotypes (for concatenated cyt *b* and d-loop data). Numbers on nodes are the bootstrap support values for clades. Haplotype abbreviations are from Table 3.2. Haplotypes shown in blue are supported as a clade with 100% bootstrap support.

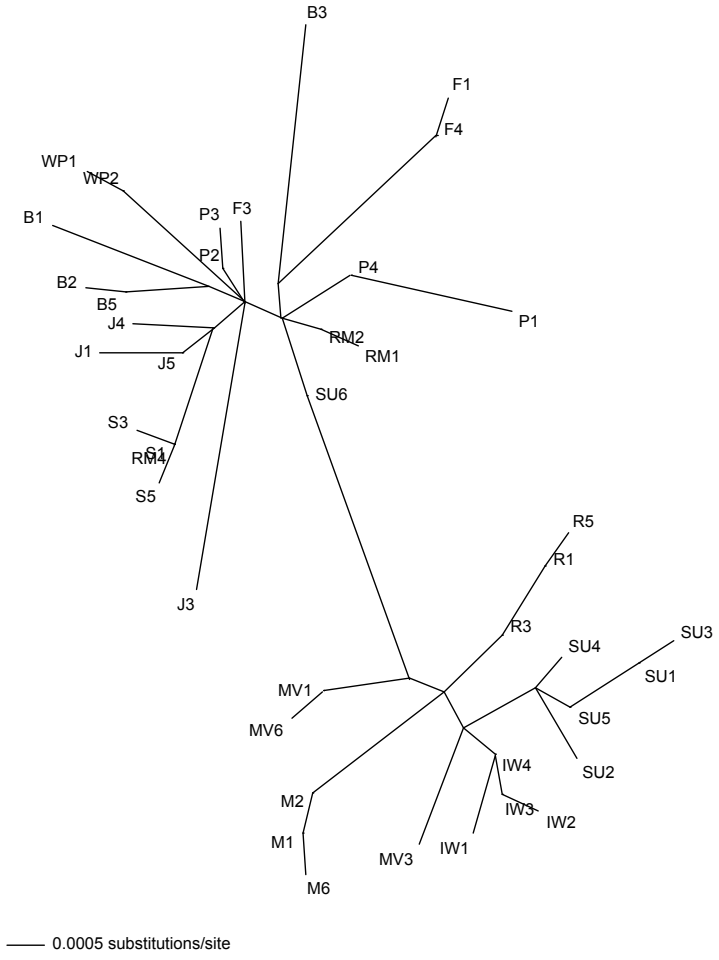


Figure 3.2: Unrooted maximum likelihood tree showing the phylogenetic relationship between 42 bank vole mtDNA haplotypes (for concatenated cyt *b* and d-loop data). Haplotype abbreviations are from Table 3.2. The phylogram was generated in PAUP* using a HKY85 + G + I model of sequence evolution (see text) with molecular clock not enforced, tree score = 3247.2.

3.3.3. Phylogeographic analysis

The MJ network built using the entire sequence length of 1863 bp revealed two separate lineages of haplotypes with the inferred basal haplotypes of each group separated by at least nine mutational steps (Fig 3.3). Group one showed a star-like phylogeny containing haplotypes from Wales (Ramsey, Skomer, mainland Pembrokeshire), Jersey, France and Bedfordshire and one haplotype sampled in Surrey (the same group recovered by the maximum parsimony analysis). Group two

contained the Scottish samples (Mull, Raasay and Morvern), the majority of haplotypes sampled in Surrey and all haplotypes from the Isle of Wight (Figure 3.3). In this analysis the frequencies of haplotypes that are common to more than one animal are depicted by increasing node size and the small red nodes (median vectors) represent hypothetical intermediate haplotypes (i.e. haplotypes 'missing' from the data set either because they were not sampled or have become extinct from the population). Interestingly, the analysis revealed there were no 'major' haplotypes sampled; none of the sampled populations contained the inferred ancestral haplotypes presumed to be basal to the two separate lineages (represented by median vectors in Fig. 3.3) and no haplotypes were common between sites.

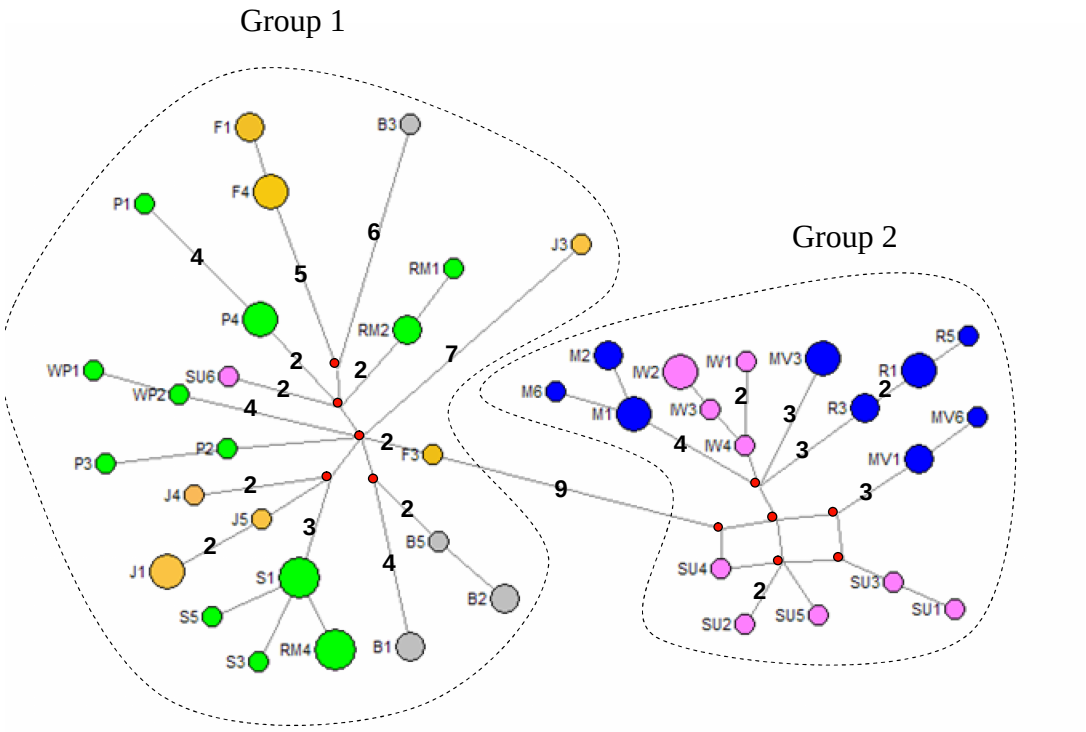


Figure 3.3: Median-joining network tree of bank vole mtDNA haplotypes (for concatenated cyt *b* and d-loop sequence data). The size of the node is proportional to the frequency of haplotypes. Numbers of mutations (>1) are indicated on branches. Red nodes are hypothetical intermediate haplotypes. Haplotype abbreviations are from Table 3.2. Nodes are coloured according to geographic distribution; Wales - green, Scotland - blue, Channel Islands and France – orange, southern England – pink, Bedfordshire – Grey.

Several of the island populations contained a small number of closely related haplotypes whereas there tended to be a greater mix of more divergent haplotypes present within mainland sites (with the exception of the Morvern population). However both Ramsey and Jersey islands contained groups of relatively divergent haplotypes, separated by at least eight and nine mutational steps respectively.

Whilst there was some geographically meaningful clustering to the network tree (e.g. the separate grouping of Welsh and Scottish haplotypes), there was little evidence to support the theory that island haplotypes were more closely related to haplotypes found on the nearby mainland than those from other populations. For example, haplotypes present on the Isle of Mull were at least seven mutational steps away from haplotypes sampled from the nearby mainland site of Morvern, but only a minimum of five mutational steps away from those sampled on the Isle of Wight. This lack of overall geographic trend was supported by results of the AMOVA (Table 3.3). When geographic groupings (island sites with nearest mainland; see Section 3.2.2) were submitted to the analysis, amongst population within group variation (Φ_{SC}) was far greater than between group variation (Φ_{CT}) (contributing 41.65% and 28.63% of the total variation respectively). Rearranging these groups did little to change these statistics except when Bedfordshire samples were omitted from the 'southern England' group, when among group variation decreased and between group variation increased (31.74% and 38.72% of the total variation respectively).

Table 3.3: Analysis of molecular variance (AMOVA) based on concatenated cytochrome *b* and control region mtDNA sequence data from geographical groupings of British bank voles.

Source of variation	Variance components	% variation	*P* value	Φ statistics
Among groups	1.777	28.63	<0.02	Φ_{CT}=0.286
Among populations within groups	2.585	41.65	<0.001	Φ_{SC}=0.584
Within populations	1.845	29.72	<0.001	Φ_{ST}=0.286

When islands with named subspecies of voles were mapped onto the MJ network (Figure 3.4), it was clear that the four island populations that show characteristics of island syndrome were not closely linked. In fact, the Raasay and Mull haplotypes appeared to be of a different lineage to those of the Jersey and Skomer haplotypes. Furthermore, both the Raasay and Mull haplotypes were more divergent from each other than they were to the haplotypes sampled from Isle of Wight. Interestingly the haplotype common to three of the voles sampled on Ramsey Island was genetically very similar to that of the commonest haplotype found on Skomer Island, with only one mutational difference.

Bandelt *et al*. (1999) recommend exploring the sequence data in the MJ network by increasing the value of epsilon (which essentially increases the number of median vectors) when the maximum length of links is large or sampling is sparse. This should expose any homoplasies in the data set as well as breaking up large, but potentially misleading, sub-network blocks. Thus the data were reanalysed using a value of ε = 10, the minimum value of ε that effectively differs from ε = 0 for this data set. The network that was produced from this analysis was not substantially different in appearance to Figure 3.3 (Appendix Fig. 3.2); the two main groups identified by previous analyses were still supported and little information on the relationships of

haplotypes within these groups was added. However, vast numbers of median vectors were inserted in between the two main groups, corroborating the ambiguity of the phylogenetic relationship between the two lineages.

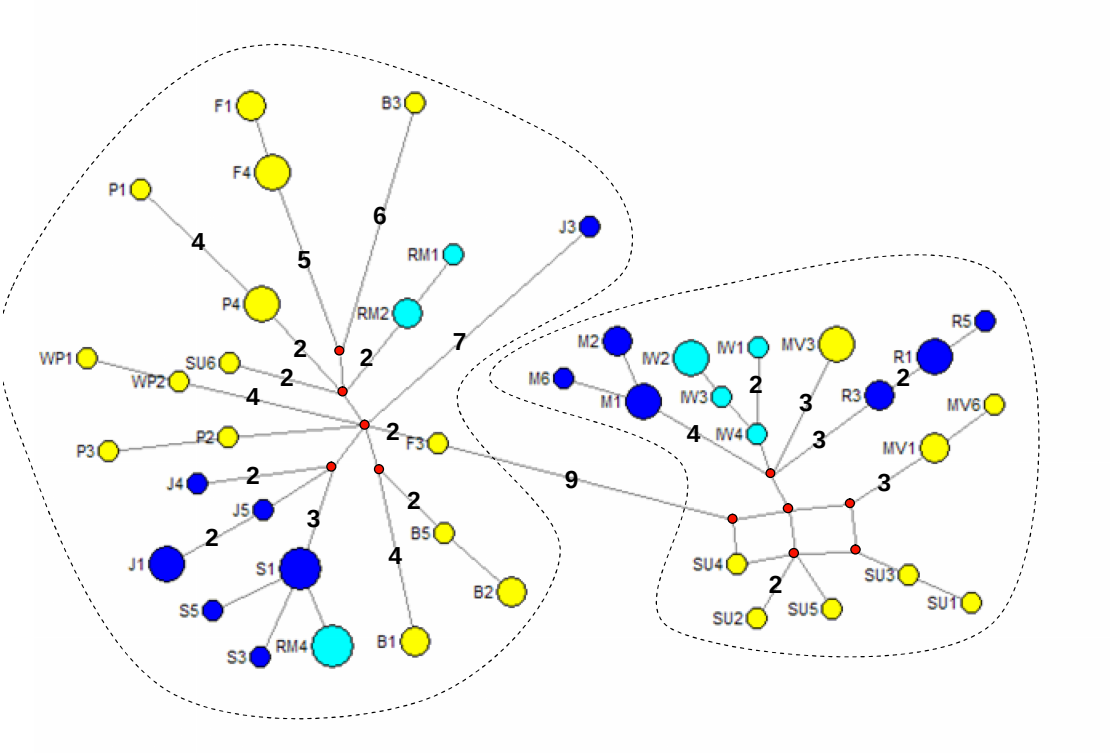


Figure 3.4: Median-joining network tree from Fig. 3.3 recoloured to show mainland populations – yellow, island populations with subspecies – dark blue, other island populations – turquoise.

When cyt *b* sequences from this study were combined with sequences from the published literature, MJ network analysis revealed sampled haplotypes were closer to those from the hypothesised western phylogroup than those from other proposed phylogroups (Eastern, Italian, Spanish, Balkan, Ural) as suggested by Deffontaine *et al*. (2005) (data not shown). MJ analysis of downloaded sequences from the western phylogroup failed to add much information about possible ancestral haplotypes of the British voles sampled during this study but the existence of the two phylogroups, as

suggested by previous analyses, was still supported (Fig. 3.5). Sampled haplotypes were also distinct from the Carpathian group suggested by Kotlik *et al*. (2006) (data not shown).

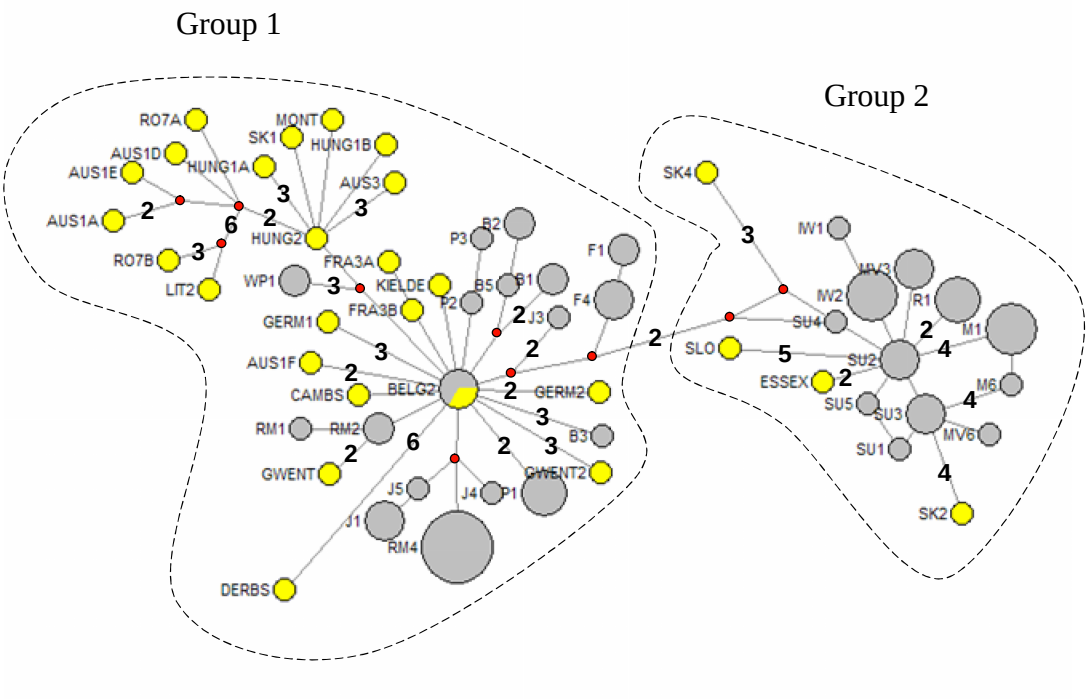


Figure 3.5: Median-joining network tree of bank vole mtDNA haplotypes (for 916 bp of cyt *b* gene), showing relationships between voles sampled during this study (in grey) and previously published sequences from the western phylogroup identified by Deffontaine *et al.* (2005) (in yellow). Haplotype abbreviations are from Table 3.2. for this study and given in Appendix Table 3.1 for published sequences. Group lines show phylogroups identified by previous analyses are still apparent even with a reduced data set.

3.3.4. Divergence time

When corrected for ancestral polymorphisms, the mean K_3P distance between the two major phylogroups for cyt *b* sequence data was 0.594% (Table 3.4). Using a molecular clock rate of 3.6% K_3P distance per My (95% CI: 3.45-3.75%), the divergence time between these phylogroups was estimated to be 165,000 years ago (95% CI: 158,000 – 172,000).

Table 3.4: Mean percentage genetic divergences within and between the two major phylogroups of bank voles sampled during this study. Corrected and uncorrected distances for concatenated and cyt *b* sequence data are given. P_a and P_b are within group distances, P_{ab} is between group distance and P_{net} is the net genetic distance between groups A and B (see Section 3.2.5).

	P_a	P_b	P_{ab}	P_{net}
Cyt *b*	0.449	0.333	0.953	0.562
Cyt *b* (K3P corrected)	0.458	0.328	0.987	0.594
Cyt *b* and d-loop	0.412	0.342	0.859	0.482
Cyt b and d-loop (HKY85 + I + G corrected)	0.442	0.361	0.971	0.570

3.4. Discussion

3.4.1. Precolonisation history of British voles

Previous phylogenetic analyses suggested that extant bank vole populations colonised most of Western Europe, including the British Isles from refugia in central Europe after the Last Glacial Maximum (Deffontaine *et al*. 2005, Kotlik *et al*. 2006). Data from this study provide further support for this theory but show that extant lineages present in the British Isles consist of two major phylogroups that split approximately 165,000 years ago. If this date is correct (see below for further discussion of the accuracy of molecular dating), the separation of the two lineages probably occurred during a glacial event during the Wolstonian Stage of the Pleistocene (Yalden, 1982). However, whether the two lineages survived the last glacial cycle in the same or different glacial refugia is less certain. The lack of mixed haplotypes (i.e. from both major phylogroups) in all populations except for Surrey would perhaps indicate separate 'waves' of colonisation from different refugia, with haplotypes of later colonisers replacing those before. This pattern has also been observed in British water voles (*Arvicola terrestris*). Piertney *et al*. (2005) showed that extant water vole lineages in Scotland seem to have arisen from an Iberian glacial refugium, whilst

those in England and Wales are probably derived from an eastern European lineage. They suggest that the two waves of colonisation were either separated spatially, with different routes into Britain or temporally, with the second wave of colonisers replacing the first. Alternatively, if haplotype frequency in bank vole lineages is maintained by some form of assortative mating, then theoretically the same phylogeographic pattern could be observed in British populations even if the two lineages expanded from a single glacial refugium.

Deffontaine *et al*. (2005) and Kotlik *et al*. (2006) suggest southern France, northwestern Italy, the western Balkans and the Carpathian region as possible glacial refugia for the western phylogroup. This hypothesis is supported by the findings of bank vole remains in the northern Carpathians and northern Moldovia, which have been dated to the height of the LGM (Nadachowski *et al*. 2003 in Deffontaine *et al.* 2005, Pazonyi 2004). The lineages sampled during this study were distinct from the Carpathian group identified by Kotlik *et al*. (2006), which were hypothesised to have split from the rest of the western lineage at the height of the last glacial cycle. However, in a more recent study, the Carpathian lineage was not supported as a monophyletic group and phylogenetic analyses included this group in the Eastern lineage, rather than a distinct clade within the western lineage (Deffontaine *et al.* 2009). Thus the ancestors of the British voles sampled are unlikely to have survived in the same refugium as this group, but may have been isolated in nearby refugia.

Fløjgaard *et al*. (2009) used modern day species distributions to model likely glacial refugia for several European rodent species during the LGM according to suitable climatic conditions. The power of the models was assessed by comparing predicted

distributions of extant populations in Siberia with actual distribution data and then models with the best predictive power were selected for LGM projections. For bank voles, Fløjgaard *et al*. (2009) predicted that large areas of southern central Europe, including southern England, stretching to the northern Balkans would have been climatically suitable. The discrepancy between phylogenetic data and predicted suitable refugia may be simply a consequence of the uncertainty of climatic conditions during the LGM. However, the models assumed bank voles are a boreal species. Whilst this species' current range does include cold climates with coniferous forests (e.g. in Fennoscandia), the majority of its range lies in temperate regions (Shore and Hare 2008). Thus it is possible that the population ancestral to the western lineage were temperate dwelling and consequently not so well adapted to cold climates. The refugia suggested by Deffontaine *et al*. (2005) and Kotlik *et al*. (2006) included regions that would have contained mixed coniferous and deciduous forest (Willis *et al*. 2000) and therefore may have been eminently more suitable locations for voles to survive the coldest epoch of the LGM.

3.4.2. Accuracy of molecular clocks

Using a calibration point of an estimated separation time of 4.25 Mya between *Myodes rufocanus* and *M. glareolus* (from Matson and Baker 2001), Deffontaine *et al.* (2005) calculated a molecular clock rate of 3.6% K_3P distance per My for the bank vole cyt *b* gene. They used this rate to calculate the divergence time between European bank vole lineages, producing an estimate of 0.27 Mya. However, there is potentially a large problem with using such estimations to time the divergence between extant bank vole lineages because the molecular clock rate is not linear and changes with time. Studies that calculate molecular clock rates on timescales of <1

million years can produce rates an order of magnitude higher than those calculated over longer timescales (Ho and Larson 2006). This problem arises because mutation rates, defined as 'the instantaneous rate at which nucleotide changes occur in the genome', tend to be much faster than substitution rates, defined as 'the rate at which mutations are fixed in the population' (Ho and Larson 2006). This is because many of the mutations that occur within a genome are eliminated by selection or drift and therefore do not ever become fixed in the population. Thus, when molecular clocks are generated for timescales of less than 1-2 million years, it is generally the mutation rate that is calculated, whereas clocks generated for timescales over 1-2 million years are in fact using the substitution rate. The disproportion between mutation rates and substitution rates mean that the molecular clock rate calculated by Deffontaine *al.* (2005) is likely to greatly overestimate the genetic distance between the two phylogroups identified during the course of this study. This possibly hugely inflated the time divergence estimates between groups. Nevertheless, given the calculated divergence time of 165,000 years ago, even if the true divergence time is an order of magnitude lower than the estimated date, the balance of probability is that the split between these two lineages at least occurred before the last glacial cycle. Currently, no molecular clock rate has been published for the bank vole d-loop gene. However, it would be interesting to see whether divergence estimates from this region of mtDNA would produce a congruent timing to that of the cyt *b* gene.

One of the key findings of this study is that the four island populations said to show characteristics of island syndrome have evolved from two entirely separate mtDNA lineages, probably separated by over 100,000 years of evolution. Therefore, the occurrence of island syndrome in British bank voles can clearly not be explained

simply by phylogeographic history. This finding is unsurprisingly in agreement with Corbet's argument against the relict theory proposed by Hinton and Barrett-Hamilton (1913 in Corbet 1961). Moreover, these data suggest that morphological traits such as increased body size have evolved relatively rapidly in island populations. Such rapid changes have previously been shown to occur in mammals. For example, Lister (1989) estimated that red deer (*Cervus elaphus*) on Jersey shrunk to one sixth of their original body weight over a period of less than 6000 years. The house mice (*Mus musculus*) of the Faroe archipelago appear to have developed their distinct genetic, morphometric and skeletal traits in under 1000 years (Berry, 1992). Furthermore, the body size of white tailed deer (*Odocoileus virginianus*) released onto Anticosti Island, Canada has diminished by 50% in just over 100 years (Simard *et al*. 2008).

3.4.3. Haplotype diversity amongst British voles

Haplotype diversity amongst British bank voles was relatively high in that no haplotypes were discovered that were common to more than one population when the entire sequence data set was considered. These findings are in contrast to other mtDNA studies of British small mammals (stoats - Martínková *et al*. 2007, shrews - White and Searle 2008, water voles - Piertney 2005). Whilst this may partially be an artefact of using concatenated sequence data from both the mtDNA cyt *b* gene and the d-loop gene, diversity was still relatively high when only cyt *b* data were considered. This level of haplotype diversity is almost as great as that experienced by the western phylogroup across its entire range. Deffontaine *et al*. (2005) sampled 64 bank voles from 22 sites across 12 countries and detected 36 unique haplotypes from sequencing 1011 bp of the cyt *b* gene. In this study, 993 bp of cyt *b* yielded 30 unique haplotypes from 62 British animals across 10 sites, with a further three haplotypes discovered

from the six French animals (Table 3.2). However, perhaps this level of diversity is to be expected when sampling insular populations that may have been isolated from mainland conspecifics for long periods of time. Sampling on the mainland was largely restricted to coastal sites near to islands populated by voles, thus a less sporadic sampling regime may have yielded the 'common' haplotypes presumed to be basal to the two lineages.

The complex mtDNA phylogeny of British bank voles, as revealed by the MJ network analysis, is more characteristic of a naturally colonizing population than a population that has been introduced by humans (Martínková *et al*. 2007) and is consistent with land bridge colonisation from Europe. For example MJ network analysis of mtDNA sequences from pygmy shrews in Ireland produced a much simpler star-like phylogeny with two major haplotypes that were sampled in multiple locations (McDevitt *et al*. 2009). Previous genetic evidence had already suggested that pygmy shrews probably arrived in Ireland via boat from southwest Europe rather than coming across a land bridge from mainland Britain (Mascheretti *et al.* 2003). The Irish pygmy shrew phylogeny constructed by McDevitt *et al*. (2009) supports this theory because it is typical of introduced populations. These populations tend to proliferate from few founding members resulting in a starlike phylogeny, indicative of rapid population expansion (Wang *et al*. 2004).

3.4.4 Colonisation of offshore islands by bank voles

Unfortunately, the absence of clear geographic structuring within the two major lineages makes it impossible to discuss the probable origins of most of the island populations without resorting to wild speculation. However it is extremely likely that

that all island populations (with perhaps the exception of Jersey) are derived from populations on the British mainland. The lack of well supported clades within the two major phylogroups also made it fruitless to attempt to time the divergence between island and mainland populations. Thus, analyses of mtDNA sequences were neither able to definitively confirm or refute the theory that most island populations are the result of human-mediated introductions. When the entire sequence data were considered there were no haplotypes common to more than one population and when cyt *b* data only were analysed there was still very little geographic signal in the data. This absence of strong geographic signal probably resulted from a loss of haplotypes during the LGM followed by rapid population expansion from glacial refugia, as discussed by Deffontaine *et al*. (2005).

The pattern observed in insular populations of British bank voles is less clear than the phylogeographic pattern observed by White and Searle (2008) in Hebridean populations of common shrews. These authors found that while most sampled haplotypes were generally endemic to specific islands, the majority appeared to have evolved from one of the haplotypes sampled on the mainland. They hypothesised that shrews colonised the Hebridean islands via ice bridges at the end of the LGM *c*. 11,000 years BP. The shrews on Raasay were presumed to have come directly from the mainland via ice bridges but may have 'island-hopped' from neighbouring Skye. However, this is an unlikely route for the passage of voles onto Raasay because extant lineages are presumed not to have survived this period in Britain (Yalden, 1982). Thus it is probable that any such ice bridges had melted by the time voles expanded into Scotland. Furthermore, natural colonisation across ice bridges would undoubtedly have led to colonisation by field voles first (able to exist in more northerly climes) and

these species are not present on Raasay. The absence of bank voles of neighbouring Skye (which is geographically much nearer to the mainland) also argues against the theory of natural colonisation from the mainland.

Similarly, the absence of field voles on Skomer, Jersey and Ramsey suggests that bank voles on these islands did not colonise via land or ice bridges. Furthermore, whilst other 'natural' events (e.g. swimming or rafting) cannot be completely ruled out as a colonisation source, the frequent human habitation of these islands from the Neolithic period onwards would suggest that human-mediated introduction still remains the most likely route of bank vole colonisation onto these islands. Conversely, the presence of *Microtus* on the Isle of Wight and Mull indicate that colonisation by bank voles may have occurred via land bridges. Unfortunately, analyses of mtDNA sequences failed to provide sufficient data to address this hypothesis further.

Unlike most of the other islands, and contrary to the typical pattern observed on islands (Berry 1986), Jersey and Ramsey Islands contained a mix of genetically distant haplotypes. This pattern could arise through three potential scenarios; (i) colonisers of mixed haplotypes reached the island at the same time and neither lineage was eliminated through drift; (ii) the islands have been subjected to more than one wave of colonisers and rather than displacing existing haplotypes, the latter managed to coexist; (iii) haplotypes rapidly evolved on these islands from a basal haplotype that was either not sampled during the course of this study or is now extinct. Given the level of genetic variation present throughout Britain and the western lineage (Deffontaine *et al*. 2005) the third scenario is extremely unlikely and as such does not

warrant further discussion. Regarding the first two hypotheses, on a small island such as Ramsey (*c*.2 km^2), both scenarios would suggest relatively recent colonisation events because genetic drift is likely to have a greater effect on smaller populations, thereby causing the extinction of one lineage given enough time (Frankham 1998). However, in the case of Jersey (112 km) the first scenario would perhaps indicate natural colonisation of the island from mainland France, because survival of two distinct haplotype lineages becomes increasing unlikely with the small founding population size usually associated with human-mediated introduction events. However, land bridge colonisation is not consistent with the lack of presence of other small mammal species (e.g. field voles) on the island and given the habitat preferences of *Microtus* it seems unlikely that *Myodes* would survive whilst the latter subsequently became extinct (Yalden 1982). Thus, the more likely scenario is that bank voles were later introduced to Jersey, aided by human transportation, and possibly more than once. Furthermore, the close relationship between haplotypes present on Jersey and those in the rest of the western phylogroup unsurprisingly argue against the survival of this species *in situ* during the LGM.

The relationship between Skomer and Ramsey was probably one of the most interesting findings of this study and warrants particular attention. Four of the voles sampled on Ramsey exhibited a haplotype very similar to that of the Skomer voles, whilst three other individuals showed much more distant haplotypes. The lack of similar haplotypes on the mainland may indicate that transfer of voles has occurred at some point between the two islands. This is entirely possible because it seems both islands were rented in the 1800's by John Summers and his descendents for farming purposes (http://www.rosemoor.com/IPFiles/Nationalpark/skomer.html). Whilst bank

voles are not typically commensal species, the accidental transport of voles in amongst farming materials is not unlikely and commensal introduction to Ireland has already proven the most likely route for this species (Stuart *et al*. 2007). Alternatively, Ramsey and Skomer may have been colonised by similar ancestral haplotypes that have now become extinct on the mainland (or were not sampled during the course of this study) and a subsequent wave of colonisation brought additional divergent haplotypes to Ramsey Island.

Moreover, the genetic similarities between Skomer and Ramsey voles raises the major question, if both populations have the same genetic propensity, why do Skomer voles exhibit island syndrome when Ramsey voles do not? Differences in selective pressures operating on the islands in relation to the possible causes of island syndrome are discussed in Chapter 8, however it would be interesting for future research to use polymorphic nuclear DNA loci (microsatellite variation) to see how genetically close the two populations are and whether gene flow has occurred between the islands within the last 200 years as hypothesised. Analysis of nuclear markers may also help to further resolve relationships between other insular and mainland populations throughout the British Isles (particularly if better sampling coverage of the mainland was achieved) thus answering some of the questions left outstanding by this study.

3.5. Summary

1. This study investigated the phylogeographic origins of six insular populations of bank voles present on the small islands surrounding the British coastline.
2. Using sequence variation from the mtDNA cytochrome *b* and control region genes, phylogenetic trees for these populations and nearby mainland populations were constructed using maximum likelihood, maximum parsimony and median-joining network analyses.
3. Two major phylogroups were uncovered by these analyses and these lineages were estimated to have diverged approximately 165,000 years ago. The correctness of this timing and the use of molecular clocks for recent genetic divergence events (<1 million years) are discussed.
4. The data support hypotheses of postglacial expansion into Britain and the surrounding offshore islands from central European glacial refugia.
5. The insular subspecies of Mull and Raasay were found to belong to a separate lineage to those of Skomer and Jersey, clearly demonstrating that phylogeographic history alone cannot account for the occurrence of island syndrome in these populations.
6. The lack of clear geographic sub-structuring within the phylogenies made it difficult to pinpoint the origins of island populations but similarities between haplotypes on Ramsey and Skomer indicated possible, recent (*c*.200 years ago) transference of animals between these islands.
7. Whilst analyses of mtDNA sequences were not able to directly confirm the theory that most island populations are the result of human-mediated introductions, the lack of *Microtus agrestis* on Raasay, Skomer, Jersey and

Ramsey suggests that bank voles on these islands did not colonise via land bridges.

8. Analysis of polymorphic nuclear loci is recommended for future research to help answer unresolved questions about the possible origins of these populations.

Chapter 4: Population biology

4.1. Introduction

Much research has been carried out on the population biology of bank voles, both in Britain and Europe (Alibhai and Gipps 1985, Crespin *et al.* 2002, Stenseth *et al.* 2002 and reviewed by Shore and Hare 2008). Populations in northern Europe tend to exhibit multiannual cycles of 3-5 years duration (Huito *et al.* 2004), whereas British and central and southern European populations exhibit annual cycles (e.g. Petrusewicz 1983, Alibhai and Gipps 1985). Annual cycles vary, but often exhibit a spring decline phase, and an autumn/early winter increase phase (Alibhai and Gipps 1985). Densities vary throughout the annual cycle depending in particular on food availability and habitat and may reach densities of ~70 voles/ha on mainland Britain (John Gurnell pers. comm.). In contrast, densities of voles on Skomer have been reported to reach 475 voles/ha (Healing *et al.* 1984), and generally, small mammal populations living on islands frequently differ in demography, reproduction and behaviour compared to mainland populations (Adler and Levins 1994). Therefore, I made a comparative live trapping study of bank voles on four islands (Skomer, Mull, Raasay and Jersey) and the mainland (Orielton) to provide insights into the reasons why island syndrome occurs. In particular, I test the following hypotheses:

1. Island vole populations exhibit higher densities than mainland vole populations;
2. Increased vole densities on islands result from density compensation (i.e. total rodent biomass within island and mainland populations is approximately equal) rather than excess density compensation (i.e. total rodent biomass is greater within island populations than mainland populations);

3. Island vole populations exhibit higher survivorship than mainland vole populations;
4. Island vole populations have shorter breeding seasons than mainland vole populations;
5. Island voles are more catchable than mainland voles because they are less aggressive (Adler and Levins 1994), less fearful and have lower predation risk.

These traits are predicted to increase with island isolation and to decrease with island area (Adler and Levins 1994).

With respect to Skomer, previous research has suggested that Skomer voles are closely linked to the presence of bracken (Fullager *et al*. 1963, Healing *et al.* 1983). However, the distribution of bracken on the island is patchy. Thus, in 2005/6 I designed a study using sets of small grids of traps to monitor vole densities and movements between the patches to see how the absence of bracken affected the animals and their population dynamics and thus may potentially affect dispersal. As a control, a similar set of small grids was established in continuous bracken habitat the following year. The specific hypotheses I addressed were:

1. Movement of voles is reduced in patchy habitat and the absence of bracken restricts movement between patches;
2. Patchy habitats support fewer voles than continuous habitat (Healing, 1984);
3. Animals tend to disperse out of suboptimal habitats;
4. Survival is lower in patchy habitats.

4.2. Methods

4.2.1. Trapping protocol

4.2.1.1. Monitoring trap grids

Live-trapping grids were established at the four island sites and Orielton on the mainland in Pembrokeshire in areas of habitat deemed to be most suitable for bank voles (see Chapter 2 for full site details). Wherever possible, trapping grids were set up as 60 x 60 m 'moving' grids (see Page 89) with 10 m spacing between the main points. However, due to habitat constrictions, this 60 m x 60 m formation was not possible in all sites. Table 4.1 gives the exact dimensions of each grid with the effective trapping area allowing a 5 m boundary strip.

Live trapping was conducted over a two-year period from November 2005 to November 2007. In order to estimate pre-breeding season and post-breeding season densities, consecutive visits to all five sites were made in spring and autumn of 2006 and 2007. For logistic reasons it was only possible to visit three of the five sites in autumn 2005. Additional visits were made to Orielton and Skomer in early and late summer of 2006-2007. Full details of trapping sessions are given in Table 4.2.

Grids were trapped for four consecutive days and checked twice a day, shortly after dawn and again before dusk, apart from the 4th day when there was no evening trap round. On four occasions, time constraints prohibited a full trapping session thus only five or six rounds were conducted instead of the typical seven (Table 4.2). On one further occasion (Orielton, July 2007) trapping was abandoned on the morning of the 3rd day after 25% of the traps were found broken open, probably by a fox.

Table 4.1: Trapping grid dimensions for each field site. Total grid area allowing a 5 m boundary strip is given. For Skomer experimental grids (E1 and E2) dimensions of individual mini-grids are given along with total grid area.

Site	Grid size (m)	Grid area (m^2)
Jersey	65 x 65	5625
Mull	55 x 75	5525
Raasay (1)	35 x 45	2475
Raasay (2)	35 x 45	2475
Orielton	65 x 65	5625
Skomer T	65 x 65	5625
SW	45 x 35	2475
SP	25 x 25	1225
SR	70 x 0	800
SB	15 x 45	1575
SGRN	15 x 45	1575
SGRY	25 x 25	1225
SY1	25 x 35	1575
SY2	25 x 35	1575
Skomer E1		12025
SGRN2	15 x 45	1575
SR2	15 x 35	1375
SY2(2)	15 x 35	1125
SY1(1)	15 x 35	1125
SW2	35 x 25	1575
SP2	25 x 25	1225
SGRY2	25 x 25	1225
SB2	15 x 45	1575
Skomer E2		10800

Two Longworth live capture traps (Penlon Limited, Abingdon, Oxfordshire; Chitty and Kempson 1949) were placed within 1 m of each trap point on the grids. A moving grid system was employed, meaning that traps were moved 5 m at the end of each day in a north, south, east and west direction respectively (giving a total effective grid area of 65 x 65 m on a 60 x 60 m grid). This method reduces the effects of trap position on captures (Stanford 1995), as well as providing finer scale resolution when looking at trap-revealed movements of individuals (Gurnell & Gipps

1989). In compliance with the Wildlife and Countryside Act 1981, nest boxes of traps were fitted with 12 mm holes to allow shrews to escape. Traps were baited with whole oats or wheat and fresh carrot, and hay was provided for bedding material. Traps used on Jersey were not fitted with 'shrew holes' so blowfly pupae were also provided as a food source for these animals. Traps that were transported between sites were washed after each trapping period with sterilisation fluid and then thoroughly rinsed. Although chemical cleaning of traps may discourage some animals from entering (Gurnell and Flowerdew 2006), this approach was deemed necessary to safeguard against the spread of disease to potentially sensitive island populations.

Captured animals were weighed to the nearest 0.5 g with a Pesola spring balance, sexed, and their breeding condition noted. Females were recorded as imperforate, perforate, lactating, pregnant (if embryos were palpable) or have bred. Males were recorded as having abdominal testes or scrotal testes of small, medium or large size. To enable identification of previously caught animals within a trap period; wood mice and field voles were given a generic fur-clip. Bank voles were marked with a uniquely numbered ear tag (Mitchel Surgical Staples; Le-Boulange-Nguyen and Boulenge 1986) inserted through the tragus. A 2 mm tissue sample was taken from the ear of small number of animals from each site (and all animals caught on grids Skomer E1 and E2) using a sterile biopsy punch (Steifel Laboratories, High Wycome, Buckinghamshire). This procedure was carried out under Home Office Project Licence Number: PPL 70/6302. Tissue was stored in 70% ethanol.

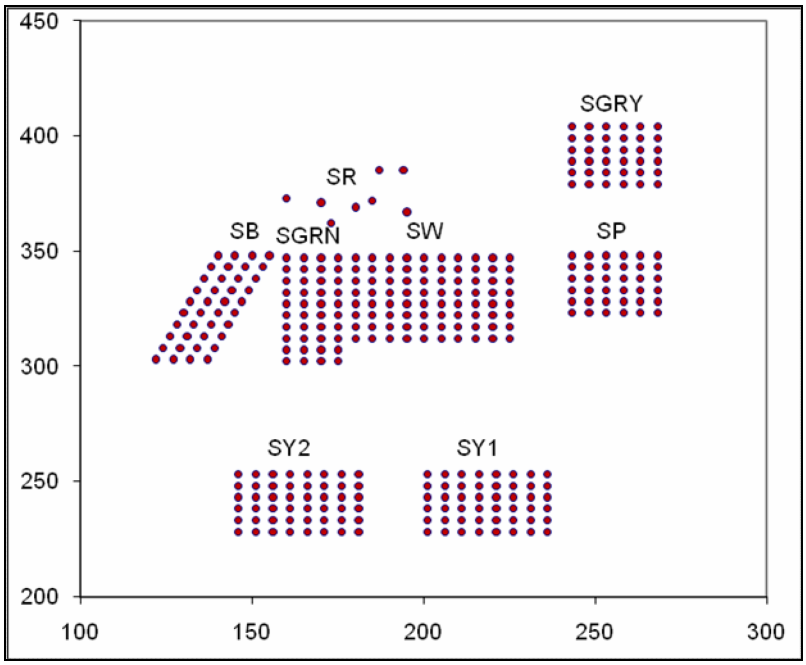
Table 4.2: Details of trapping sessions conducted during the 2005-2007 field season.

Year	Season	Code	Site	Dates of trapping	No. trap rounds	No. trap nights
2005	Autumn	05-4	Jersey (1)	2nd-5th Oct	7	686
			Skomer T	9th-12th Oct	6	540
			Skomer E1	13th-19th Oct	14	2660
			Orielton	2nd-5th Nov	7	686
2006	Spring	06-1	Skomer T	21st-23rd Mar	5	490
			Skomer E1	21st-26th Mar	10	1900
			Orielton	30th Mar-2nd Apr	7	686
			Mull	24th-28th Apr	7	672
			Raasay	1st-4th May	7	560
			Jersey (2)	15th-18th May	7	686
	Early summer	06-2	Skomer T	5th-8th Jun	7	686
			Skomer E1	4th-9th Jun	10	1900
			Orielton	19th-22nd Jun	7	686
	Late summer	06-3	Orielton	12th-15th Aug	7	686
			Skomer E1	17th-23rd Aug	14	2660
	Autumn	06-4	Mull	5th-8th Sept	7	672
			Raasay	11th-14th Sept	7	560
			Jersey (2)	11th-14th Oct	7	686
			Orielton	17th-20th Oct	7	686
			Skomer T	24th-27th Oct	7	686
			Skomer E1	29th Oct-5th Nov	14	2660
2007	Spring	07-1	Jersey (2)	6th-8th May	7	686
			Mull	16th-19th Apr	7	672
			Raasay	23-26th Apr	7	560
			Orielton	3rd-6th Apr	7	686
			Skomer E2	26th-31st Mar	10	1280
	Early summer	07-2	Orielton	21st-24th May	7	686
			Skomer E2	28th May-2nd Jun	10	1280
	Late summer	07-3	Orielton	11th-13th Jul	5	490
			Skomer E2	16th-20th Jul	10	1280
	Autumn	07-4	Mull	12th-14th Sept	5	480
			Raasay	16th-19th Sept	7	560
			Jersey	4th-7th Oct	7	686
			Orielton	14th-17th Oct	7	686
			Skomer E2	22nd-29th Oct	10	1280

4.2.2. Experimental trap grids

In order to study the relationship between Skomer voles and bracken cover, two sets of experimental grids were established on Skomer Island in addition to the standard control grid (Grid T). Grid E1 was comprised of a series of small grids spanning an area of naturally patchy bracken habitat and was trapped during the 2005-2006 field season (Fig. 4.1a). Grid E2 was a replicate grid system (slightly modified to fit the habitat) established in an area of continuous bracken to enable direct comparisons and was trapped during the 2007 field season (Fig. 4.1b). These grids were trapped in two stages so that animal movement between areas would not be entirely restricted by the provision of traps. Half of the mini-grids were trapped for 3 or 4 consecutive days (5-7 trap rounds) and then traps were moved to the remaining mini-grids for a further 3-4 days. To avoid bias, the order in which mini-grids were trapped was also varied for each trapping period. For density and biomass analyses the total area of each set of mini-grids with a 5 m boundary strip were summed and treated as two single grids.

(a) Grids E1 2006



(b) Grids E2 2007

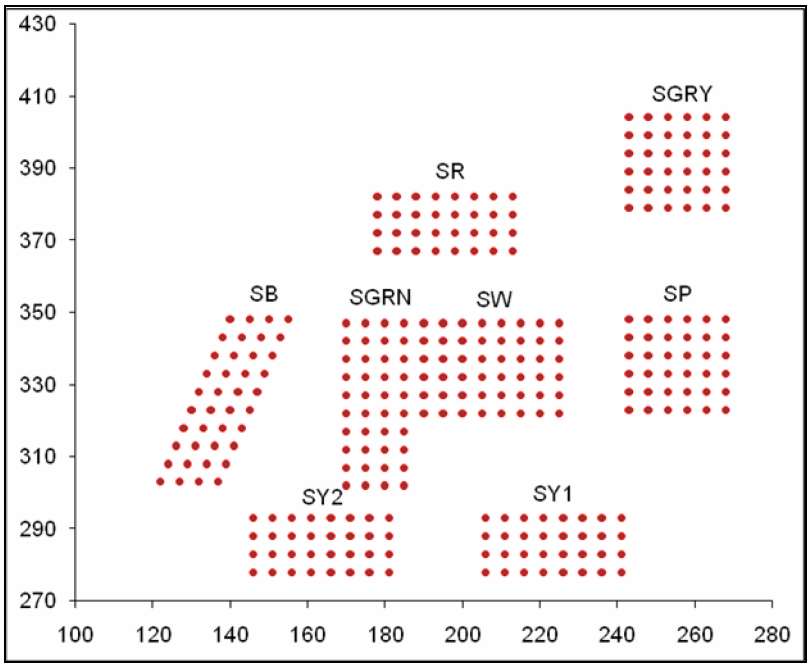


Figure 4.1: Layout of experimental grids on Skomer (a) Grids E1 (trapped 2006), (b) grids E2 (trapped 2007) - x, y coordinates in metres.

4.2.3. Estimates of density and biomass

Within small mammal populations, individuals do not all have the same probability of capture, and factors such as age, sex, breeding condition and social status have been shown to influence the probability of a given animal entering a trap (Montgomery 1987). Therefore, only a proportion of the population is likely to be captured during a given trapping period. Whilst there are several mathematical models available that aim to produce estimates of 'true population size' from capture-mark-recapture data (e.g. Jolly-Seber), the assumptions of these models are frequently violated during small mammals studies and the estimates produced are particularly unreliable when the number of captured individuals is low and recaptures are infrequent (Krebs *et al*. 1986). Thus, in this study, population size was estimated for each site and trapping period using the number of unique individuals captured (M_t) during trap period (t).

This method is considered more robust than the commonly used minimum number of animals alive (MNA), which also includes animals presumed to be present but not captured (i.e. those caught in previous and subsequent trapping sessions) (McKelvey and Pearson 2001; Pocock *et al*. 2004). M_t were converted into estimates of species number per hectare (ha^{-1}) allowing boundary strips of 5 m around all grids. Effective grid areas for each site are given in Table 4.1.

An estimate of bank vole biomass and total rodent biomass were produced for each site for each trapping period. Biomass values were calculated for each grid with a 5 m boundary strip, using the weights of individual animals at first capture and then converted to the standardised unit of grams per hectare. On the rare occasions where weight data were missing for an individual of a particular species and sex, mean weights for that site and trapping period were used for the estimation of population biomass.

In the instances where grids were only trapped for three days instead of four (Table 4.2), density and biomass values were adjusted using approximations of the number new animals likely to be caught on the fourth day. These data were estimated using the mean proportion of new animals captured on the fourth day for all other visits to that site, for each species.

During the 2007 field season on Skomer Island, equipment and time constraints prohibited the trapping of both the control grid (Grid T) and the experimental grids (Grid E2). Thus, density and biomass data for this site were taken from Grid T for 2005-2006 and Grid E2 for 2007. Only one individual was captured over 10800 m^2

on Grid E2 in March 2007, therefore density and biomass figures for spring 2007 were taken from the May 2007 trapping period instead. Because of the topography of the trapping area on Raasay, trapping was carried out on two grids (see Chapter 2). There was very little difference in numbers of animals caught on the two Raasay grids so data from the grids were combined for subsequent analyses.

4.2.4. Breeding seasons and sex ratios

The proportion of animals breeding during each trapping period was examined for all sites. Males were considered 'breeding' if they had enlarged scrotal testes (medium or large) and 'non-breeding' if they had small scrotal testes or abdominal testes. Females were recorded as 'breeding' if palpably pregnant or lactating, 'perforate' or 'non-breeding' if imperforate. Females that had obviously previously produced offspring but were not currently in breeding condition were excluded from this analysis (i.e. those that were not pregnant or lactating but had hairs plucked from around the nipples). Data from Skomer Grids T and E1 were combined for this analysis. Sex ratios of animals in spring 2006 and 2007 were examined with Chi-squared Goodness of Fit tests to see if they deviated significantly from 50-50 ratios.

4.2.5. Estimates of survival

The loss of marked animals between trapping periods may either result from movement away from the given trapping area or mortality of individuals. Thus the persistence of marked individuals within a given area may actually refer to survivorship or residency. However, because intervals between trapping events were relatively long during this study (> 2 months) it is probable that most marked animals

'lost' from the population suffered mortality and thus the persistence of marked animals between trapping periods is subsequently referred to as 'survival'.

Survival of voles between trapping periods was calculated for Orielton and the three Skomer grids. Animals that had lost ear tags were easily recognisable by a small tear in the ear tissue (these animals were subsequently re-tagged in the other ear). Animals with lost tags were assumed to have survived from the preceding trapping session and were included in the survival analysis. On the rare occasions when the animal ears showed signs of recent trauma, animals were assumed to have lost-tags within that trapping session and were not included in the survival analysis.

4.2.6. Vegetation

Surveys of the major vegetation types at each main trap point (10 m spacing) were conducted after each trapping period at each site. The percentage cover of the main species within a 1 m^2 quadrat was recorded at each point, along with the height of the leaves of the tallest ground layer of vegetation at the four corners of the quadrat (this included both dead and alive plants). Because of major time constraints during each trapping period, it was not possible to identify plants, such as grasses, down to species level. In order to estimate likely predation risk from avian predators, a 'vole-sized' piece of carrot was thrown randomly (over the shoulder) near each main trap point and the percentage visible from 1 m directly above was recorded. In sites where leaf litter provided an extensive layer of cover, the depth of this layer was also recorded and in particularly wet sites (Mull and Raasay), water level was also recorded on a scale of 1-4; where 1 = dry ground and 4 = water ≥ 1 cm above ground

level. Vegetation data were analysed with respect to the numbers of captures at each trap point.

4.2.6.1. Vegetation and density, Skomer experimental grids

To look at effects of vegetation on the distribution of small mammals on Skomer Island, densities of mice and voles occurring on the mini-grids constituting Grids E1 and E2 were examined within each trapping period and compared to vegetation cover. Densities were calculated for each mini-grid allowing a 5 m boundary strip (see Table 4.1 for effective grid area) and the number of unique individuals captured was converted into species density estimates per hectare. Bank voles caught on more than one grid were treated as independent animals. Mice were given generic fur clips and therefore only newly captured individuals could be included in this analysis. Vegetation data were averaged for each mini-grid and the percentage cover of the most prevalent species (bracken, grass and bluebells) as well as vegetation height, leaf litter depth and percentage of carrot visible were explored with Spearman's Rank Correlation tests, to see whether any of the variables could explain the differing densities of voles and mice captured within the mini-grids. Mini-grid SR in E1 was excluded from the percentage cover of the most prevalent species analyses because the vegetation on this grid was largely composed of bramble and sedge and was therefore not comparable with other mini-grids.

4.2.7. Weather

Weather conditions, such as cloud cover, may influence capture success of small mammals. To examine the possible effects of weather conditions on density estimates, numbers of captures of animals in morning rounds only were tested with Chi-squared Goodness of Fit tests for each site, looking at the relationship between overnight cloud cover (5 categories 0-20%, 30-40%, 50-60%, 70-80%, 90-100%), and rainfall (classed as (i) none, (ii) rain, (iii) heavy rain). Traps shut due to shrews were also included in these analyses.

Analysis of weather and capture data within seasons for each site was complicated by the strong effect of trap round. Numbers of captures towards the end of each trapping period were almost always higher than at the beginning of trapping periods regardless of weather conditions. Furthermore, it was rare to experience a wider range of weather conditions within trapping period. Thus, to look at the overall effect of weather conditions on capture success, data were pooled between seasons for each site. To verify these results whilst minimising the effect of trap round, data were then reanalysed using only captures from the first two morning rounds of each trapping period.

4.2.8. Trap revealed movement

Trap revealed movement patterns for animals captured more than once were analysed using Ranges 8 ver. 1.15 software (Anatrack Ltd). Three measures or indices of movement were explored; 100% Minimum Convex Polygons (MCP) as a measure of range area (e.g. Erlinge *et al.* 1990), average location interval per individual per trap period (i.e. mean distance between captures, D) as a measure of activity on the grids,

and the proportion of movements between captures that were >20 m for individuals within each trap period. Twenty meters was taken as an arbitrary distance to distinguish 'long' from 'short' movements. Within trap-period measures were analysed with respect to age (A – adult, J – young), sex (M – male, F – female) and time period (P – month and year). MCP and D values were not normal and were log transformed to improve their normality: log MCP values were normal, Log (D+1) values were not normal but were better than untransformed values and sufficient to explore hypotheses with respect to age, sex and time. All areas and distances have been rounded to whole numbers.

Four data sets were analysed: Orielton, Skomer Grid T (2005-6), Skomer Grids E1 (2005-6) and Skomer Grids E2 (2007): these will be referred to as Orielton, Grid T, Grids E1 and Grids E2 respectively. None of the data sets were sufficiently balanced to look at interactions among age, sex and time, and, therefore, interactions between pairs of factors and the main factors were analysed using ANOVA (GLM option Minitab 15). In addition, the results for adults only have been analysed with respect to time, sex and age. Example movement plots from Ranges software are also presented.

Analyses on the effects of density of trap-revealed movement and a comparison of movement indices among sites have also been carried out. Last, a brief consideration is given to movement between trap periods.

4.3. Results

4.3.1. Island comparisons

4.3.1.1. Densities and biomass

The highest densities of bank voles occurred on Skomer Island in the autumn of 2005, 2006 and 2007 and Orielton in autumn 2007, peaking at 140 animals per hectare on both sites (Fig. 4.2). Peak densities on the other sites ranged from 43 to 49 animals per hectare. Densities of voles were generally higher in the autumn than the spring, with the exception of Jersey where densities remained fairly constant but dipped in the autumn of 2007.

Mean density of bank voles in the spring was highest on Jersey and lowest on Mull whereas in the autumn, mean density was highest on Skomer and lowest on Jersey (Fig. 4.3). The biggest change in mean density between seasons occurred on Skomer, and the smallest on Jersey (Fig. 4.4).

Bank voles and wood mice were the only rodents captured on four out of the five sites. On Mull however, field voles were also present and constituted 52% of the mean total rodent density in both spring and autumn (Fig. 4.4). Total rodent density followed a very similar pattern to bank vole density except that densities on Mull were comparatively elevated because of the high numbers of field voles. When data were averaged between years, rodent density was highest in the autumn on Skomer > Orielton > Mull > Raasay > Jersey. However it should be noted that peak densities of rodents on Jersey in spring were greater than autumn densities on Raasay.

Bank vole biomass was also generally greater in autumn than spring apart from on Jersey when the reverse pattern was observed (Fig. 4.2). Despite similarities in densities of voles on Skomer and Orielton grids (autumn 2007), vole biomass was far greater on Skomer. Furthermore, vole biomass at Orielton during this trapping period was very similar to vole biomass on Raasay (2124 g/ha and 1942 g/ha respectively), whilst the number of voles was more than double that found on Raasay (140/ha and 67/ha respectively). Mean biomass of voles in spring was greatest on Jersey followed by Skomer, Raasay, Orielton then Mull (range; 1847-378 g/ha). Conversely, in autumn mean biomass of voles was greatest on Skomer, followed by Raasay, Orielton, Mull and then Jersey (range; 3292-822 g/ha). Overall change in biomass between spring and autumn was greatest on Skomer and least on Mull (Fig. 4.4). Total rodent biomass followed a very similar pattern to vole biomass except that total biomass was greatly elevated on Mull because of the field voles. Mean total rodent biomass was greatest on Jersey in spring and lowest on Skomer, but in the autumn the reverse situation was observed (Fig. 4.4). Bank voles contributed over 50% of the total rodent biomass in both spring and autumn for all sites except for Mull (Fig. 4.3), where field voles were the dominant species.

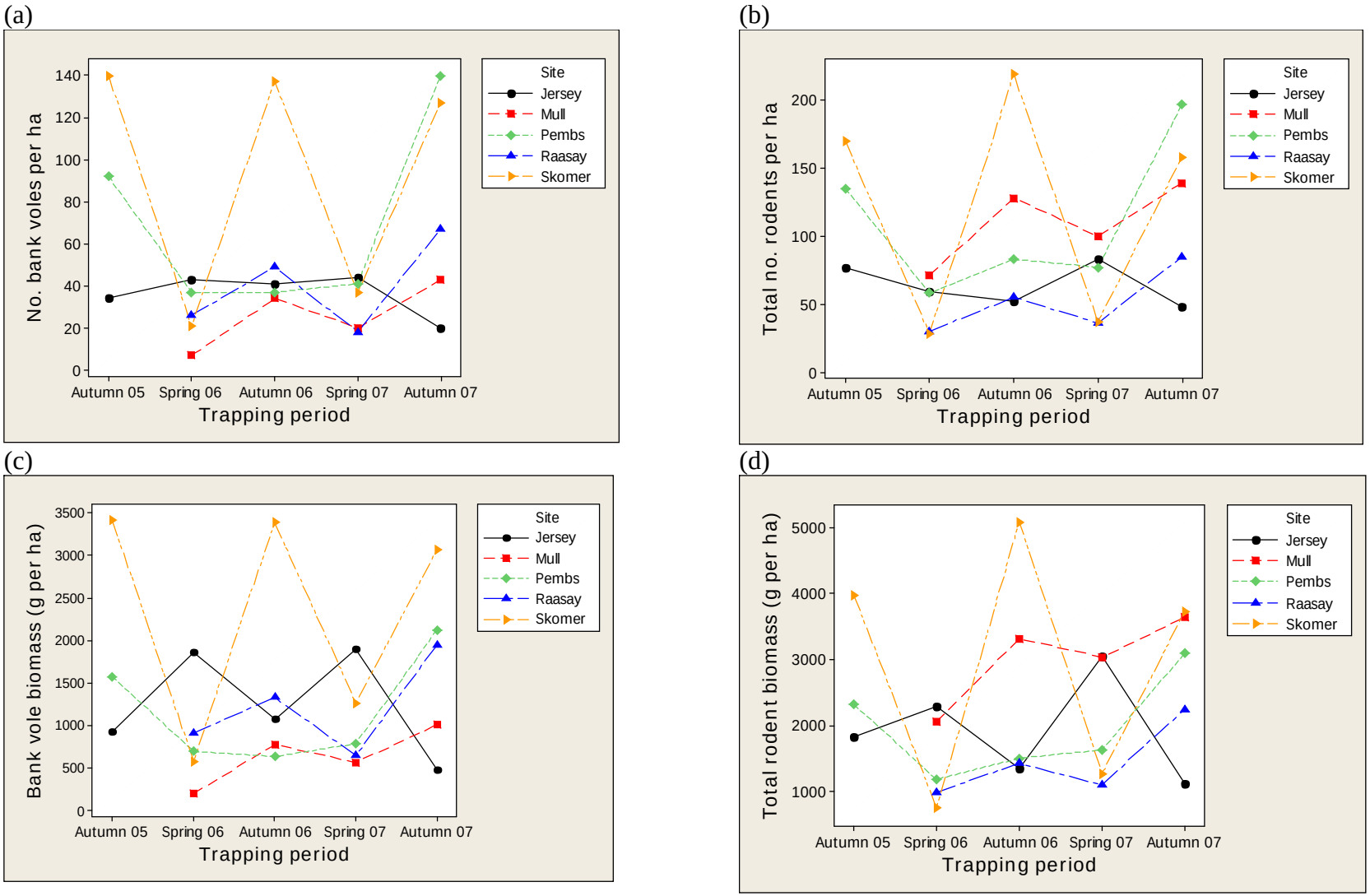
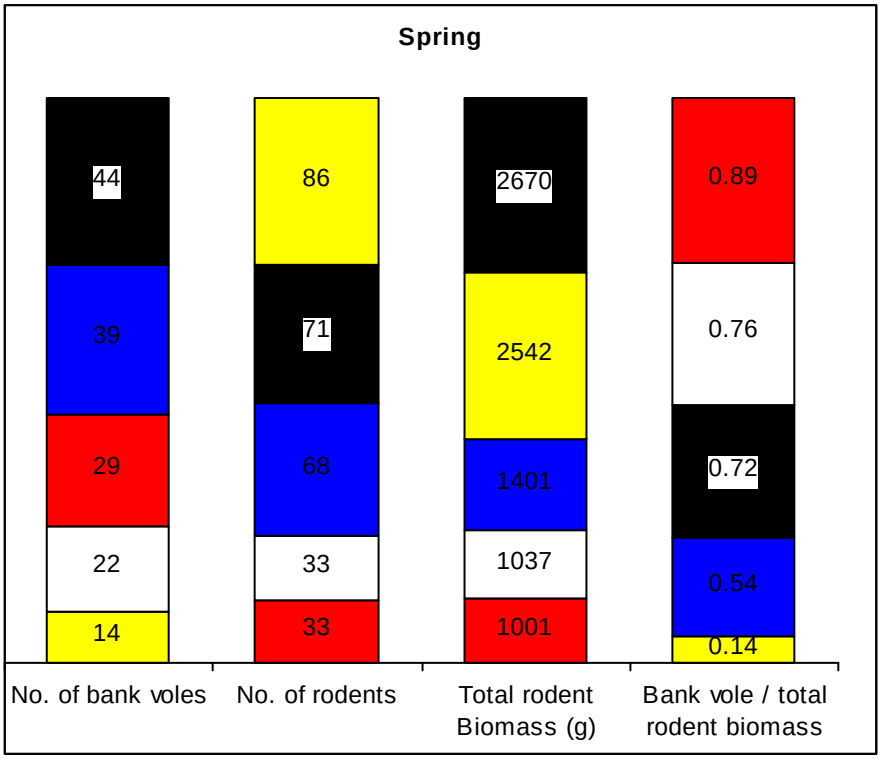


Figure 4.2: Plot of; (a) density of bank voles per hectare (b) density of rodents per hectare (c) bank vole biomass (g/ha) (d) total rodent biomass (g/ha). Animals were captured at five sites during autumn and spring 2005-2007.



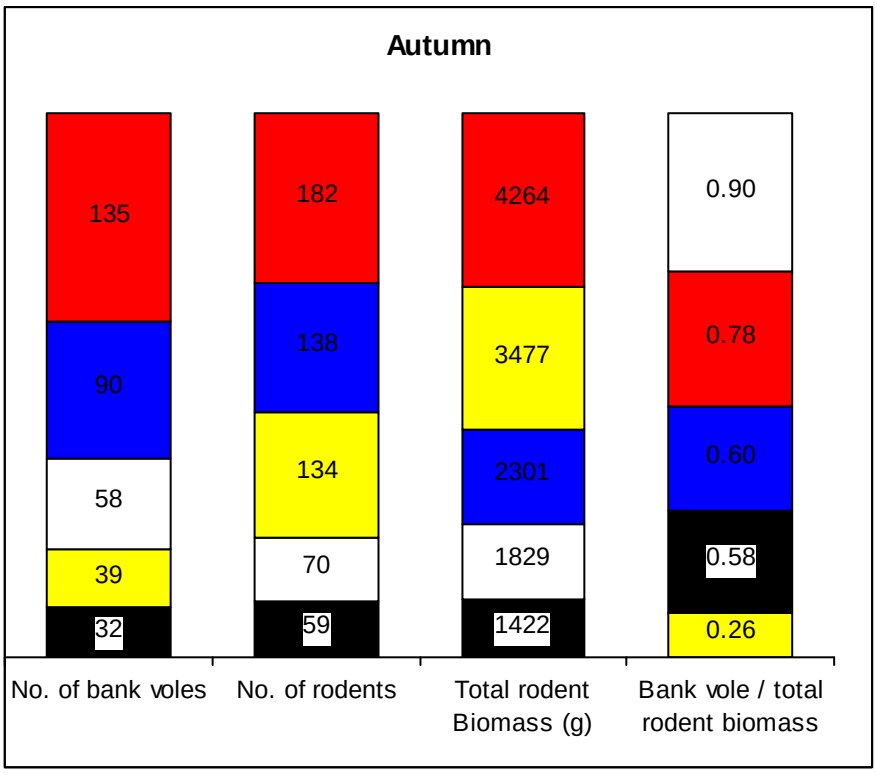


Figure 4.3: Columns 1 to 3 - mean values per hectare for spring and autumn trapping periods conducted on; Skomer – red, Jersey – black, Mull – yellow, Raasay – white and Orielton – blue. Bar proportions indicate rank values. Actual values are given on bars. Column 4 – bank vole biomass as a proportion of total population biomass.

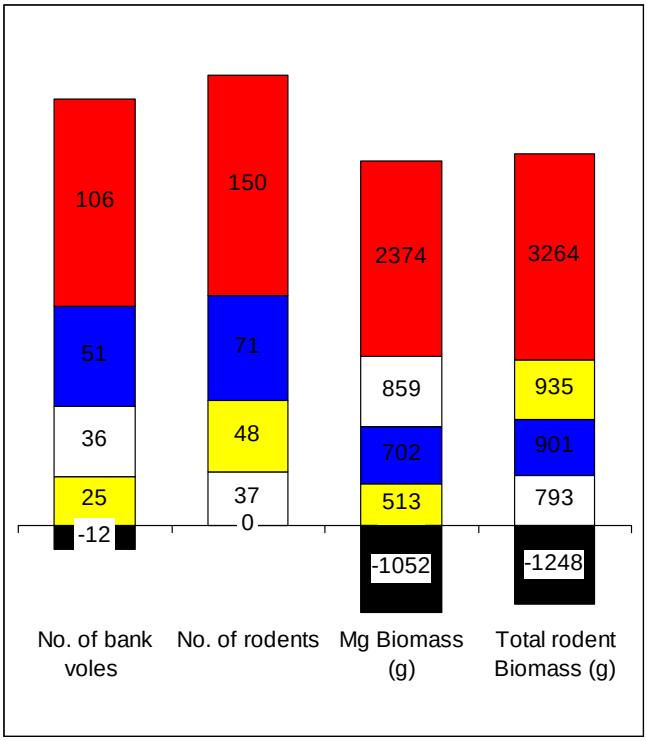


Figure 4.4: Change in mean values per hectare between autumn and spring trapping periods conducted on; Skomer – red, Jersey – black, Mull – yellow, Raasay – white and Orielton – blue. Actual values are given on bars.

4.3.1.2. Breeding season

Of the female bank voles trapped in spring 2006, the proportion of breeding animals varied markedly between sites, ranging from 100% on Jersey to 0% on Skomer (Fig. 4.5). Palpably pregnant animals were only recorded on Jersey, but on all other sites, with the exception of Skomer, a proportion of the females showed signs of sexual activity (perforate vaginas). Most male bank voles trapped during spring 2006 were in breeding condition (range; 58-100%).

In spring 2007, the majority of females caught were either breeding or perforate on three out of the five sites (Fig. 4.5). However, none of the females caught on Mull (N=4) showed signs of breeding. This is in sharp contrast with the field voles where

89% of the females captured were perforate (Fig. 4.6). Unfortunately, no females were captured on Skomer during this trap season. Once again, the majority of male voles trapped at all sites were in breeding condition.

By autumn 2006 the majority of both male and female voles on all sites were not in breeding condition (Fig. 4.5). Breeding or perforate females were only detected on Raasay and Orielton. This is in contrast to wood mice, where pregnant and lactating individuals were found on Skomer (N=3) and 83% of the female mice on Mull were perforate (N=6). In autumn 2007, a similar pattern was observed; breeding female bank voles were only caught at two sites (Mull and Raasay) and the majority of animals were not in breeding condition. Of the three sites visited in autumn 2005, breeding or perforate female voles were caught on Jersey and Skomer (Fig. 4.7). However, none of the 20 females captured at Orielton showed signs of breeding. Wood mice showed a similar pattern, except perforate females made up smaller proportion of the catch on Jersey. Both non-breeding and breeding field voles of both sexes were captured in the autumns of 2006 and 2007.

Sex ratios of adult voles captured in spring were tested to see if they deviated from 50-50 male to female ratio. The only significant difference was for animals captured on Raasay in spring 2006, when 12 males and only one female were captured (X^2=9.3, P<0.01).

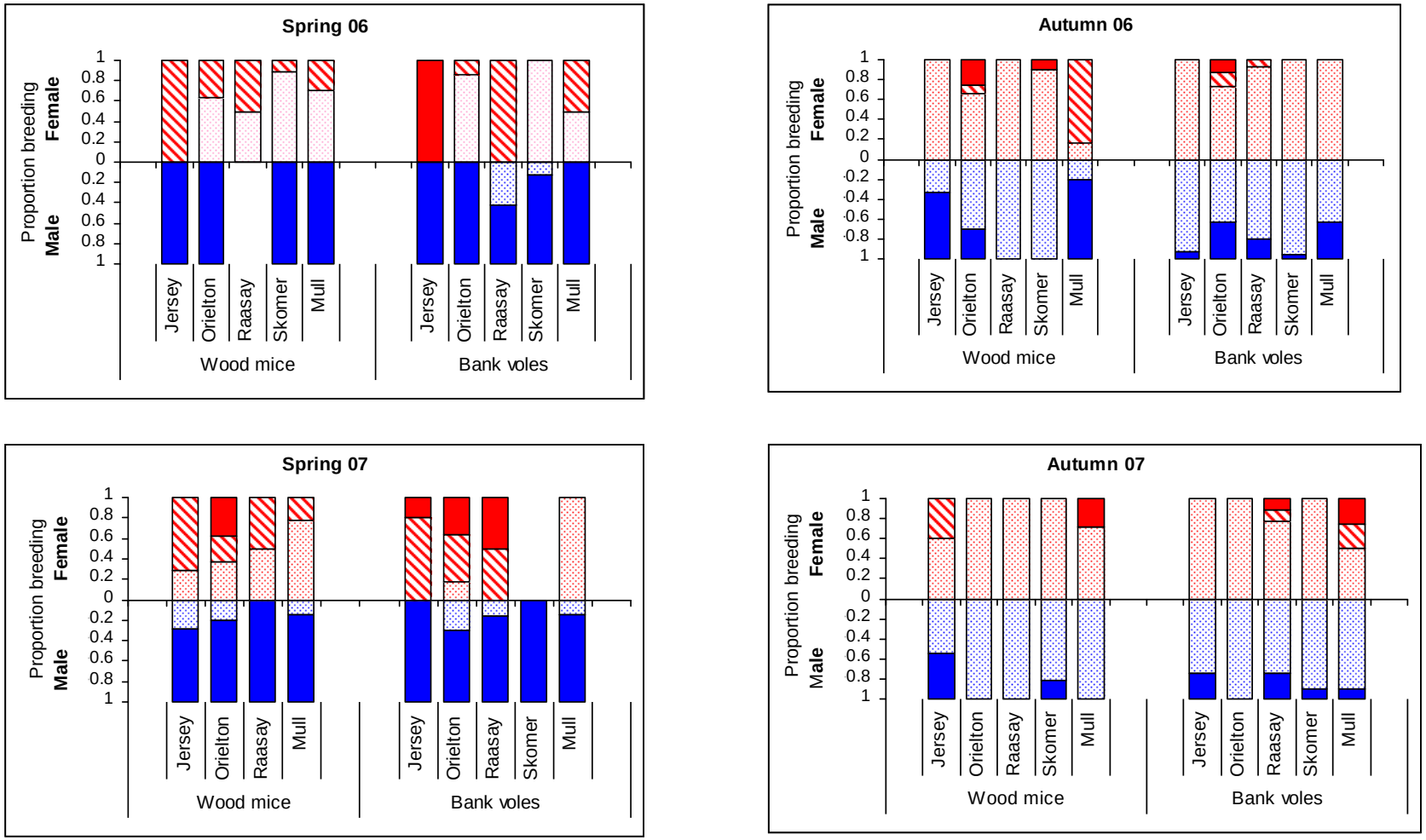


Figure 4.5: Proportion of wood mice and bank voles breeding in spring and autumn 2006-2007 at five sites; males in blue, females in red. Solid colour - breeding animals; small spots – non-breeding animals; diagonal stripes – perforate females.

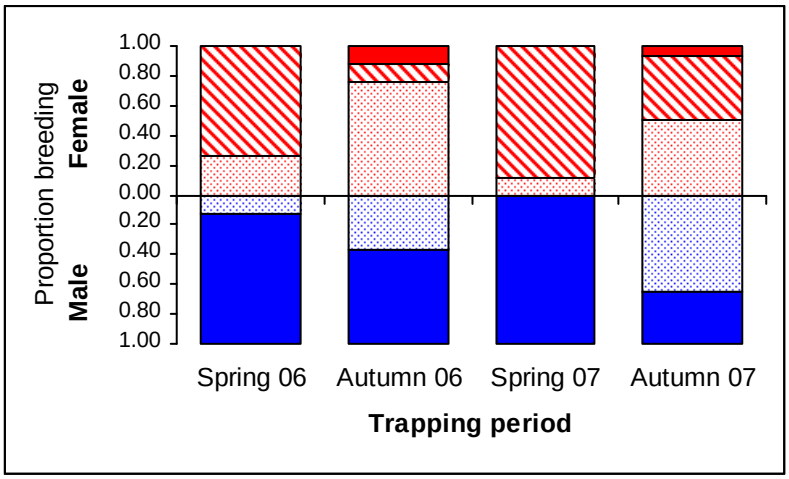


Figure 4.6: Proportion of field voles breeding in spring and autumn 2006-2007 on Mull. Males in blue, females in red. Solid colour - breeding animals; small spots – non-breeding animals; diagonal stripes – perforate females.

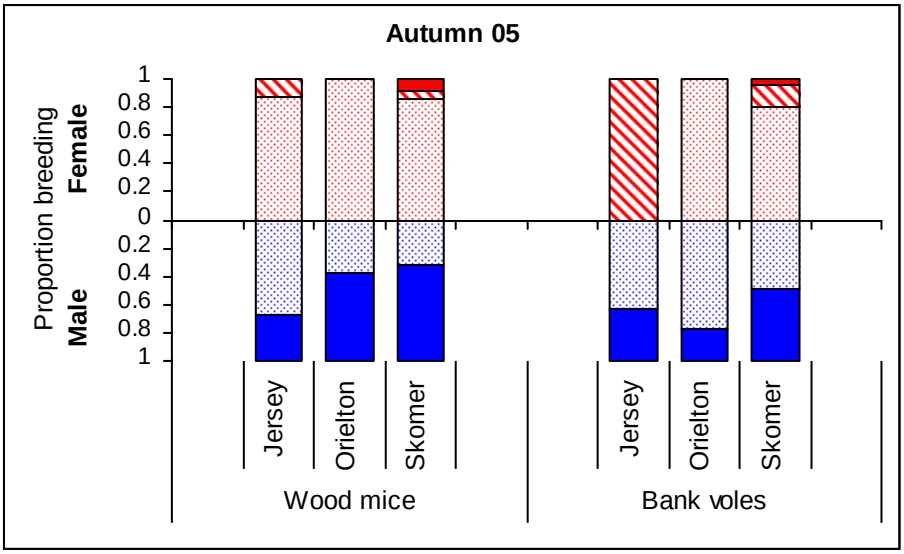


Figure 4.7: Proportion of wood mice and bank voles breeding in autumn 2005 at three sites. Solid colour - breeding animals; small spots – non-breeding animals; diagonal stripes – perforate females.

4.3.1.3. Recapture rates

When recaptures of voles were examined within trapping period for each site, rates varied between 34% and 92% of individuals (Table 4.3). The highest recapture rates were on Skomer Grid E2 in early summer 2006 and the lowest were on Orielton grid in late summer 2007. There was no association between trapping period and capture

history (single capture, recapture) at any site except Orielton, where significantly more individuals were recaptured in autumn 2005 and spring 2006, and fewer were recaptured in late summer 2007 (Table 4.3). There was a significant association between overall recapture rates and site (Fig. 4.8), with particularly high recapture rates on Skomer E1 and Jersey. Recapture rates varied between the Skomer grids, Skomer E1 > T > E2, with a particularly significant difference between E1 and E2. However, there was no significant difference in recapture rates between Skomer T and Orielton. There were no significant regression relationships between the proportion of animals recaptured and the total number of individuals at each site (Fig. 4.9). Regression coefficients were positive at Skomer E2 and Skomer T, marginally positive at Jersey and Raasay and negative at Skomer E1, Orielton and Mull. The slope of the regression line for Skomer E2 was heavily influenced by the extremely low capture numbers in spring 2007 (one individual in 1280 trap nights). Whilst recapture rates may partially be related to the proportion of traps closed in each period at each site (Table 4.4), it is notable that on Skomer T Grid, recapture rates changed very little despite over 60% of the traps being occupied during morning rounds for two of the four trapping periods. Furthermore, recapture rates on Skomer E1 were significantly higher than on Skomer E2, despite little difference in the proportion of traps shut (with the exception of autumn 2007). However, this variation in recapture rates may be partially attributable to the increased number of trap nights on Grid E1 (Table 4.2).

Table 4.3: The proportion of individuals (N) recaptured at each study site during each sampling period (years-season: 1 – spring, 2 – early summer, 3- late summer, 4- autumn). Contingency X^2 test for association between capture history (single capture, recapture) and period. Significant period cell values in bold.

Site		Period									Contingency (Period) X^2		
		05-4	06-1	06-2	06-3	06-4	07-1	07-2	07-3	07-4	X^2	df	P
Orielton	Recapt %	**81**	**76**	65	35	67	55	47	**34**	46	35.21	8	<0.0001
	N	48	21	17	23	18	20	47	53	78			
Skomer T	Recapt %	60	45	63		63					1.42	3	0.700
	N	75	11	41		79							
Skomer E1	Recapt %	76	75	92	84	63					6.78	4	0.148
	N	50	20	12	45	35							
Skomer E2	Recapt %						0	38	52	44	3.42	3	0.331
	N						1	42	86	135			
Jersey	Recapt %	75	71			86	52			55	7.37	4	0.118
	N	16	21			21	25			11			
Mull	Recapt %		75			50	27			43	3.06	3	0.383
	N		4			18	11			23			
Raasay	Recapt %		73			64	56			64	0.84	3	0.840
	N		15			25	9			33			

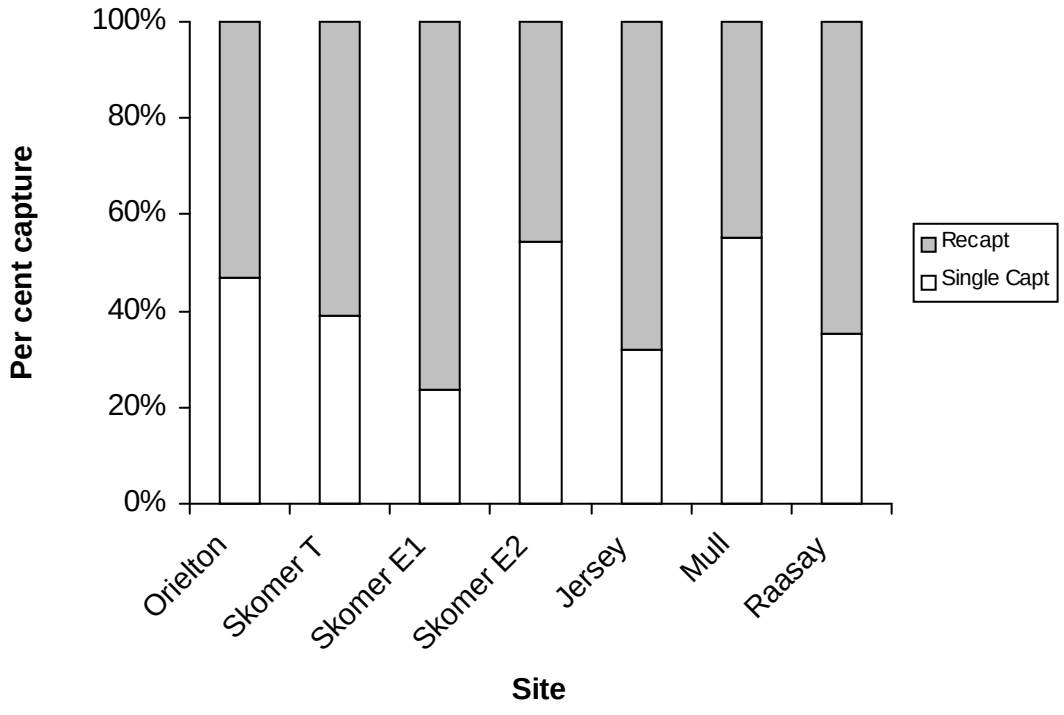


Figure 4.8: The proportion of individuals captured once and recaptured within trapping periods at each study site. There was a significant association between capture history and site, X^2_6=52.15, P<0.0001).

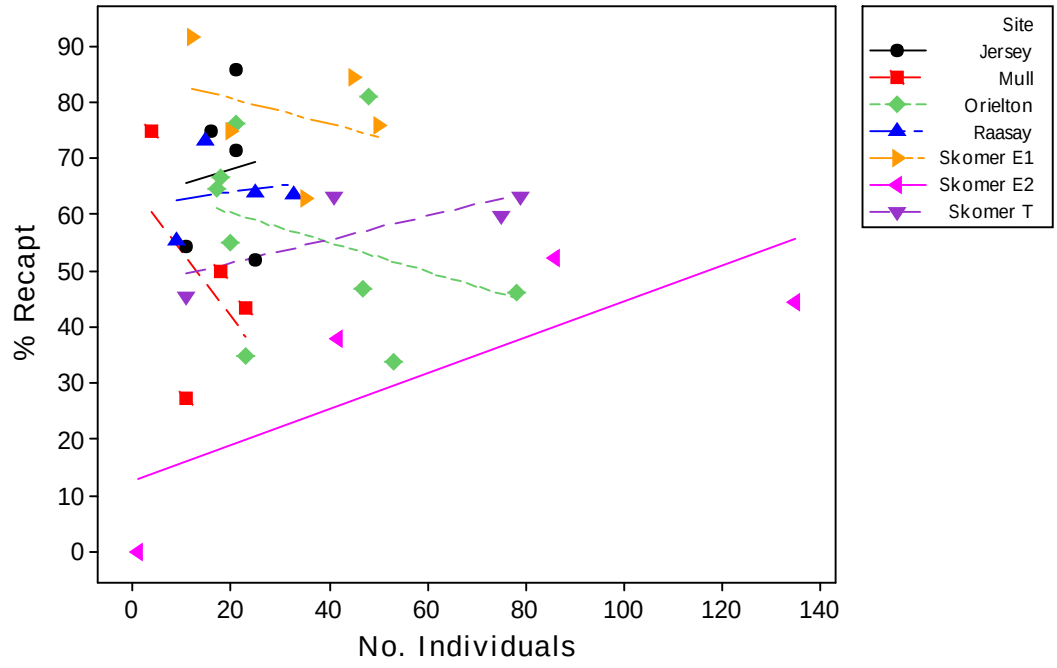


Figure 4.9: Relationship between proportion of individuals recaptured within trapping periods and the total number of individuals captured.

Table 4.4: Proportion of traps shut during morning and evening trap rounds at each site during each trapping period (years-season: 1 – spring, 2 – early summer, 3- late summer, 4- autumn). Data also include traps disturbed and thus not accessible to animals. Values > 50% in bold.

SITE	ROUND	PERIOD								
		05-4	06-1	06-2	06-3	06-4	07-1	07-2	07-3	07-4
Orielton	AM	**0.57**	0.28	0.16	0.16	0.36	0.20	0.30	0.38	**0.61**
	PM	0.03	0.03	0.04	0.06	0.02	0.10	0.22	0.04	0.07
Skomer T	AM	**0.68**	0.10	0.24		**0.80**				
	PM	0.14	0.01	0.20		0.03				
Skomer E1	AM	0.43	0.20	0.21	0.37	0.39				
	PM	0.07	0.02	0.02	0.13	0.04				
Skomer E2	AM						0.00	0.13	0.40	**0.64**
	PM						0.00	0.05	0.14	0.20
Jersey	AM	0.34	0.09			0.20	0.19			0.11
	PM	0.06	0.12			0.09	0.07			0.02
Mull	AM		0.19			0.41	0.29			0.41
	PM		0.09			0.26	0.14			0.13
Raasay	AM		0.11			0.21	0.10			0.43
	PM		0.03			0.08	0.05			0.12

As a rule of thumb, no more than 50-60% of the traps should be shut during a given trapping period because otherwise trappable unmarked animals may be unable to encounter unoccupied traps and thus, population size may be

underestimated (Gurnell & Flowerdew 2006). This assumption was violated during the morning rounds of five trapping periods. Unfortunately a restriction in the number of traps available meant that additional traps could not be added to each trap point.

4.3.1.4. Weather

Analysis of overnight cloud conditions preceding each morning trap round showed that the number of animals captured tended to be higher than expected in conditions of high cloud cover (>50%), and lower than expected in low cloud cover (<50%) This trend was significant for all populations except Jersey (Table 4.5). When only the first two morning rounds of each trapping period were analysed, the trend was even stronger ($P<0.001$ for all sites, data not shown) and the observed number of captures for low cloud cover were all less than the expected values.

Analysis of rainfall data showed that numbers of captured animals were always higher than expected on mornings following overnight rainfall. This trend was significant for all sites except for Mull (Table 4.6). When only the first two morning rounds of each trapping period were analysed, the same significant trend was observed for four of the sites, however there was no significant effect of rainfall on capture numbers for Skomer.

Table 4.5: Chi-squared analysis of number of animals captured following overnight cloud cover conditions for all morning rounds, pooled between seasons for each site. Cell X^2 values are given in normal type when the number of observed captures were lower than expected (O - E < 0), and bold when observed captures were greater than expected (O - E > 0).

	% Cloud Cover						
Site	0-20	30-40	50-60	70-80	90-100	X^2	P
Skomer	24.09	2.62	**5.23**	**12.85**	**2.30**	47.08	<0.001
Orielton	6.26	**1.17**	13.16	**5.09**	**7.06**	32.75	<0.001
Jersey	1.62	0.15	**0.03**	**1.80**	**1.25**	4.85	NS
Mull	**6.31**	27.27	1.09	5.10	**9.70**	49.47	<0.001
Raasay	2.84	1.71	**23.76**	4.89	**0.81**	34.02	<0.001

Table 4.6: Chi-squared analysis of number of animals captured following overnight rain conditions for all morning rounds, pooled between seasons for each site. Cell X^2 values are given in normal type when the number of observed captures were lower than expected (O - E < 0), and bold when observed captures were greater than expected (O - E > 0).

	Rain					
Site	None	Rain	Heavy rain	X^2	Df	P
Skomer	30.50	**18.96**	**8.16**	57.63	2	<0.001
Orielton	4.46	**4.21**	-	8.68	1	<0.001
Jersey	1.60	**8.53**	-	10.13	1	<0.001
Mull	**0.73**	**0.37**	-	1.10	1	NS
Raasay	15.45	**1.21**	**40.10**	56.76	2	<0.001

4.3.1.5. Vegetation

(a) Skomer experimental grids

A principal component analysis was carried out on the six vegetation measures common to all grids over both years: average height of vegetation AH, per cent cover of: bracken, wood sage, ground ivy and grass, and % carrot visible %C (Fig. 4.10, Table 4.7). The first two components explained 64% of the variation and the first component was defined by average height of vegetation (AH) and to a lesser extent bracken, at one end of the axis and the per cent carrot visible (%C) at the other. In the

remaining analysis the vegetation measures explored are AH and %C as both are measures related to cover for the small mammals from aerial predation.

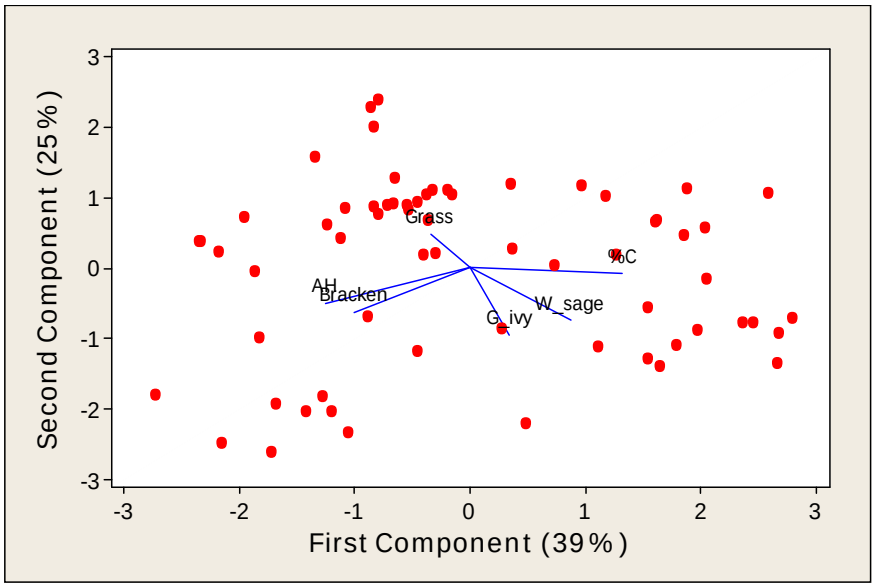


Figure 4.10: Biplot of first and second principal component of six vegetation measures (average height of vegetation AH, bracken, wood sage, ground ivy, grass and % carrot visible %C) common to E1 and E2 grids on Skomer. Scores for each trap point in red, factor loadings in blue.

Table 4.7. Results of principal component analysis of six vegetation variables on the Skomer experimental grids, 2006-7. PC1 – PC3, principal components 1 to 3 respectively.

	PC1	PC2	PC3
(a) Variance (eigenvalue)			
Eigenvalue	2.308	1.522	0.915
Proportion of total variance	0.385	0.254	0.153
Cumulative	0.385	0.638	0.791
(b) Coefficients			
Variable			
Av height vegetation	-0.54	-0.33	-0.01
Bracken % cover	-0.44	-0.41	0.00
Wood sage % cover	0.38	-0.49	-0.15
Grass % cover	-0.15	0.32	-0.92
Ground ivy % cover	0.15	-0.62	-0.35
% carrot visible	0.57	-0.05	-0.06

Over both 2006 and 2007, numbers of bank voles on the experimental grids were significantly positively associated with AH and significantly negatively associated with %C, supporting the notion that the voles preferred to move in areas where there was more field cover (Table 4.8a, Fig. 4.11). This was not the case for wood mice, although there was a positive association between wood mouse and bank vole numbers. There was also a significant negative association between AH and %C. Interestingly, the cover effects were stronger in 2006 (Table 4.8b) than 2007 (Table 4.8.c).

Table 4.8. Pearson correlation matrices for mean % carrot visible (%C), mean height of vegetation (AH), and total numbers of bank voles (Mg), wood mice (As) captured at trap points across all trap periods on experimental grids on Skomer. Pearson r (above diagonal) and P value (below diagonal)

(a) Both grids, 2006 and 2007

Variables	AH	%C	As	Mg
AH		**-0.693**	0.231	**0.642**
%C	**< 0.0001**		0.128	**-0.487**
As	0.067	0.312		**0.365**
Mg	**< 0.0001**	**< 0.0001**	**0.003**	

(b). Grids E1, 2006

Variables	AH	%C	As	Mg
AH		**-0.838**	**0.397**	**0.638**
%C	**< 0.0001**		-0.338	**-0.533**
As	**0.025**	0.059		**0.739**
Mg	**0.000**	**0.002**	**< 0.0001**	

(c). Grids E2, 2007

Variables	AH	%C	As	Mg
AH		-0.251	**0.385**	**0.542**
%C	0.166		0.025	-0.243
As	**0.030**	0.892		**0.575**
Mg	**0.002**	0.179	**0.001**	

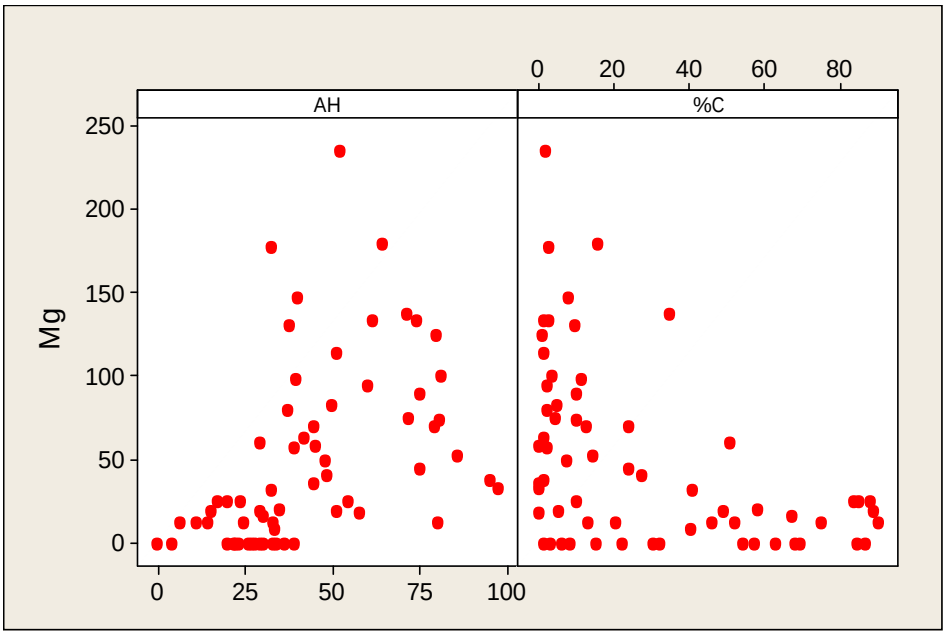


Figure 4.11: Scattergrams for captures of bank voles (Mg) against mean vegetation height (AH) and % carrot visible (%C) – for years 2006 and 2007.

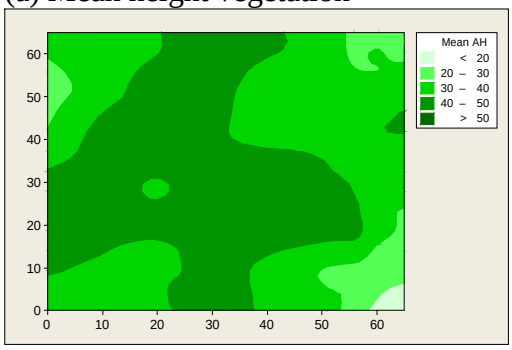
(b) Orielton

There was not a great deal of variation in per cent of carrot visible (%C) (mean CV=55%, N=8) or average height of vegetation (AH) (mean CV=21%, N=6) at trap points across the different sample periods at Orielton, and these vegetation measures have been pooled across trap periods.

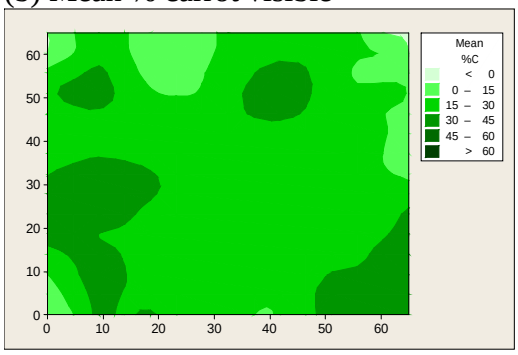
Overall, there were higher numbers of vole and mouse captures at points towards the "left side" of the grid than the "right side" (Fig. 4.12). However, this was not particularly associated with the two vegetation measures. In fact, there were lower correlations between numbers of rodents and the two vegetation measures at Orielton than on Skomer (Table 4.9). Numbers of mice and voles were not associated with %C, and there was only a weak association between AH and vole numbers and a moderate one between AH and mouse numbers. Estimates of shrew numbers were also weakly correlated with the vegetation and rodent variables at Orielton (Table

4.9). It seems that cover was not so important in influencing capture success at Orielton than on Skomer.

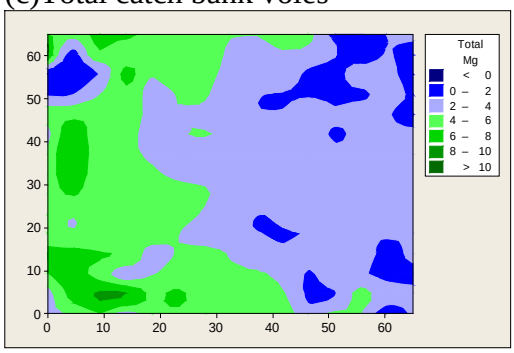
(a) Mean height vegetation



(b) Mean % carrot visible



(c)Total catch bank voles



(d)Total catch wood mice

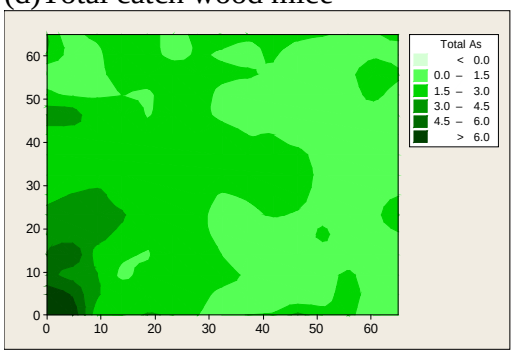
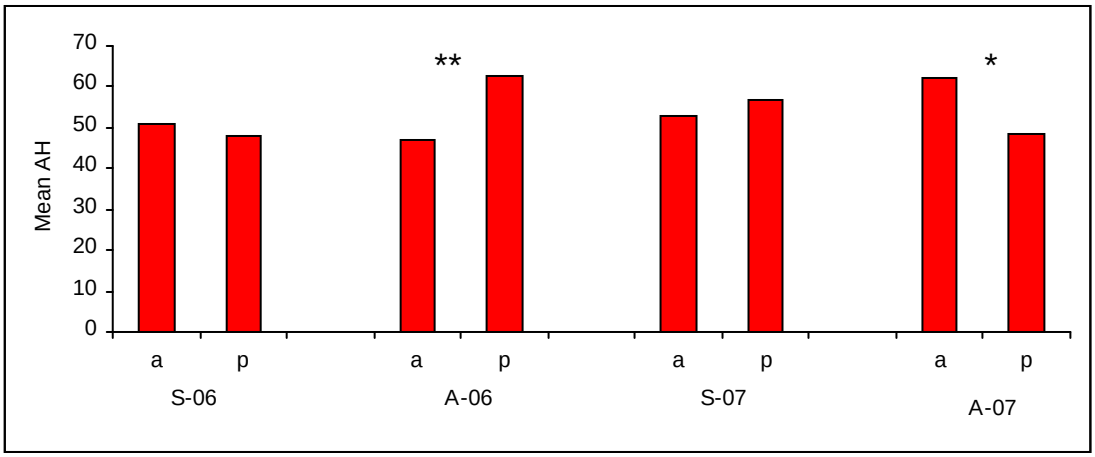


Figure 4.12: Contour maps for (a) mean height vegetation, (b) mean % carrot visible, (c) total catch of bank voles and (d) total catch of wood mice pooled for all sampling periods at Orielton.

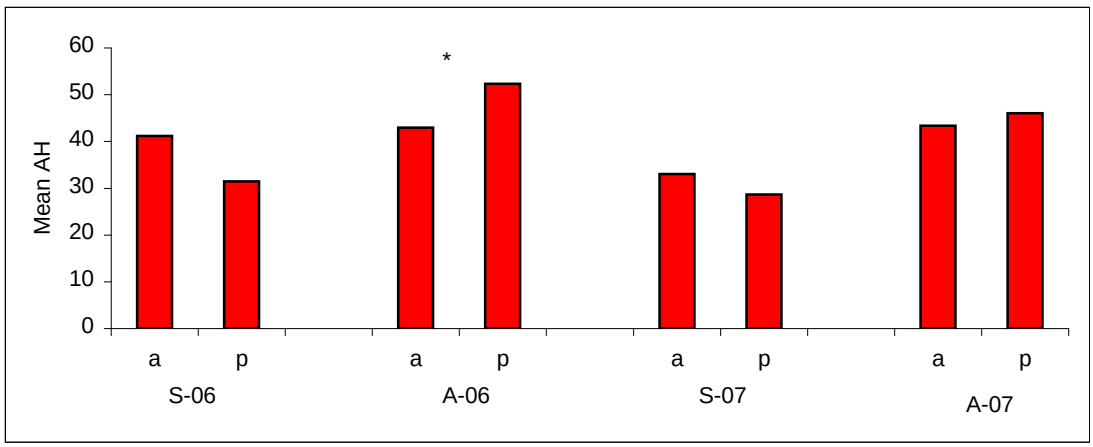
Table 4.9: Spearman's rank correlation matrix for mean % carrot visible (%C), mean height of vegetation (AH) and total numbers of bank voles (Mg), wood mice (As) and shrews (S) captured at trap points across all trap periods at Orielton. AH, Total As and Total S not normally distributed. Spearman r (above diagonal) and P value (below diagonal)

Variables	Mean %C	Mean AH	Total As	Total Mg	Total S
Mean %C		**-0.218**	0.035	-0.003	**-0.154**
Mean AH	**0.002**		**0.158**	**0.212**	**0.289**
Total As	0.628	**0.027**		**0.556**	**0.251**
Total Mg	0.963	**0.003**	**< 0.0001**		**0.457**
Total S	**0.032**	**< 0.0001**	**< 0.001**	**< 0.0001**	

(a) Jersey



(b) Mull



(c) Raasay

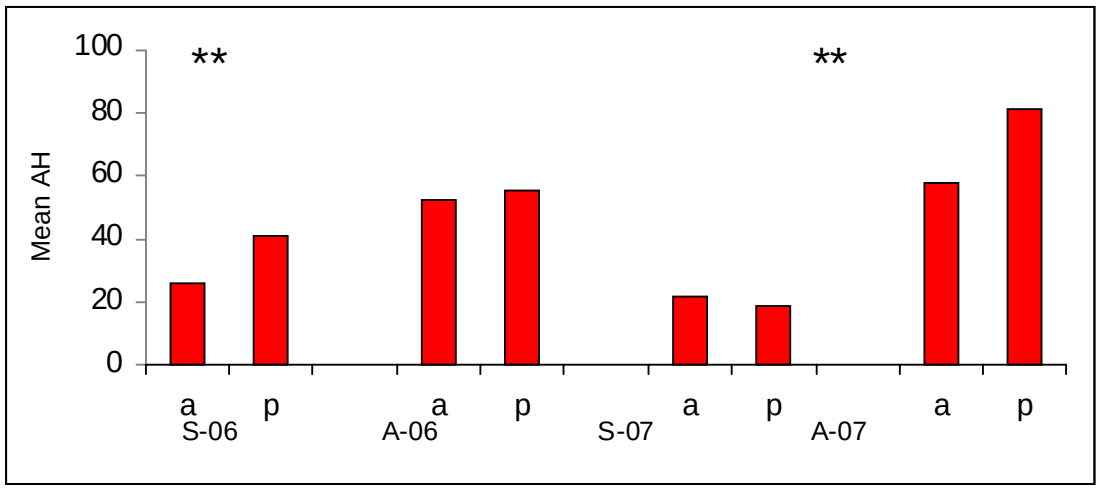
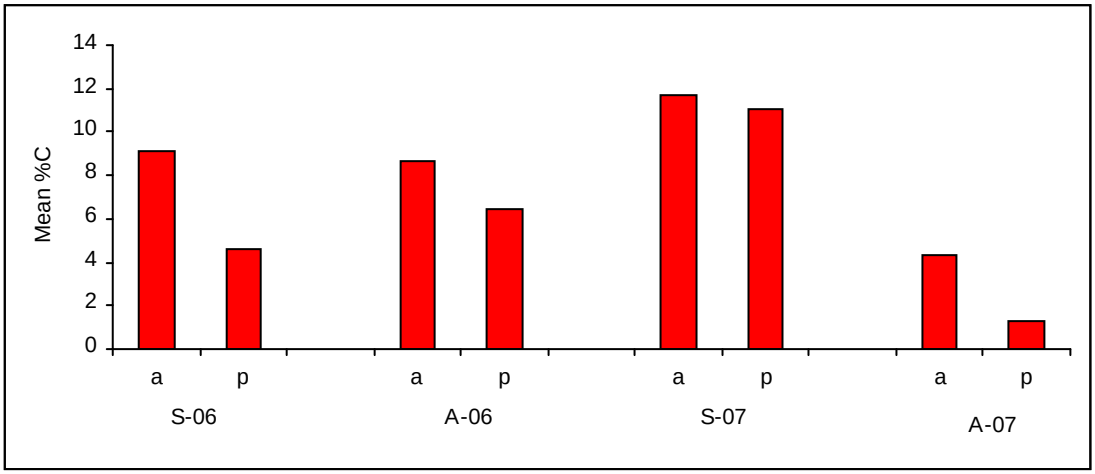
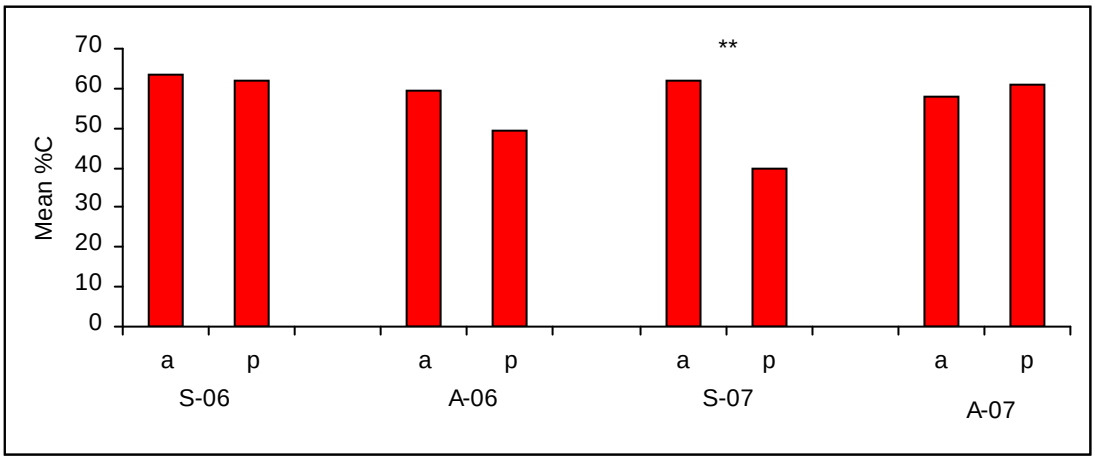


Figure 4.13: Mean height of vegetation (AH) at non-capture [a] and capture [p] trap points for (a) Jersey, (b) Mull and (c) Raasay. ** = significant difference (P<0.05) between a and p, Mann Whitney test. X-axis labels: S = spring, A – autumn, numbers refer to years

(a) Jersey



(b) Mull



(c) Raasay

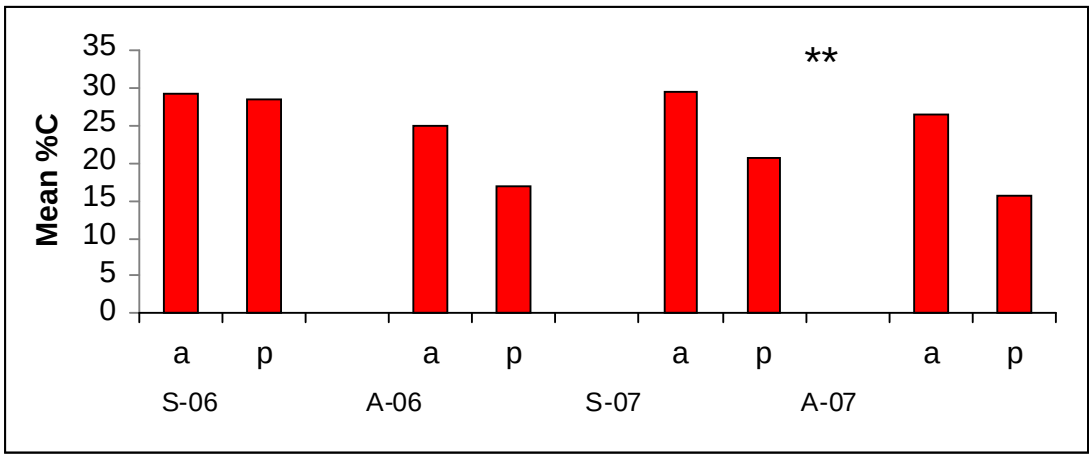


Figure 4.14: Mean % carrot visible (%C) at non-capture [a] and capture [p] trap points for (a) Jersey, (b) Mull and (c) Raasay. ** = significant difference (P<0.05) between a and p, Mann Whitney test. X-axis labels: S = spring, A – autumn, numbers refer to years.

(c) Jersey, Mull and Raasay

For Jersey, Mull and Raasay, mean AH and mean %C have been compared for trap points that captured voles and those that did not capture voles for each season and year of study (Fig. 4.13 and 4.14 respectively). Most differences, but not all, indicated that the voles preferred ground cover (i.e. high AH, lower %C). Notable exceptions to expectation were AH values in spring in both 2006 and 2007 on Mull, and in autumn 2007 on Jersey. Other than these exceptions, there did not appear to be any distinct patterns among sites and seasons.

4.3.2. Skomer and Orielton

4.3.2.1. Density and biomass, Skomer and Orielton

On grids Skomer T, Skomer E2 and Orielton 2007, bank vole densities were lowest in the spring and climbed steadily throughout the year, reaching a peak in autumn (Fig. 4.15a). However, on grid Skomer E1, the density of voles changed very little between March and May, reached a peak in August and then fell in autumn. In 2006 on the Orielton grid, numbers of voles remained fairly constant throughout the year. Interestingly, densities of voles on Skomer Grid T and the Orielton grid in 2007 were similar throughout the year.

Densities of wood mice followed a slightly different pattern to that of voles; changing very little between March and May/June and reaching a peak in October/November on all sites. In contrast with other sites (and Orielton 2007), numbers of mice caught at Orielton in 2006 decreased by 40% between June and August, although low capture numbers may have been affected by weather

conditions (see Section 4.3.1.4).

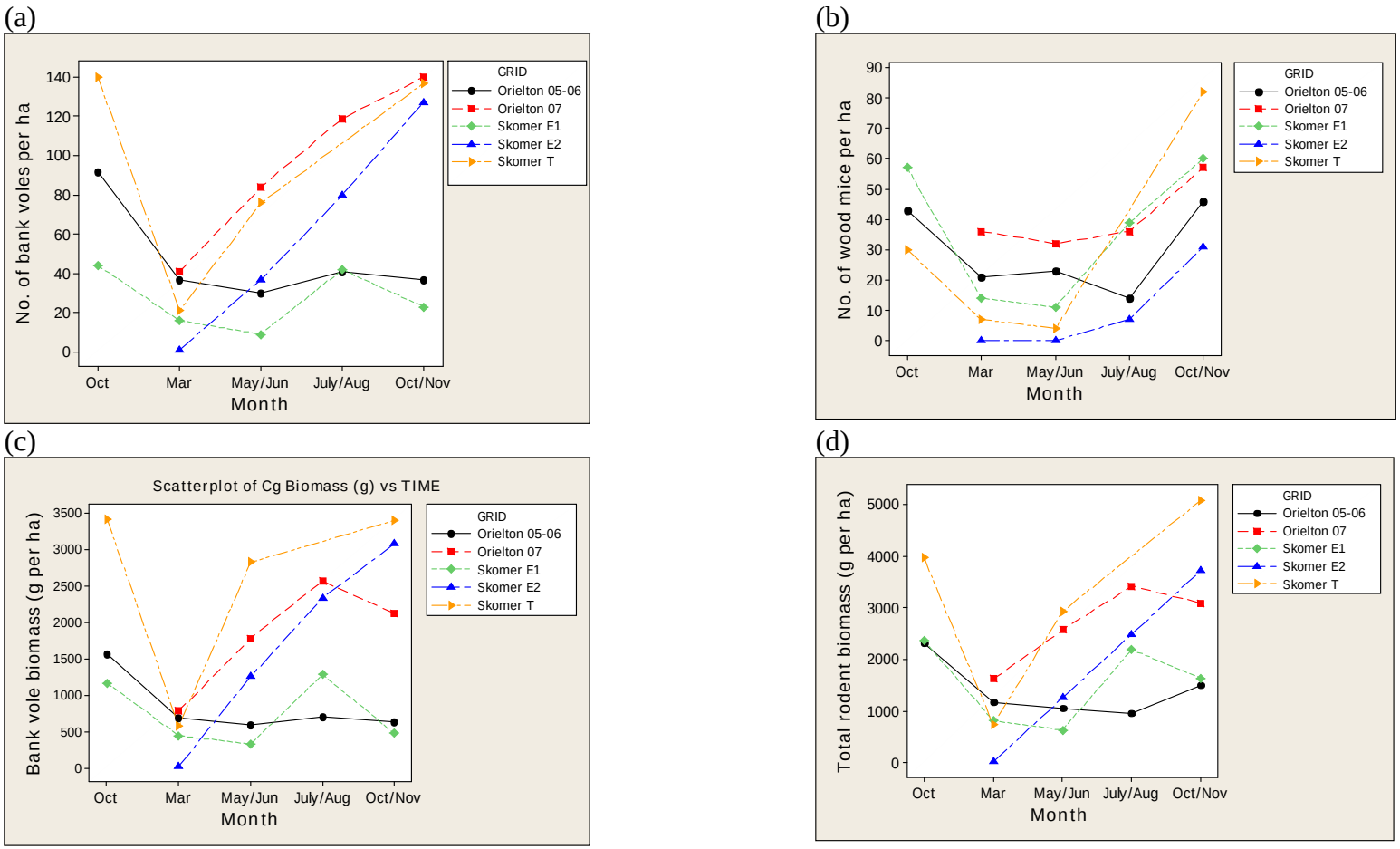


Figure 4.15: Plot of; (a) density of bank voles per ha (b) density of wood mice per ha (c) bank vole biomass (g/ha) (d) total rodent biomass (g/ha) captured throughout the year on Skomer Island and Orielton (mainland Pembs) during 2005-2007 field season.

Bank vole biomass followed a similar pattern to vole density with two exceptions; vole biomass decreased in autumn 2007 at Orielton despite a corresponding increase the in number of voles; biomass of voles was notably greater on Skomer Grid T than on the Orielton grid in 2007 in May/June and Oct/Nov. Mean bank vole biomass was greatest on Skomer T, followed by Orielton 2007, Skomer E2, Orielton 2005-2006 and Skomer E1 respectively. Total rodent biomass generally increased throughout the year on all sites, being lowest in spring and higher in autumn. However, on grids Skomer E1 and Orielton 2007, biomass peaked in July/August. Mean total rodent biomass was highest on Skomer Grid T, followed by Orielton 2007, then Skomer E2, Skomer E1 and lowest on Orielton 2005-2006.

4.3.2.2. Breeding

There was very little difference in the proportion of voles breeding in each trap period on the two Skomer grids trapped in 2005-2006 (grids T and E1) (Figure 4.16). Therefore, from this point forward, these grids are not independently discussed. In autumn 2005 all female and 77% of male voles captured at Orielton were immature. However, on Skomer 51% of male voles were still in breeding condition, 14% of females were perforate and a few were pregnant or lactating (4%). In March 2006, perforate females were captured at Orielton but all female voles captured on Skomer were imperforate. Similarly, all males captured at Orielton during this period were in breeding condition but 14% of the male voles on Skomer were immature. By June 2006, the large majority of voles captured at both sites were in breeding condition and proportions of pregnant or lactating females were almost identical (45% and 44%). A single juvenile female (9 g) and was captured on Skomer along with an immature adult female (28 g) but several young-of-the-year immature animals were captured at

Orielton. By August 2006, immature young-of-the-year were prevalent at both sites but the majority of animals captured were breeding adults. In October 2006, all female voles and 96% of male voles captured on Skomer were immature. However, 38% of males captured at Orielton were in breeding condition, 13% of females were perforate and a further 13% were pregnant or lactating.

In March 2007, only one breeding male was captured on Skomer. At Orielton, 70% of the males captured were in breeding condition, 45% of the females were perforate and 36% were pregnant or lactating. Body weights ranged from 12-22 g with mean of 16.9 g (both sexes). However, no animals with juvenile pelage were captured indicating that breeding probably started in early spring rather than continuing over the entire winter period. By May/June, all females captured on Skomer were perforate or pregnant/lactating. Only 12% of males captured were immature but body mass data (not shown) indicated that these were overwintered animals and not young-of-the-year. Conversely, the few immature animals captured at Orielton in June were predominantly juveniles. By the latter half of July immature males made up 26% of the trapped population at both sites. However, immature females made up 50% and 14% of the female population captured at Orielton and Skomer respectively. In contrast to the previous years, breeding had stopped on both sites by October; 100% of captured females were immature and only 10% of males captured on Skomer showed signs of breeding condition.

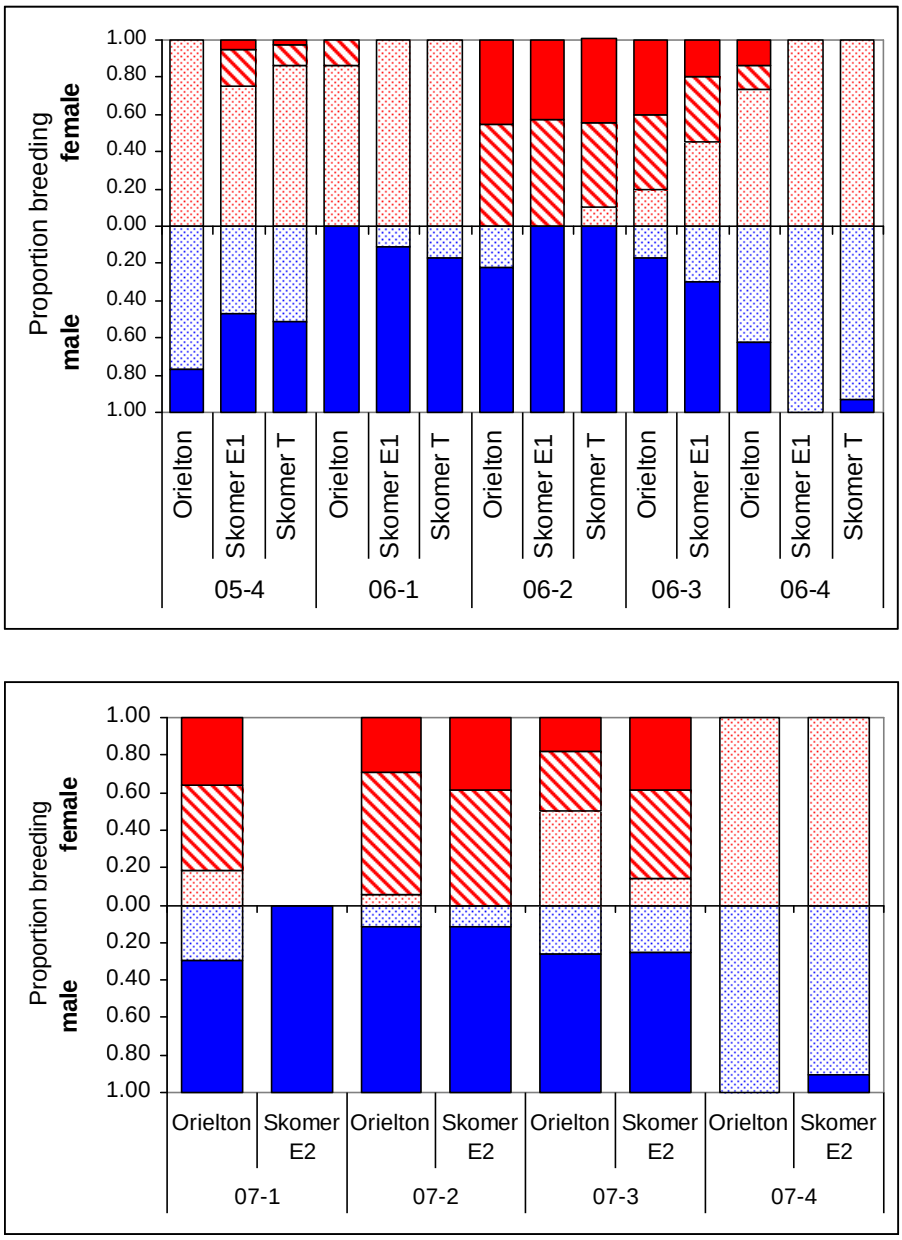


Figure 4.16: Proportion of bank voles breeding during 2005-2006 field season (top) and 2007 field season (bottom) at Skomer and Orielton (years-season: 1 – spring, 2 – early summer, 3- late summer, 4- autumn); males in blue, females in red. Solid colour - breeding animals; small spots – non-breeding animals; diagonal stripes – perforate females.

4.3.2.3. Loss of ear tags

Unfortunately, the proportion of marked animals that had lost ear tags was high on all four of the intensively trapped grids. Animals that had lost tags were easily identifiable by a 'tag-shaped tear' in the ear and on Skomer grids, a hole where a 2 mm biopsy tissue had been taken. Only on very rare occasions did Skomer animals show signs of a tissue biopsy without the corresponding distinctive ear tear or *vice versa*, indicating that this method was adequate to identify previously marked animals.

At Orielton, proportions of marked animals with lost tags ranged from 20%-90% between trapping periods, with the highest rate of tag loss occurring over the winter period 2005-2006. Whilst high rates of tag loss during this period may have been partially caused by operator inexperience in inserting tags during the first field trip, tag loss was again extremely high the following winter (71% of marked animals caught in spring 2007 had lost tags). On the Skomer grids, tag loss was greatest between the late summer and autumn trapping periods when 88%, 88% and 84% of marked animals had lost tags on Grid T, E1 and E2 respectively. The highest rates of tag loss for other trapping periods were 43%, 54% and 45% respectively. The loss of tags will affect estimates of survival considered below, and the results must be treated with caution.

4.3.2.4. Survival

The proportion of animals surviving from previous trapping sessions at Orielton ranged from 0.21 to 0.51 (Fig. 4.17). Survival between trap periods was generally

highest between spring to early summer and early summer to late summer, then lower in the autumn. However, the proportion of animals surviving until autumn 2007 may have been affected by fox predation of animals in traps in the previous July. Nevertheless, survival did not seem exceptionally low when compared to the previous year. Over winter survival in 2006-2007 was nearly double that of the previous winter.

On the Skomer grids, survival also tended to be highest during the summer trapping periods, although survival on Grid T in the autumn was exceptionally high when compared to other sites (Fig. 4.18). However, although trapping on Grid T in late summer was not conducted as part of this study, the area was trapped in August by Dr. Tim Healing as part of a long-term population study. Thus, survival estimates for the following autumn are likely to be artificially inflated. Very few animals were captured in spring 2006 on Grid T and this undoubtedly affected survival estimates. More marked animals were captured in early summer than the total number of animals captured in spring on this grid, thus spring survival estimates were adjusted to include animals that must have been marked the previous year. Overall survival seemed slightly higher on the Skomer grids than on the mainland Orielton grid although the high percentage of animals with lost ear tags obviously decreased the precision of survival estimation.

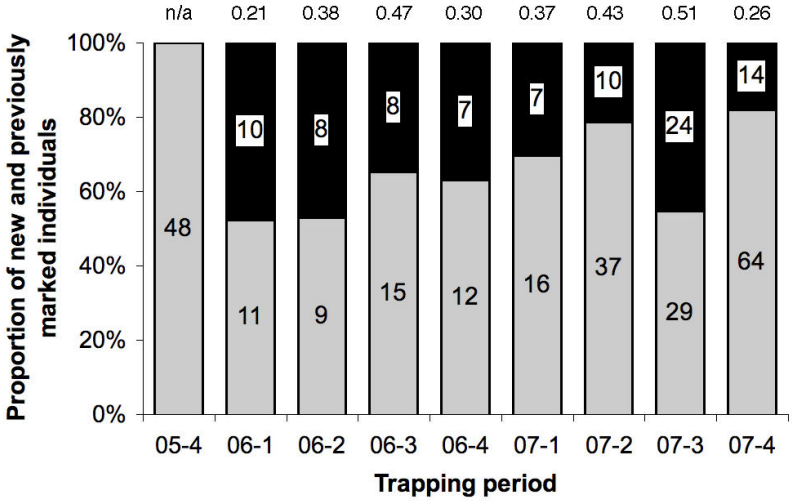


Figure 4.17: Proportion of bank voles newly captured (grey)/recaptured from previous trapping periods (black) at Orielton, Pembrokeshire. Actual numbers of voles are given within bars. Proportion of animals survived from previous trapping period given on tops of bars. Trapping periods; years-season (1 – spring, 2 – early summer, 3- late summer, 4- autumn).

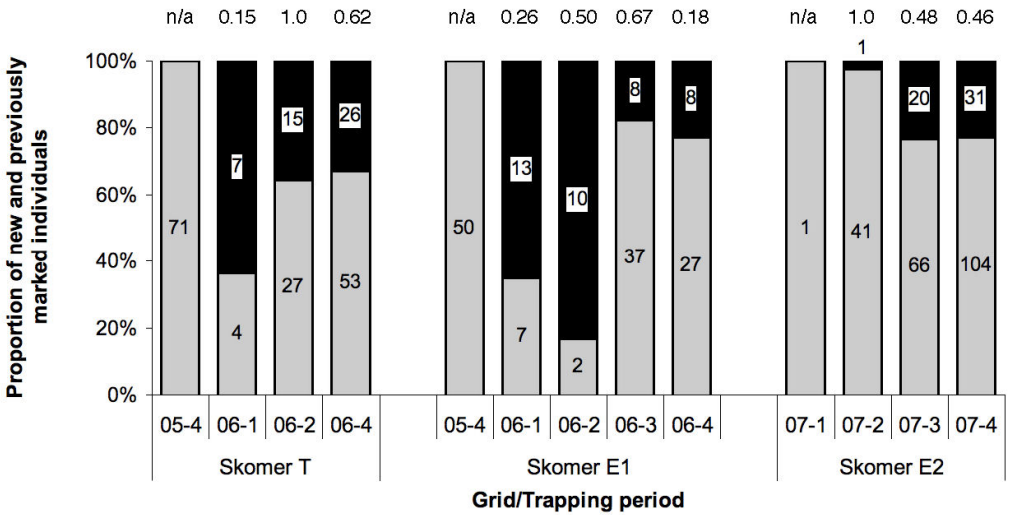


Figure 4.18: Proportion of bank voles newly captured (grey)/recaptured from previous trapping periods (black) for three grids on Skomer Island. Actual numbers of voles are given within bars. Proportion of animals survived from previous trapping period given on tops of bars. Trapping periods; years-season (1 – spring, 2 – early summer, 3- late summer, 4- autumn).

4.3.3. Trap revealed movement

Movements on the Skomer experimental grids E1 and E2 are considered here alongside movements recorded on Skomer grid T and the Orielton grid. Fig. 4.19 illustrates movements on the grids.

4.3.3.1. Range areas (MCP)

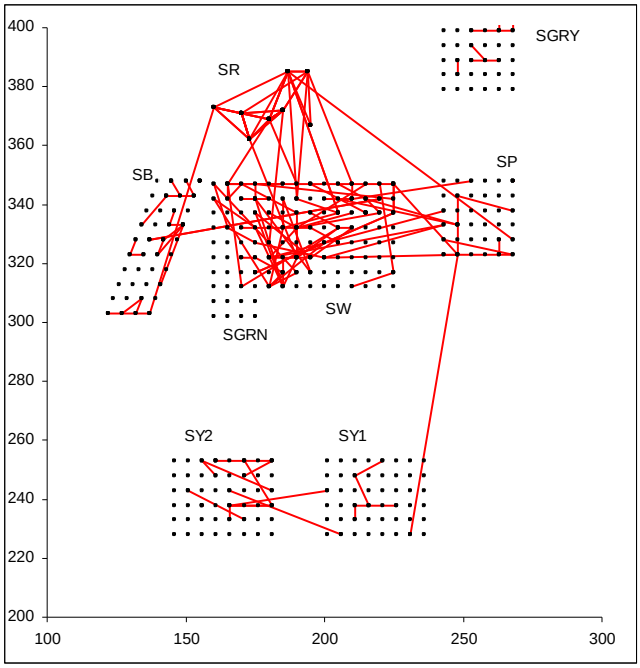
Consistent patterns across all four grids were difficult to identify; only the key points will be highlighted here. Adult male ranges tended to be similar to or larger than females and juveniles on all grids (Appendix Tables 4.1, 4.2). Range areas were largest in May/June on Grid E1 (males), Grid E2 (males), Grid T (both sexes) and Orielton (males only). Male ranges were also large in August on Grid E1 and October 2006 at Orielton. Taking just adults for all periods, Grids T and E1 tended to be larger than grids E2 and Orielton (Fig. 4.21). However, there were no significant differences in median MCP among grids for males (H_3=3.09, P=0.377), but there were significant differences for females (H_3=19.02, P<0.001) with grid T being significantly larger than the other three grids (Dunn's post hoc procedure).

4.3.3.2. Grid activity (D)

Indices of grid activity (D) were positively skewed on all grids, with few high values being recorded. There were few significant differences in the data (Appendix Tables 4.3, 4.4). Again, in general males had higher values than females, except on Grid T where females in May and June had higher values than males. As with MCP areas, Grids T and E1 tended to be larger than grids E2 and Orielton (Fig. 4.22). Again, there were no significant differences among grids in male activity indices (H_3=2.86,

P=0.413) but there were for females (H_3=19.15, P<0.001) with grid T being higher than the other three grids (Dunn's post hoc procedure).

(a) Grids E1



(b) Grids E2

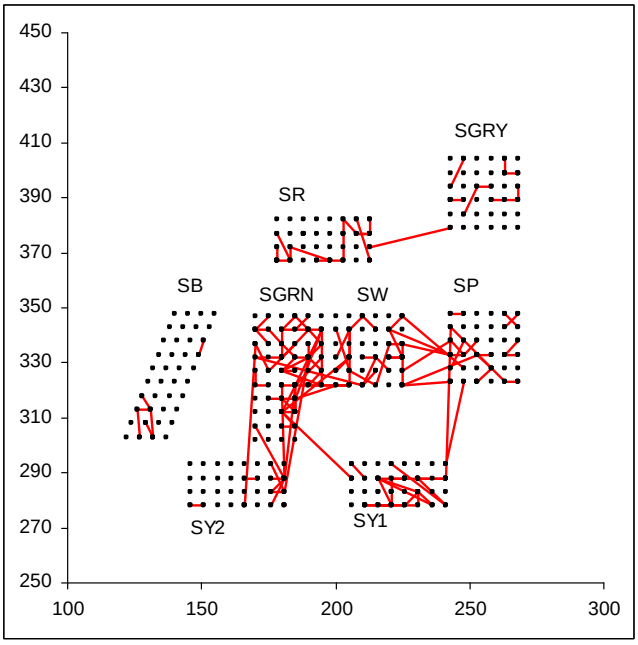
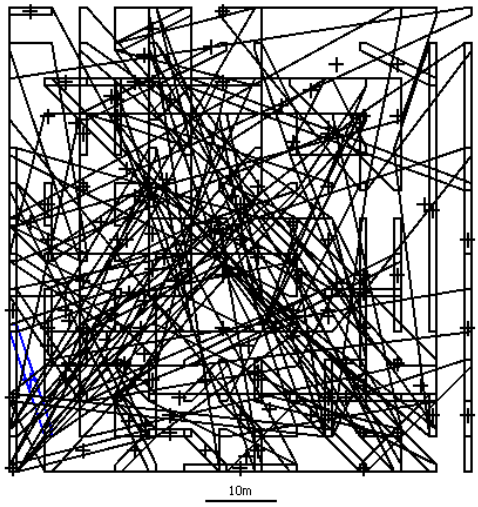


Figure 4.19: Illustrations of inter-trap movements on Skomer Grids E1 and E2 (Ranges 8 ver. 115). Axes scales are in metres.

(a) Grid T – MCP all records



(b) Orielton – MCP all records

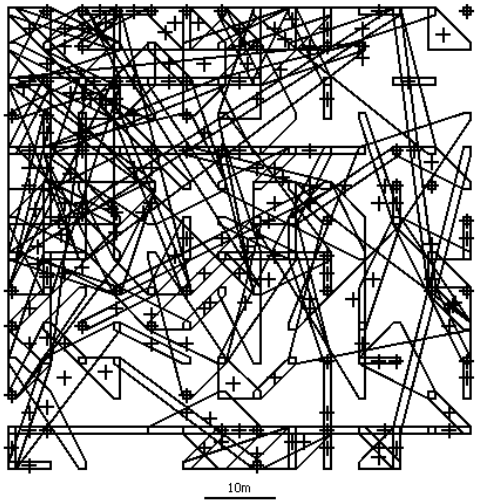


Figure 4.20: Illustrations of movements on Skomer Grid T and Orielton (Ranges 8 ver. 115).

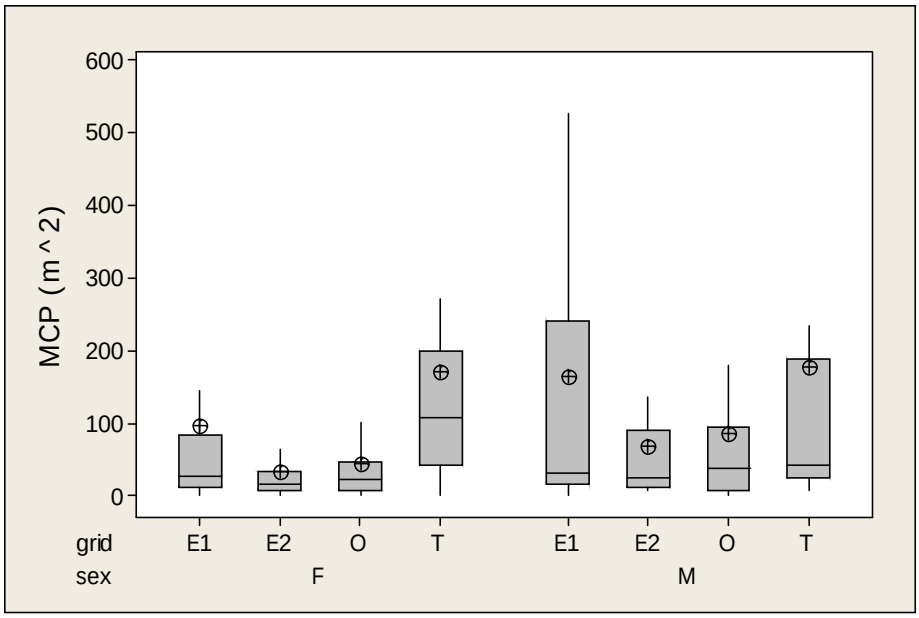


Figure 4.21: Boxplots (without outliers to improve appearance) for minimum convex polygons (MCP) for adult males and females on each grid. E1, E2 and T are Skomer grids, O = Orielton.

Movements on experimental E1 revealed three clusters of grids, SGRY grid at the top of the site in the north east, five in the centre, SB, SGRN, SW, SR and SP and two in the south of the site, SY1 and SY2. No voles moved across open ground between SGRY and the other grids – a minimum span over open ground of 31 m. Only one moved from the central cluster to the SY grids – a minimum span of 71 m (see Fig. 4.19a). There were a lot of short movements within the central cluster of grids, including the grid SR, which mainly consisted of bramble. Minimum gaps separating these grids ranged from 5 m to 18 m. In 2007 on grids E2, again most movement was within the central grids, but also between these grids and the SY grids, which were closer than in 2006. Minimum distances between these grids were 9 m to 18 m. However, there was little or no movement from or to SB, SR or SGRY (minimum gaps 15 m to 31 m) grids despite being within the same patch of bracken as the other grids (see Fig. 4.19b).

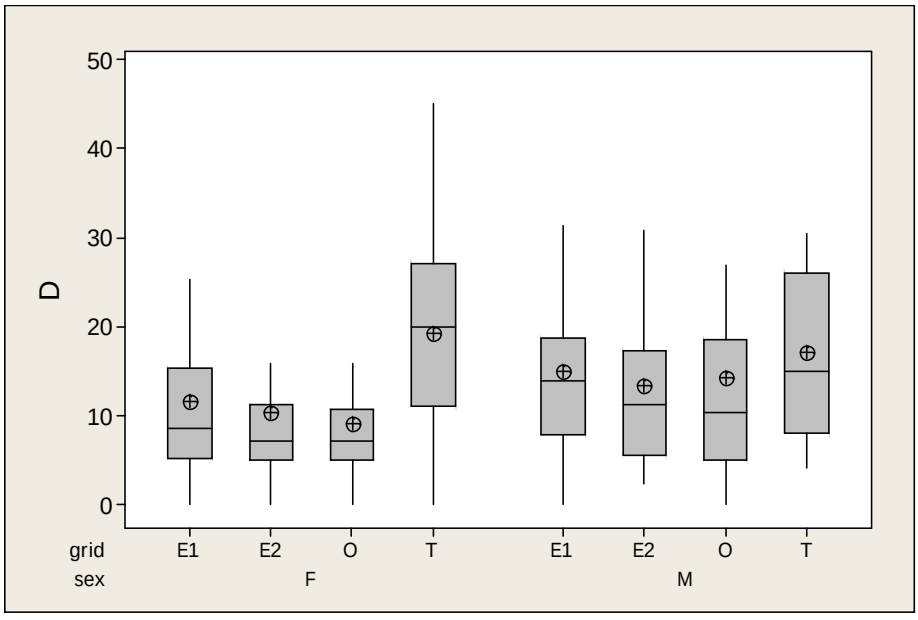


Figure 4.22: Boxplots (without outliers) for grid activity indices (D) for adult males and females on each grid. E1, E2 and T are Skomer grids, O = Orielton.

4.3.3.3. Relationship between MCP and D

MCP and D were significantly correlated on all grids (E1 r_s=0.84, P<0.001, N=86; E2 r_s=0.80, P<0.001, N=120; Grid T r_s=0.73, P<0.001, N=125; Orielton r_s=0.70, P<0.001, N=168; all grids r_s=0.80, P<0.001, N=369; Fig. 4.23). However, there was an increase in variance with scale (Fig. 1.3) and correlations varied considerably between periods without any obvious pattern – these results have not been included.

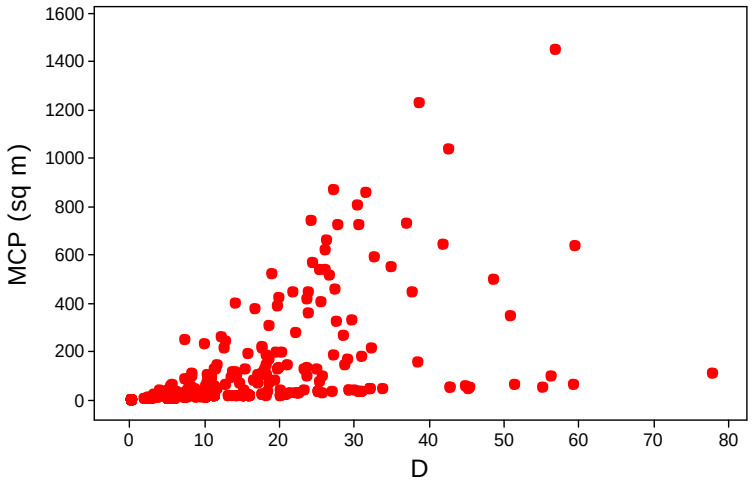


Figure 4.23: Relationship between MCP and D across all sites, r_s=0.80, P<0.001, N=369.

4.3.3.4. Long movements

Although adults (19%) made proportionally more long moves than juveniles (13%) and males (19%) more than females (14%), neither of these were statistically significant (age $F_{1,66}$=3.5, P=0.66, sex $F_{1,66}$=2.48, P=0.120 – data arcsine transformed) (Fig. 4.24). However, there was a significant difference among grids ($F_{3,64}$=6.13, P=0.001) with grid T having significantly more long distance movements than grid E2 and grid O (Tukey post-hoc test), but not larger than grid E1 (grid E1,

mean 20%, stdev.12.3%, N=16, grid E2, mean 11%, stdev.11.9%, N=11, Orielton, mean 12%, stdev.12.8%, N=29, grid T, mean 28%, stdev. 6.4%, N=12). (No interactions were significant in a three-way ANOVA with factors: grid, age and sex.)

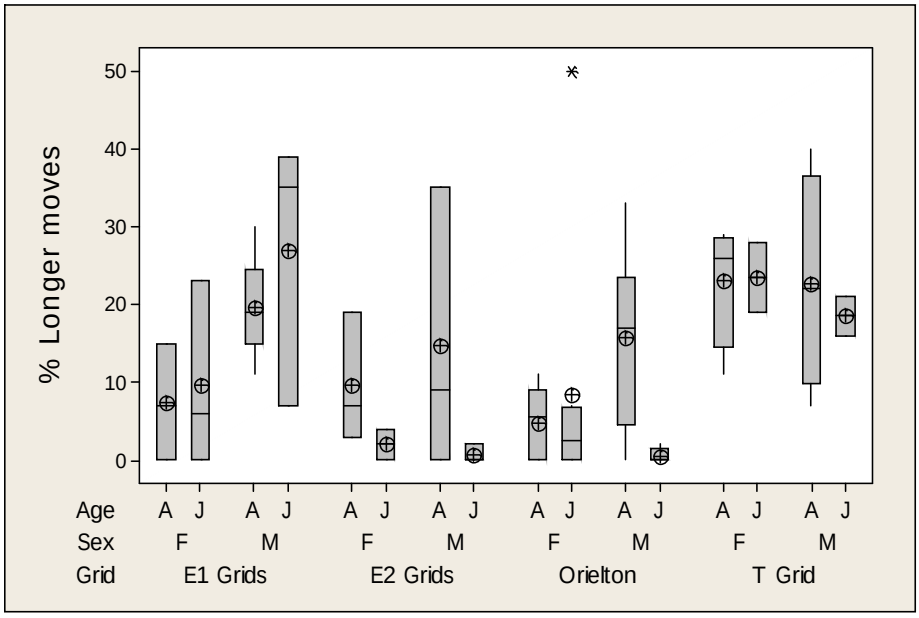


Figure 4.24: Boxplots (without outliers) of the proportion of long moves for each age, sex and grid.

4.3.3.5. Movement and density

Exploratory rank correlation analyses were carried out between mean and median MCP and D values (as indices of movement) and bank vole numbers (M), bank vole biomass, wood mouse M, rodent M and rodent biomass (as indices of abundance) for each sampling period on each grid. Means gave slightly stronger correlations than medians, and so these are the results that are presented in Table 4.10. There were significant negative correlations for all measures of abundance for Orielton except numbers of wood mice (Table 4.10, Figs. 4.25, 4.26). Grids E2 also showed negative correlations between measures of abundance and both MCP and D, but slightly positive correlations were observed for grids E1 and T.

Table 4.10: Spearman's rank correlation between (a) range area, MCP, and (b) grid activity indices, D and mean species abundance^{-ha} (M) and biomass^{-ha}. Mg = bank vole, As = wood mouse. M was calculated using the number of unique individuals captured.

(a)

Population parameter	All (N=21)		Orielton (N=9)		Skomer (N=8)	
	r_s	P	r_s	P	r_s	P
Mg M	-0.235	0.303	**-0.916**	**0.001**	-0.571	0.151
As M	-0.043	0.855	-0.450	0.230	0.286	0.501
Rodent M	-0.213	0.353	**-0.798**	**0.014**	-0.524	0.197
Mg Biomass (g)	-0.09	0.696	**-0.950**	**0.000**	-0.452	0.267
Rodent Biomass (g)	-0.073	0.752	**-0.832**	**0.008**	-0.548	0.171

(b)

Population parameter	All (N=21)		Orielton (N=9)		Skomer (N=8)	
	r_s	P	r_s	P	r_s	P
Mg M	-0.268	0.24	**-0.823**	**0.008**	-0.452	0.267
As M	-0.196	0.393	-0.483	0.194	0.071	0.882
Rodent M	-0.327	0.149	**-0.882**	**0.003**	-0.452	0.267
Mg Biomass (g)	-0.093	0.688	**-0.849**	**0.006**	-0.286	0.501
Rodent Biomass (g)	-0.168	0.464	**-0.866**	**0.005**	-0.500	0.216

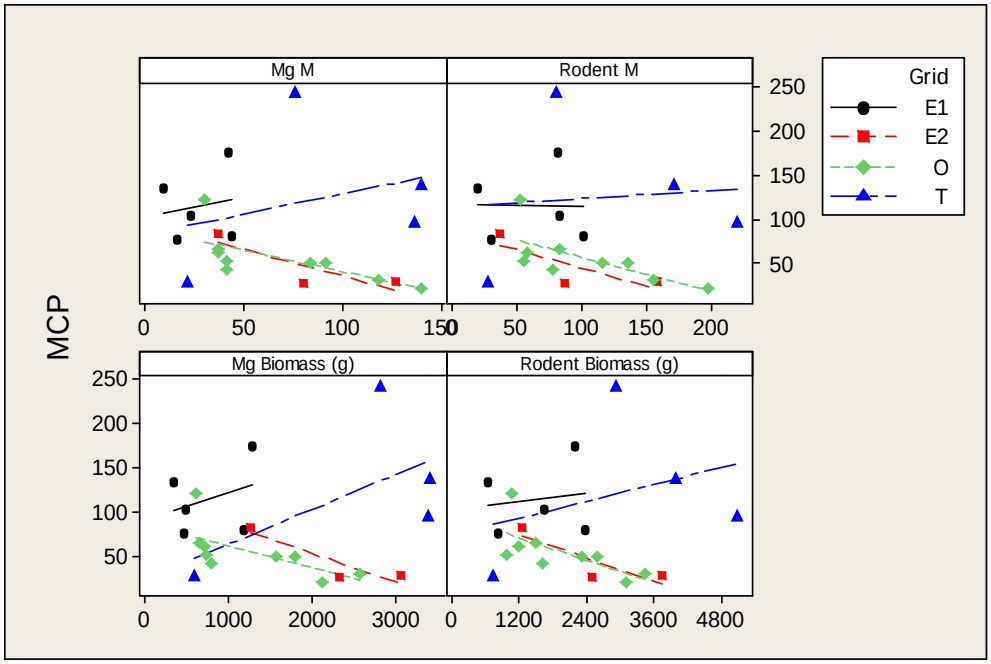


Figure 4.25: Scattergrams with trend lines for each grid between range area (MCP) and numbers of bank voles (Mg M), numbers of rodents (Rodent M), bank vole biomass (Mg biomass g) and rodent biomass (rodent biomass g).

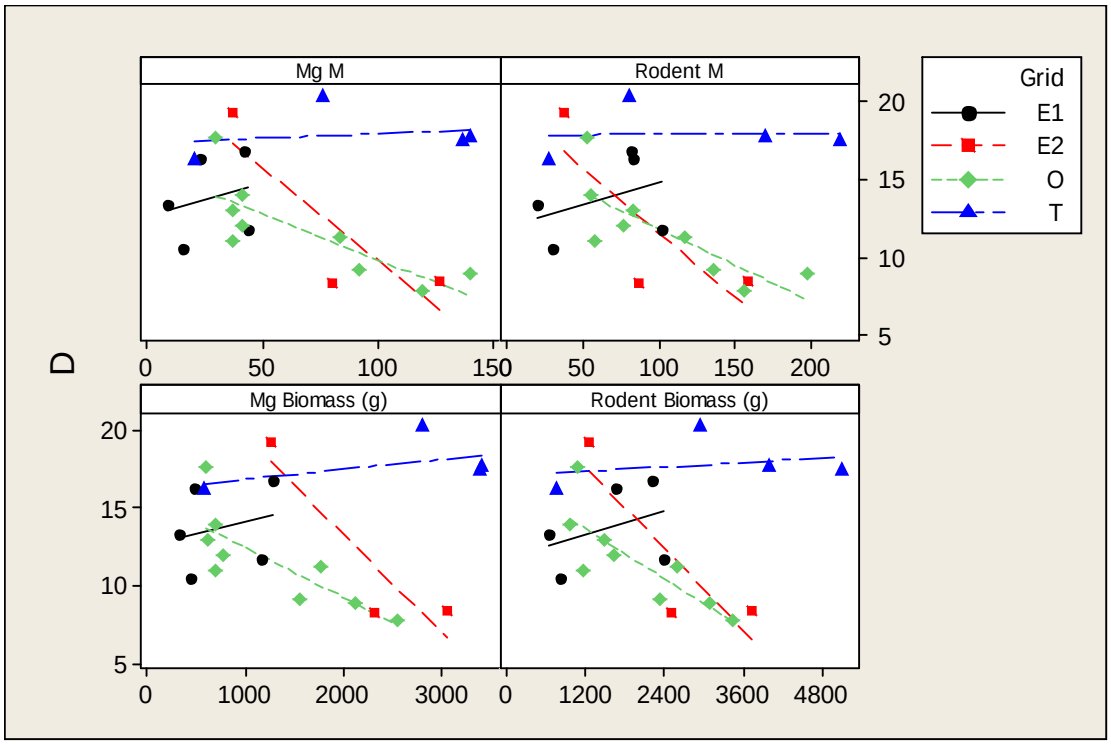


Figure 4.26: Scattergrams with trend lines for each grid between grid activity indices (D) and numbers of bank voles (Mg M), numbers of rodents (Rodent M), bank vole biomass (Mg biomass g) and rodent biomass (rodent biomass g).

4.3.3.6. Movement between trap periods.

There were few recorded movements between trapping periods (Table 4.11). Sixty-eight per cent of moves were < 20 m, and there were only three moves of longer than 50 m: 98 m by an adult female between May and July on E2, 70 m by an adult male between March and June on E1 and 65 m by an adult female between May and June on Grid T.

Table 4.11: Movement between trapping periods for each site.

Grid	Mean	CV (%)	Median1	IQR1	Min	Max	N
Grid T	30	66	30	34	11	65	7
Grids E1	16	140	9.5	7.5	0	70	8
Grids E2	20	138	11	14	0	98	11
Orielton	15	83	11	20	0	40	13

4.4. Discussion

4.4.1. Do island populations reach higher densities than mainland populations?

This study found no evidence to support the theory that island populations of bank voles reach abnormally high densities in comparison to mainland populations. This result was somewhat surprising, particularly on Skomer Island and Jersey where grids were established in sites where some of the densest concentrations of these animals had previously been found. In an island wide-survey conducted by Magris (2000), densities of voles were commonly found to be highest in areas of heathland and grassland and vole densities on the heathland grid used during this study have previously been found to exceed 200 animals per hectare. Long term studies conducted on Grid T on Skomer Island (1971-1983) have recorded densities of up to 475 voles/ha, and peak densities during this 13-year period only twice dipped below 200/ha (Healing, 1984). Thus, it can perhaps be concluded that for reasons unknown, voles on Skomer Grid T (and possibly the Jersey site) were experiencing 'population lows' during the years of this study. To my knowledge there have not been any previous demographic studies of voles on the islands of Raasay and Mull. Therefore it is not possible to tell whether the densities recorded by this study were typical of these populations. However, the presence of field voles on Mull and their significant contribution to overall rodent abundance suggests that it is unlikely that bank voles on this island would reach greatly elevated densities when compared to mainland populations (see below). The equivocalness of the data from this study compared to previous demographic studies highlights the problem of relatively short-term

population studies; it is very difficult to tell what the 'norm' is for a given population without sustained trapping effort over a number of years.

In contrast, the mainland control site at Orielton seemed to experience relatively elevated densities of bank voles in comparison to that previously recorded in British broadleaf woodlands, particularly in autumn 2007. Summer and winter density data from a 34-year study of bank voles captured in Wytham Great Wood, Oxfordshire (see Alibhai and Gipps 1985) showed maximum densities reached or exceeded 75/ha in only three years with the highest density attained at 110/ha. However, the Wytham Woods study used a 1.6 ha grid whilst the Orielton grid trapped during the course of this study covered less than 0.5 ha. Thus, 'edge-effects' may have resulted in slightly inflated densities from employing a smaller trapping area (Flowerdew 1976). Nevertheless, other studies have reported much lower densities. For example, long-term studies of woodland rodent communities in a southern English oak wood reported peak densities of bank voles at ~75/ha and rodents at ~158/ha (John Gurnell, pers. comm.; Gurnell 1981, 1985). Small mammal surveys in broadleaf and mixed woodlands in sites across Britain (1982-1992) produced mean density estimates of 12-24 voles/ha in May-June and 11-34 voles/ha in November-December (Flowerdew *et al*. 2004). Perhaps the reason the Orielton grid was able to support such high numbers of voles was because of the relatively dense ground vegetation. Orielton is home to the only substantially sized area of woodland in Pembrokeshire and deer are conspicuously absent from the site. Studies in Wytham Woods, Oxfordshire have showed a decline in the number of bank voles over the last 50 years and this is thought to be related to a significant decrease in the amount of ground cover

vegetation, specifically bramble; increasing numbers of deer on this site are thought to be a major factor contributing to this decline (Flowerdew and Ellwood 2001).

Bank voles were the most numerous rodents on all sites except for Mull, where field voles were the dominant species. Peak densities of rodents reached 219 animals/ha on Skomer Island, 197/ha at Orielton, 139/ha on Mull, 85 on Raasay and 83/ha on Jersey. Thus, total rodent density was also far less than has been previously reported for Jersey and Skomer (Healing 1984, Magris 2000). Furthermore, it should be noted that in years of peak densities, numbers of field voles on mainland sites can reach in excess of 400/ha (Lambin 2008). Therefore, even in years of peak bank vole numbers, overall rodent densities on these islands may not be significantly higher than those achieved in mainland Britain.

The competitive dominance of field voles over bank voles has been well documented (Shore and Hare 2008) and removal experiments have shown that the presence of field voles can negatively impact on maturation, survival and territory size of female bank voles (Eccard and Ylönen 2002, Eccard *et al*. 2002). The role of competitors in relation to 'island syndrome' is discussed in more detail in Chapter 7. However, it should be noted that on islands that lack *Microtus*, bank voles seem fairly ubiquitous across most habitats with moderate to dense vegetative cover. For instance, on Jersey bank voles appear to reach their highest densities in grassland habitats (up to 369/ha Magris 2000). Peripheral trapping in order to obtain genetic samples suggested that bank voles on Raasay were also probably more abundant in untouched grassland habitats than in the scrubby woodland habitat trapped during the population study.

Thus, if competitive exclusion prohibits bank voles from reaching high densities in mainland grassland habitats, bank voles may simply take over the niche of *Microtus* on islands when this behaviourally dominant species is absent.

4.4.2. Is there evidence of higher population biomass on islands than on the mainland?

Overall I found little evidence to show that islands supported an increased biomass of rodents compared to the mainland, apart from perhaps on Skomer Island. However, this is somewhat unsurprising considering that greatly elevated densities of rodents on the island sites were not recorded. Nevertheless, peak biomass of rodents on Skomer Island did reach over 1.5 times that of the peak rodent biomass reached at the mainland site of Orielton. Thus perhaps it should be concluded that in years of peak vole density, rodent biomass on these islands would probably greatly exceed that reached by an equivalent mainland site in woodland or scrub dominated habitat. As a rough estimate, using mean body weight data for Skomer voles in autumn from this study (Chapter 5, Table 5.4) and assuming a density of 475 per hectare (from Healing 1984) biomass of voles on Skomer could reach excesses of 120,000 g per hectare in peak years. Magris (2000) reported total biomass of small mammals on Jersey to exceed 34,000 g per hectare in some habitats. This is an order of magnitude larger than the maximum biomass found on Jersey during the course of this study. However, following on from the niche expansion in the absence of *Microtus* hypothesis (Section 4.4.1.), using mean weights of field voles in years of peak density from Ashford (2006) and Loughran (1998) and assuming peak densities of 400/ha (Lambin 2008), indicates that field vole biomass on the mainland could reach

over 10,000 g/ha during some years. This suggests that peak rodent biomass on *Microtus*-free islands probably does not greatly exceed that of productive mainland habitats, but that species contributions to overall biomass may change dramatically when a subset of small mammal fauna is present.

4.4.3. Are island populations more stable than mainland populations

It has been suggested that island populations of rodents may achieve more stable densities than mainland conspecifics (Adler and Levins 1994). However the definition of 'greater stability' in this context is unclear. For example, on Crabapple Island in Poland, the lack of a multiannual cycle in bank vole populations (which is common in some northerly populations of bank voles e.g. Hansson and Henttonen 1985, Norrdahl and Korpimäki 2002) was used to describe 'greater stability' of this population (Bujalska 1985). Conversely, other studies have used this term to describe constant peak densities in summer populations (*Microtus californicus* on Brooks Island, California; Lidicker 1973) or year round constant densities (*Microtus breweri* on Muskeget Island, Massachusetts; Tamarin 1978 in Gliwicz 1980). Thus 'stability' is subsequently discussed in the context of the regularity of population cycles and general fluctuations in vole numbers.

Multiannual cycles are not apparent in populations of bank voles in Britain (Shore and Hare 2008) and one may expect populations on the surrounding offshore islands to follow the same trend. For example, annual densities commonly peak in the autumn and declining in the spring (Alhibai and Gipps 1985). However, the causes of multiannual cycles in mainland bank vole populations have been the subject of

much debate, with limitations on dispersal, variability in food conditions, changes in territorial behaviour of breeding females and predation pressure all suggested as possible contributing factors (Hansson 1979, Bondrup-Nielsen and Ims 1988, Erlinge *et al*. 1983, Hansson and Henttonen 1985, Jedrzejewski and Jedrzejewska 1996, Löfgren 2006). Therefore, since selective pressures may differ between mainland Britain and populations on the surrounding islands, it is possible that these factors could result in multiannual cycles in insular populations. However, vole density data from this study revealed a standard annual cycle pattern in all populations except for Jersey, where densities remained fairly constant throughout spring and autumn. However, autumn densities of voles on this site may have been underestimated due to reduced trappability of younger voles (see Section 4.4.6). Whilst annual population fluctuations were much less pronounced on Mull and Raasay than Skomer or Orielton, there seems little evidence to suggest that these island populations differ from the British mainland in terms of annual cycles. However, the lack of long-term data on Mull, Raasay and Jersey makes it difficult to definitively confirm the absence of multiannual cycles. Nevertheless, on Skomer Island evidence from a 30-year population study has shown that voles exhibit irregular annual fluctuations in density (Healing 1984; Healing pers. comm.). Further, by comparing density estimates from previous population studies with the data from this study, it is clear that densities of voles on Skomer and Jersey fluctuate greatly between years (see Section 4.4.1). Since similar unpredictable fluctuations in density can also occur in British mainland populations (Gurnell unpublished; Appendix Fig. 4.1) it seems reasonable to suggest that Jersey and Skomer populations are no more ‘stable’ than those on mainland Britain. If this is the case, then Gliwicz’s (1980) proposal that stability in confined

populations may be attributable to density-dependant changes in individuals' behaviour remains debatable. Since the dispersal of animals in insular populations is restricted and only a subset of competitors and predators are present compared to mainland populations, she argues that changes in reproductive behaviour and dispersal in response to resource availability may lead to greater stability in island populations. Conversely, if individuals are unable to respond appropriately to increased population densities in the absence of dispersal opportunities, numbers may build up to exceed the carrying capacity of the environment and subsequently crash, a phenomenon known as the 'fence effect' or 'Krebs effect' (Krebs *et al*. 1969, MacArthur 1972). However, long-term data from Healing (1984) seem to suggest that Skomer voles can maintain relatively high densities for sustained periods, contrary to predictions of the Krebs effect. Thus the population 'low' noted by this study may have resulted from an entirely different phenomenon than those previously discussed. For example, it is possible that an outbreak of disease may have caused the crash in vole numbers witnessed by this study. In which case, the population may ordinarily persist at high densities and extrinsic factors may be the cause of periodic population crashes.

It is notable that island-wide studies on Skomer and Jersey have shown that densities of small mammals are not consistent across habitats (Fullager *et al.* 1963, Healing *et al* 1983, Magris 2000, this study). Previous studies demonstrating 'greater stability' in island populations have largely been restricted to much smaller islands than those investigated during the course of this study (e.g. Crab-apple Island – 4 ha, Brooks Island – 26 ha, Muskeget Island – 120 ha). Thus it is possible that in larger islands

with heterogeneous habitat a source-sink dynamic works in a similar way to that of the mainland. However, Healing (1984) did not find support for the source-sink hypothesis from experimental removal studies of voles on Skomer. It is clear that further studies on the dynamics populations of small mammals on islands of different sizes and habitat composition are required to understand what limits (or regulates) numbers.

4.4.4. Factors affecting capture success

Factors such as weather conditions, trap availability and differential trappability amongst individuals can potentially affect density and biomass estimates produced by small mammal live-trapping studies (Gurnell and Flowerdew 2006). Thus, the influences of these variables are reviewed below, with particular reference to any 'anomalies' apparent in the population data gathered during this study.

4.4.4.1. Weather conditions

There was a significant association between capture rates and overnight cloud conditions, suggesting that surface movement of small mammals was greater during nights with high cloud cover (> 50%). This pattern is commonly observed during small mammal studies because of an increased predation risk associated with clear, moonlit nights (see Griffin *et al.* 2005 and references therein). However, increased capture rates after overnight rainfall was probably an effect of the association between high cloud cover and rainy conditions. Whilst low overnight cloud cover was rarely observed for an entire trapping period, there were a few occasions when weather conditions could have influenced vole density estimates. For example, low

numbers of captures observed at Orielton in August 2006 could have been attributable to low overnight cloud cover, which was < 30% for the first three morning trap rounds. However, densities of voles were quite low for that entire year suggesting weather conditions probably did not result in greatly underestimated densities. Furthermore, captures of new individuals did not seem particularly low when compared to other trapping sessions conducted at this site during 2006, although this was mainly due to an influx of new animals captured on the last morning following an increase in overnight cloud cover.

Low overnight cloud cover probably affected density estimates for the autumn 2007 trapping session on Jersey, when cover was 0% for three out of four nights and only 30% on the fourth night. Subsequent trapping of this grid directly after the population study had finished revealed a large number of unmarked animals that were presumed present but not captured during the standard four day trapping procedure. This capture of unmarked animals was concurrent with increased overnight cloud cover.

Exceptionally low capture numbers were observed during March 2007 on Skomer Grid E2. Whilst this could perhaps be attributed to low cloud conditions for four out of the eight trap nights, it is more probable that surface movements of animals were restricted at this time of year due to sparse vegetation cover.

Interestingly, despite a subset of nocturnal predators present on the four islands (Chapter 2) response to overnight cloud cover did not change between island and

mainland populations. Thus the presence owls on these islands (for example, little owls (*Athene noctua*) and barn owls (*Tyto alba*) on Skomer, long-eared owls (*Asio otus*) and barn owls on Jersey) may be sufficient to maintain nocturnal predator avoidance behaviour on these islands.

4.4.4.2. Trap availability

On several occasions the number of occupied traps during a trapping session exceeded 60%. This may well have led to the underestimation of rodent densities for Skomer and Orielton because unmarked animals may not have encountered open traps (Gurnell and Flowerdew 2006). Regrettably, a restriction in the number of available traps meant that further traps could not be added to each point on these occasions. High occupancy rates were particularly a problem on Skomer Grid T in autumn 2006 when the percentage of traps shut was 80%. A lack of available traps may also partially explain the discrepancy between the densities of voles observed during this study and those of Healing (1984). The high densities recorded by Healing (1984) were achieved by trapping the grid in stages, covering two lines at a time and using up to 10 traps per point. Whilst this method is undoubtedly superior for obtaining accurate density estimates, limited time and resources prevented the use of this technique for this study.

4.4.4.3. Trappabilty

The significant association between recapture rates of voles and site suggests that animals from different populations are not equally trappable, particularly because there was no consistent relationship between the total numbers of captures and

recapture rates. These potential differences in animal responses to traps may have affected density estimates. For instance, recapture rates were comparatively high on Jersey and Skomer Grid E1 and low on Mull and Skomer Grid E2. Furthermore, the imbalance in recapture rates between the Skomer grids shows that even within a population, trappability may be dependent the habitat.

Krebs (1989) suggested three reasons why unequal catchability may occur in populations of small mammals: (i) animals may behave differently in the vicinity of traps with some individuals readily entering whilst others exhibit 'neophobia' and behave is a more cautious manner; (ii) previous experience of traps may affect trappability, with some animals becoming 'trap-addicted' because of food provisions placed in traps whilst others may develop an avoidance response to traps; (iii) the position of traps may result in unequal opportunities for capture, for example social interactions such as territorial behaviour may reduce an animal's access to a trap.

It is possible that the high recapture rates observed on Jersey resulted from an age-related trappability bias in this population, where younger voles were frequently uncatchable. The smallest vole captured on Jersey during the course of this study weighed 17 g and in total only four animals weighing less than 20 g were captured during three autumn trapping sessions in 2005-2007 (data not shown). Whilst this may have been a trap sensitivity issue, the concurrent capture of juvenile mice and shrews (with a minimum weight of 7.5 g), in traps without shrew holes, suggests that trap treadles were sufficiently sensitive to capture smaller voles. This may suggest that young animals were less likely to enter traps than adult animals. If this is the

case, the proportion of unmarked animals may have been significantly underestimated thus artificially inflating recapture rates.

An age-related trappability bias may also explain the apparent lack of an annual density cycle in the Jersey population (Section 4.4.2). Spring trapping on Jersey commenced in May and autumn trapping was conducted in September/early October. Although the breeding season for voles on Jersey was clearly underway by May (see Section 4.4.7.), no juvenile voles were captured during this period so 'spring' capture numbers were not inflated. However, if 'trap-shyness' is exhibited by the younger animals, this may have led to underestimated autumn densities because voles from litters produced in mid to late summer may not have been captured. Whilst, juvenile voles (identified by pelage properties) were very rarely captured in any populations, the presence of shrew holes in traps combined with low sensitivity of some trap treadles make it probable that animals of less than 10 g would escape (J. Gurnell, pers. comm.). Thus it is difficult to say whether an age-related trappability bias exists in other populations.

There was a significant association of recapture rates between trapping periods at Orielton, with significantly higher recapture rates in autumn 2005 and spring 2006 and lower recapture rates in late summer 2007. The latter incident is most easily explained because trapping was aborted after five trap rounds when a large proportion of the traps appeared to have been depredated by a fox (see Section 4.2.1.1). Thus, the shortened trapping session probably accounted for lower recapture rates. This incident may also explain the lower survival estimates of voles

on this site the following autumn. Conversely, the perceived excess of recaptures autumn 2005 and spring 2006 is more difficult to interpret. It is notable that numbers of voles were much lower in 2006 than following year, when animals appeared to have been breeding in very early spring. Since extended breeding seasons in small mammals can occur when conditions such as food supplies are sufficient (Alihbai and Gipps 1985) and there is a strong association between trappability and availability of natural food supplies in some species (e.g. Gurnell 1996) it may be possible that limited natural food resources in autumn 2005-spring 2006 made animals easier to trap. However, given that food availability was not measured during the course of this study, it is not possible to substantiate this theory.

Following on from this idea, it is possible that animals may be more prone to enter traps in suboptimal habitat than preferred habitats where food resources may be less limited. This is one potential explanation for the increased recapture rates experienced on Skomer Grid E1 relative to Grid E2 and Grid T. However, the suboptimality of this habitat (indicated by relatively low densities of voles) may be more far related to increased predation risk than it is to food availability. Sparse vegetation on this grid system probably increases the risk of predation by avian predators (Fullager *et al.* 1963) thus confining animals to patches of bracken where the mini-grids were situated. Thus, it is probable that animals in this habitat were more likely to encounter traps than in Grid E2 where areas of continuous bracken cover probably allowed more frequent movements between mini-grids. Alternatively, variability in recapture rates of voles on the Skomer grids may simply be a consequence of an increase in trapping effort on Grid E1 relative to Grid E2.

A significant sex bias was detected in the number of voles captured in spring 2006 on Raasay, with 12 males captured and only a single female. Whilst this suggests females are not very trappable at this time of year and therefore population estimates for this trapping session may be greatly underestimated, the same bias was not observed the following spring.

Vegetation height and cover was found to effect capture success although interestingly, the effects were much more pronounced in some populations that others. For example, vegetation appeared to heavily influence the captures of Skomer voles but not those at Orielton. The habitat preferences of bank voles in relation to ground vegetation cover are well documented (Shore and Hare 2008). However, I suggest the variability in response to vegetation cover between populations may be best explained by varying predator pressure between sites. The habitat on Skomer is very open and as such, the ground layer of vegetation provides the only cover from avian predators. Skomer voles are particularly susceptible to avian species such as kestrels (*Falco tinnunculus*) and buzzards (*Buteo buteo*), which are plentiful on this island. Conversely, voles inhabiting woodland areas, such as the population at Orielton, have a further layer of cover to protect them from avian predators, the canopy. Furthermore, mainland voles are likely to experience significant pressure from ground predators such as weasels (*Mustela nivalis*), which themselves use vegetation cover to avoid detection by other predators (McDonald and King 2008). Thus the benefits of sticking closely to areas of dense cover may not be so advantageous in a woodland population. It is also true that the voles at the

Orielton site may well use leaf litter cover in the absence of ground vegetation and thus movement may not be correlated with average vegetation height (AH).

The differences in trappability of animals are a serious problem in studying small mammals and large proportions of the population could potentially go unrecorded if animals continuously exhibit an unwillingness to enter traps (Taylor *et al.* 1981). Anecdotal evidence from sand traps positioned adjacent to Longworth traps on Jersey, suggested a great deal of small mammal activity may occur in the immediate vicinity of traps without resulting in a capture (Fig. 4.27)!

Figure 4.27: Sand trap left overnight outside Longworth trap on Jersey autumn 2005. Multiple small mammal footprints can be seen but the trap positioned at this point and the neighbouring trap were empty.

4.4.4.4. Are island populations more trappable?

Recently many behavioural studies have started to consider the role of personality in activity patterns of animals suggesting that traits such as boldness/shyness may be maintained in a population by life history trade-offs such as productivity, fecundity and survival (reviewed by Biro and Stamps 2008). Meta-analyses have demonstrated that in many species boldness is linked to increased reproductive success but decreased survival (Smith and Blumstein 2008). Thus in island populations where the risk of predation may be lower than in comparable populations, one may expect to see an increase in the 'boldness' of animals. Whilst there was a significant association between recapture rates and site during this study, disentangling this information to make assumptions about personality traits of a population is difficult. Elevated recapture rates, for example, could result from two opposing scenarios; (i) the majority of individuals within a population exhibit an increased willingness to enter traps (ii) a large proportion of the individuals in the population rarely enter traps but a small proportion of individuals readily do so (see Gurnell 1976, 1982, Taylor *et al.* 1981). The first scenario implies increased boldness at the population level whilst the second implies boldness of some individuals but increased shyness at the population level. Because there is no way to estimate the size of the 'uncatchable' population, recapture data alone cannot be used to judge the boldness/shyness of a population. Thus it is not possible to answer the question of whether island voles are more trappable than mainland conspecifics. Nevertheless, there are several interesting questions that can be raised with regards to island populations and personality and this subject area definitely warrants further research (this topic is further discussed in Chapter 7).

4.4.4.5. Do island populations have shorter breeding seasons than mainland populations?

Rodent populations on islands may experience shorter breeding seasons and/or delayed maturation when compared to mainland conspecifics. This pattern has been reported in *Microtus breweri* on Muskeget Island, Massachusetts (Tamarin 1977), *Peromyscus maniculatus* on Samuel and Saturna Islands British Columbia, (Sullivan 1977), *Myodes glareolus* on Skomer Island (Jewell 1966, Healing 1984) and *Myodes glareolus* on Jersey (Bishop and Delany 1963). It has been hypothesised that changes in reproductive strategies in insular populations may be linked to density regulation mechanisms (Adler and Levins 1994). Thus, it follows that breeding seasons in island populations may show some plasticity according to current population trends. Indeed, studies of reproductive strategies of Skomer voles by Coutts and Rowlands (1969) reported that the breeding cycle was non distinct from the mainland population during the years of their study (contrary to findings of Jewell 1966 and Healing 1984).

This study was not specifically designed to study reproduction in island populations and as such, there were several problems in attempting to estimate the length of breeding seasons. Firstly, because of the logistics of trapping five geographically distant sites for periods of at least four days, trapping lasted for a significant proportion of the spring and autumn months. This caused a considerable problem when comparing breeding data between sites because the first site in 'spring' had been trapped in March and the last site was trapped in May. A similar conundrum existed for autumn data. Secondly, the non-destructive sampling methodology

employed by this study made it difficult to ascertain whether females were truly 'breeding' (i.e. in stages of litter production) rather than just showing signs of sexual activity (i.e. had perforate vaginas) unless they were palpably pregnant or lactating. Conversely, the aforementioned studies all employed dissection techniques to check breeding status in a proportion of the captured animals and thus must be considered more accurate. Thirdly, three out of the five populations were only trapped twice a year in spring and autumn, thus if breeding had not already started/finished by the time trapping occurred there was no way to tell how long the season persisted.

Since very few juveniles were captured in traps calculating probable conception dates for the first young-of-the-year was not possible for most sites. Also, the breeding status of males may not be particularly informative in terms of defining breeding seasons because testes size may not reflect epididymal spermatozoa, especially at the beginning and end of the season. Therefore, the discussion of 'breeding seasons' below will refer to female maturation status.

Despite the aforementioned caveats, there was still a great deal of evidence to suggest that breeding seasons on islands were not especially different to that of mainland populations. For example, during the trapping session on Jersey in May 2006, all female voles captured showed signs of litter production (i.e. were palpably pregnant or lactating). This suggests that the breeding season on this island had reached a peak by this time. This is entirely consistent with the May peak in pregnancy rates experienced by southerly populations of bank voles on the British mainland (Delany and Bishop 1960). The following May however, fewer females (20%) showed

definite signs of litter production but all showed signs of sexual activity and therefore some may well have been in the early stages of pregnancy. Autumn data suggested the breeding season had finished by October in 2006 and 2007 but may have continued later in 2005. These data are in contrast to the findings of Bishop and Delany (1963) who reported delayed breeding in the Jersey population, starting in late April or early May, reaching a peak in litter production in June and continuing into October.

On the Scottish Islands, there was evidence of litter production occurring on Raasay from April through until at least the middle of September. Defining the breeding season on Mull was more problematic because pregnant and lactating bank voles were only captured on Mull in early September 2007. However, perforate animals were captured in late April 06 suggesting the breeding had probably commenced. Unfortunately, very few bank voles were captured in spring of either year, therefore these data may be unrepresentative of the island as a whole. It is notable that the breeding season of the bank voles on Mull does not appear to be synchronous with that of the field voles, the majority of which most showed signs of sexually activity in April of both years of trapping. Delany and Bishop (1960) reported no distinction in breeding seasons for both of these island populations in comparison to Scottish mainland populations, but concluded that breeding appears to start later in Scotland than southern England, beginning in May, reaching a peak in June and declining rather abruptly thereafter. However, the data from this study suggest that breeding can begin earlier on Raasay.

Comparisons between the breeding status of Skomer voles and those from Orielton showed that the two populations were remarkably synchronous throughout most of the 2.5 years of the study. The minor exceptions to this were in October 2005 when a small proportion of voles on both Skomer grids showed signs of litter production but breeding at Orielton had apparently stopped by this period, and the following year this trend was reversed. The most notable difference between the two populations was in spring 2007, where nearly 40% of females were showing signs of litter production on the mainland site during the first week of April. Unfortunately, no female voles were captured during the equivalent visit to Skomer making it difficult to ascertain whether breeding also started early that year in this population.

Using the data from this study and comparing it to that of previous studies (Delany and Bishop 1960, Bishop and Delany 1963, Jewell 1966, Coutts and Rowlands 1969, Healing 1984) it seem clears that there may be a great deal of plasticity in the breeding seasons amongst island populations. This study found no evidence to suggest that breeding seasons were shorter than in mainland populations on any of the islands but it is notable that elevated densities of animals were also not recorded during the years of this study. Gliwicz (1980) suggested that enclosed populations, such as those on islands, may be able to maintain densities by self-regulation. If this is correct then perhaps restricted breeding seasons are a mechanism for regulating numbers in years of high density. Thus it follows that in years of low density, breeding patterns may more closely mirror those of mainland populations.

4.4.4.6. Is survival higher on islands than the mainland?

It has been suggested that increased densities experienced by some insular populations may be a result of higher survival rates due to decreased predation (Adler and Levins 1994). Delany and Bishop's (1960) studies looked at age structure of Jersey, Mull and Raasay populations using teeth to age individuals. They found, for example, that older individuals tended to survive much later on these islands. However, unfortunately the substantial time-lag between trapping periods meant that it was not informative to produce survival comparisons for Mull, Raasay and Jersey and there was no means to age the animals accurately enough to carry out an age structure or cohort analysis. Thus survival data only included a comparison between the mainland Pembrokeshire population at Orielton and Skomer Island populations on the three trapping grids (E1, E2 and T).

The biggest complication in measuring survival was that many of the animals lost ear tags between trapping periods and thus only the proportion of marked or unmarked animals could be calculated rather than tracking the survival of individual animals through the seasons. Survival seemed to be greatest in both populations from spring to late summer and then decreased in late summer to autumn. This is perhaps consistent with the death of adults after the breeding season but may simply indicate the dispersal of juveniles away from their natal grounds. Overall survival was probably slightly higher on the Skomer grids than it was on the mainland site. However, survival estimates for Skomer Grid T may have been slightly inflated in comparison to other grids because of trapping by other small mammal workers.

4.4.4.8. Skomer and habitat patches

Previous work on Skomer suggested that the distribution of voles was closely linked with the presence of bracken (Fullager *et al*. 1963, Healing *et al.* 1983) which is patchily distributed over the island (Chapter 2). The small experimental trapping grids were designed to see how the voles respond to bracken patches: in 2006 each grid was in a patch of bracken (grids E1), and the patches were separated by open habitat. In 2007 the same pattern of grids was set up in continuous bracken to see if voles moved through bracken in the same way as across open patches (grids E2). The study was not designed to look at long range dispersal (see Stenseth and Lidicker 1992), and indeed only three movements of > 50 m (98 m, 70 m and 65 m) were recorded between trap periods. In fact, over two thirds of all movements recorded between trap periods were < 20 m, with an overall mean of 16 m for grids E1 and 20 m for grids E2. From the current and previous studies carried out on Skomer (e.g. Healing 1984), it is clear that dense bracken patches are favourable habitat, but further studies would need to be carried out to assess what could be described as suboptimal habitat.

Of course, looking at population averages rather than individual movements can be misleading with respect to individual variability and selection (Bowers *et al*. 1996). Some individuals moved between grids in 2006, but most of these were between the closest grids in the middle of the study area. Thus, the evidence suggests that voles occasionally crossed open habitat, but it was rare. Some small grids appeared isolated in 2007, despite all grids being within the same habitat patch. Again, most moves were short. In all, the results suggest an absence of bracken over a gap of say > 20 m

restricts movement between patches, and that voles within bracken patches have small ranges. In fact, they appear reasonably sedentary. It would be interesting to know more about the social organisation of voles within bracken patches, especially in relation to increases in population density. For example, could a permanent move of, say 30 m, by a young animal away from its natal site be considered a dispersal movement. Healing (1984) has suggested that a social fence operates (Hestbeck 1982) at high densities. Further specific studies would be needed to detect longer dispersal movements, although this would be labour intensive and with a low return if live trapping methods were used.

With respect to animal densities, grids E1 (patchy habitat) seemed unable to support as many voles as grids E2 (continuous habitat) as previously suggested (Healing 1984). However, why these patches are able to support fewer voles is still questionable. It is hypothesised that patchy bracken may greatly increase the risk of predation from avian predators. Unfortunately, the data from this study were not sufficient to address the question of whether survival was lower in patchy habitat.

4.4.4.9. Movement of voles on Skomer and at Orielton

The spatial scale of sampling devices can affect the results obtained. The trapping scale in these studies was 5 m, but traps were rotated around a 5 m square after each night of trapping to reduce the trap position effect. Stanford (1996) was one of the first people to use this technique and found that inter-trap movement was larger on grids that moved traps than those where they were not moved. Ranges at both Orielton and Skomer were small compared to those recorded in the literature (Table

4.12) and this may be partly due to the methods used. However, comparisons between Skomer and Orielton are interesting. Female ranges were smaller than males at both sites, but Skomer female ranges were considerably larger in each season compared to Orielton (Table 4.12). There was a negative relation of range size and animal density at Orielton but not overall on Skomer (although a negative relationship was evident for grids E1). Gurnell and Gipps (1989) found a weak negative relationship between movement and density from their studies on woodland rodents in Surrey. The reasons for the lack of a density effect on Skomer, particularly grids E2 and T, maybe because densities did not reach high levels during the time of the study.

There was a correlation between range area and D, the mean distance moved between captures, used here as an index of grid activity. D values tended to be higher on grids T and E1 than Orielton and grids E2, but these differences do not appear large. Above ground activity on Skomer does appear restricted during the winter/early spring months (this study, Healing 1984), and this is probably related in part to vegetation cover. At other sites and other times of the year on Skomer, the evidence from the vegetation analysis suggests, as expected, that voles move in areas with more ground cover, and there was no particular differences between the island and the mainland sites. Further, it was hoped that the analyses on trappability grid activity and movement might reveal difference in personality, e.g. aggression, boldness (see Stamps 2007, Boon *et al.* 2008, Careau *et al.* 2008) that are associated with island syndrome. However, it would appear that further, specific studies on behaviour are required to test this hypothesis.

Table 4.12. Minimum convex polygon (MCP) range areas from the literature.

Habitat	Season	Method	MCP (m^2)		Author(s)	N	
			M	F		M	F
Decid woodland	All	Trap	1674	1292	Brown 1956	41	36
Decid woodland	All	Trap	2208	1124	Kikkawa 1966	47	34
Decid woodland	All	Magnet tracking	2000	600	Bergstedt 1966	45	24
Conif/decid plantation	All	Trap	1497	600	Crawley 1969	39	24
Decid woodland	Winter	Tracking plates	1209	1067	Cody 1982	35	33
Decid woodland	Summer	Tracking plates	1398	953	Cody 1982	37	31
Decid woodland - Alice Holt	Winter	Trap	380	261	R. Gill, J.H.W. Gipps & J. Gurnell (in Wolton and Flowerdew 1985)	19	16
Decid woodland - Alice Holt	Summer	Trap	929	271	R. Gill, J.H.W. Gipps & J. Gurnell (in Wolton and Flowerdew 1985)	30	16
Orielton	Spring	Trap	64	42	This study	25	17
Orielton	Summer	Trap	99	47	This study	13	19
Orielton	Autumn	Trap	122	32	This study	8	6
Skomer	Spring	Trap	92	56	This study	17	17
Skomer	Summer	Trap	187	97	This study	49	56
Skomer	Autumn	Trap	98	92	This study	31	19

4.5. Summary

1. This study investigated demographic parameters of four island populations and one mainland population of bank voles to see whether (i) populations conformed to patterns predicted by the 'island syndrome' hypothesis, and (ii) what the possible causes of island syndrome may be.
2. There was no evidence to suggest that voles reached abnormally high densities with respect to mainland populations. However this finding is inconsistent with previous studies, suggesting vole populations on at least two

of the islands may have been experiencing a population 'low' during the years of this study.

3. Vole biomass was not significantly greater on the islands than the comparable mainland site, with the exception of Skomer Island. However, island vole biomass was predicted to significantly exceed that of an equivalent mainland site in years of high vole density.
4. When total rodent biomass is considered, it is likely that peak biomass levels on islands are roughly equivalent to that of peak biomass in mainland sites containing field voles. This suggests support for the 'density compensation hypothesis'.
5. There was little evidence that vole populations on islands were more stable than mainland populations, although the interpretation of 'stability' in the context of small mammal studies is debatable.
6. Several factors were found to influence capture success; weather conditions, trap availability, vegetation cover and an apparent trappability bias in some populations. The influence of these factors on estimating animal densities is considered.
7. There was no evidence to suggest shortened breeding seasons on islands, contrary to previous studies. Thus, it was concluded that there is some plasticity in reproductive seasons and this is likely to be related to animal densities.
8. Survival comparisons between Skomer and the mainland site were greatly hampered by loss of ear tags and other methodological problems. However, overall survival was slightly higher on the island site.

9. Movement studies suggested Skomer voles were fairly sedentary and patchy vegetation cover along with a possible ‘social fence’ effect may restrict long distance movements.
10. Trap-revealed ranges for both Skomer and Orielton voles were smaller than published estimates although this may have partially been due to methodological differences.
11. Overall there was little difference between movements on mainland and island sites although some differences in female range size were noted.
12. Future research should focus on the personality of animals in relation to population dynamics, and how this may relate to other aspects of island syndrome.

Chapter 5: Body Size

5.1. Introduction

Island populations are often morphologically distinct from their mainland counterparts. One of the most frequently observed peculiarities amongst island races is a change in body size in comparison to mainland conspecifics. This trend has been described in many different vertebrate taxa (e.g. Lomolino 2005, McClain *et al*. 2006, Millien 2006, Meiri *et al*. 2006, 2008, Welsh 2009, Olson *et al*. 2009) and has been termed 'the island rule' (Van Valen 1973). This rule describes the tendency for small animals to evolve larger size on islands whilst large animals become smaller.

However, recent evidence suggests that the island rule does not hold true for all mammals (Meiri *et al.* 2008). These authors collated data sets on body weight from the published literature, finding that, when phylogeny was controlled, only specific clades of mammals show a significant tendency towards differential size evolution on islands. Carnivores, heteromyid rodents and artiodactyls were found to have a significant tendency towards insular dwarfism, whereas rodents of the family Muridae[1] had a highly significant ($P<0.001$) tendency towards gigantism on islands.

The frequency of differential size evolution on islands has led many to assume that it is an adaptive response to selective pressures, which may either operate during the process of immigration or following colonisation (Lomolino 2005). Some of the suggested selective pressures that may drive this pattern of evolutionary divergence (reviewed by Dayan and Simberloff 1998) are outlined below. In light of the findings

[1] Whilst bank voles belong to the family Cricetidae, this species, along with three other species from the genus *Myodes*, were included as murids in the analysis performed by these authors (under the former name *Clethrionomys*).

of Meiri *et al.* (2006), I have restricted these selection pressures to those that are of particular importance to small mammals.

For terrestrial mammals to successfully populate an island, they must first traverse an oceanic barrier and then find suitable habitat in which to live. Thus, successful migration and colonisation of islands may be heavily dependent upon an individual's energy reserves and larger individuals may be better equipped to survive colonisation attempts, resulting in a genetic bias in the founding populations (Lomolino 1985, 2005).

Island microclimates may be harsher than mainland habitats because they are often more exposed to the elements and less buffered by vegetation (Berry 2009). In small mammals this could select for a decrease in surface area to volume ratio (i.e. increased body weight) to avoid excess heat loss (White and Searle 2007). This is an extension of Bergmann's Rule which describes the general biogeographical trend that closely related animal species tend to show an increase in body size in cooler climates and a decrease in body size in hotter climates (Cox and Moore 2000). Bergmann's Rule is but one of several so-called *ecogeographical rules* (Gaston *et al*. 2007), reviewed in relation to the island rule by Lomolino *et al.* (2006).

As a consequence of incomplete colonisation and increased extinction risk, islands often have a subset of fauna in comparison to the adjacent mainland. Small mammals commonly escape predators by retreating into small holes or tunnels. Therefore, in the absence of predation risk, there may be no selective pressure to remain small (Adler and Levins 1994). Similarly, reduced interspecific competition may lead to an increase

in available resources allowing animals to grow larger (Lomolino 1985, 2005). Island populations often exist at higher densities than mainland species (Jewell 1966, Bujalska 1975, Tamarin 1978) and any effect of a decrease in competitor species is likely to be outweighed by an increase in intraspecific competition. However, long-term effects of increased density may lead to selection for a decrease in reproductive output and a corresponding increase in body size as animals are able to invest more energy in growth, instead of production of offspring (Adler and Levins 1994).

High density and competition for mates in a polygamous animal may also result in greater body size in males than females through the process of sexual selection, thus leading to sexual size dimorphism (Boonstra *et al.* 1993, Isaac and Johnson 2003, Vanpe *et al.* 2008, White and Searle 2009). However, factors such as the maternal advantages often implicit with increased body size and increased female-specific competition for resources (e.g. nesting space) can lead to reverse sexual size dimorphism in mammals, where females are larger than males (Ralls 1976). Although reverse sexual size dimorphism is reportedly a characteristic of *Myodes* species (Bondrup-Nielson and Ims 1990 in Yoccoz and Mesnager 1998), in French alpine bank vole populations males have been shown to be larger than females during the breeding season (Yoccoz and Mesnager 1998). The authors of the study hypothesised that this pattern of sexual size dimorphism was caused by higher survival in alpine populations leading to lower reproductive rates and an associated increase in sexual selection amongst males. Insular voles may also experience higher survivorship than mainland conspecifics (Adler and Levins 1994 but see Chapter 4), thus one may expect to find a similar pattern of size dimorphism in these populations.

Selection is not the only mechanism through which evolutionary divergence of insular life forms can occur. Genetic drift and founder effects are likely to exert a large influence over island races, particularly in small populations (Frankham 1998). Furthermore, if Bergmann's Rule proves to be correct and founding populations did not originate from the adjacent mainland but from more extreme climes (e.g in the case of *Apodemus sylvaticus* on St. Kilda; Berry 1969), colonising animals may appear larger than normal when viewed 'out of context'. Weight is frequently used as a measure of body size, but linear measures of structural body size (e.g. head to tail length) are often recorded, for example in museum specimens. Moreover, the relationship between weight and structural body size is frequently used as a measure of body condition (e.g. Murray 2002, Norrdahl and Korpimäki 2002, Wauters *et al.* 2007, Liker *et al.* 2008), defined by Moya-Laraño *et al.*(2008) as *the relative amount of energy reserves in the body*.

In the UK, bank voles are present on 13 offshore islands (Shore and Hare, 2008). Of these, four contain races of bank voles that are reportedly much larger in size than mainland voles and, as such, have been given subspecific status. These occur on the islands of Mull, Raasay, Jersey and Skomer (Chapter 2). Yalden (1999) suggests that many of the differences between island races and mainland populations in Britain have been exaggerated when subspecies were originally described because of small sample sizes and comparisons of animals from opposite ends of the country. Corbett (1964) showed that whilst Skomer voles seem extraordinarily large compared to voles from southern England, when compared to mainland Scottish voles, the size difference is not so apparent.

In this chapter, I examine body size differences in length, weight and condition between island populations of bank voles and their nearby mainland conspecifics, using a novel approach to gather body length data from live animals in the field. In response to the findings of previous studies, these data are used to perform tests of the following hypotheses:

1. Island subspecies are larger than mainland conspecifics;
2. Insular gigantism in voles occurs as a result of selective pressures rather than random genetic effects;
3. Reverse sexual size dimorphism (females larger than males) is not apparent in island vole populations.

Moreover, by employing a comparative approach I attempt to interpret these data in terms of possible factors leading to insular gigantism in small mammals. Specifically, I question whether the following selective pressures are responsible for the increased body size of insular subspecies of bank voles:

1. Reduced interspecific competition
2. Increased intraspecific competition
3. Reduced predation pressure
4. Bergmann's Rule
5. Climatic variables (e.g. temperature, rainfall, altitude)

I also discuss which is the best measure of body size for comparative purposes, and the complications involved in taking body size measurements and accurately determining body condition.

5.2. Methods

The structural body size of field animals was measured by taken a digital image of each animal gently held flat on a measuring board, after first trimming the tail hairs (Figure 5.1). Head-to-tail (HT) measurements were taken from digital images using the programmes 'Screen Calipers 3.2' (for preliminary analyses) and 'ImageJ 1.40g' (for final analyses). It was not found possible to effectively measure head-to-body (HB) length and tail length separately on live voles in the field.

Figure 5.1: Digital image used for taking HT measurements from live bank voles in the field.

5.2.1. Estimating measurement error and validation of methods used

(a) Method of taking the HT measurements from the digital image

In order to determine the most appropriate system of taking measurements, a small subset of the images (N = 5) were used to explore three different methodologies (all measurements were taken by one operator):

- Method 1 - HT measurements were taken directly from the photo: repeated independently three times;

- Method 2 - white landmarks were placed on tips of head and tail on image (Figure 5.1) under magnification and the distance between these landmarks was measured independently three times,
- Method 3 – white landmarks were placed on tips of head and tail of image under magnification and the distance between these landmarks measured; this process repeated three times independently.

The within individual coefficient of variation for the mean, ME%, for each method across all voles was estimated using Haldane's (1955 in Lynch *et al*. 1997) correction for small sample sizes.

$$ME\% = \frac{100(1+0.25n)s}{\bar{x}}$$

where n = sample size (5), s = standard deviation and x = mean.

The mean ME% was less than 0.3% for each method (Figure 5.2), although ME% was significantly larger for Method 1 than Methods 2 and 3 across all voles ($F_{2,27} = 12.14$, $P < 0.001$). Because of economies of time in taking HT measurements, and the small ME% value, it was decided to use Method 1 for measuring HT length in future analyses.

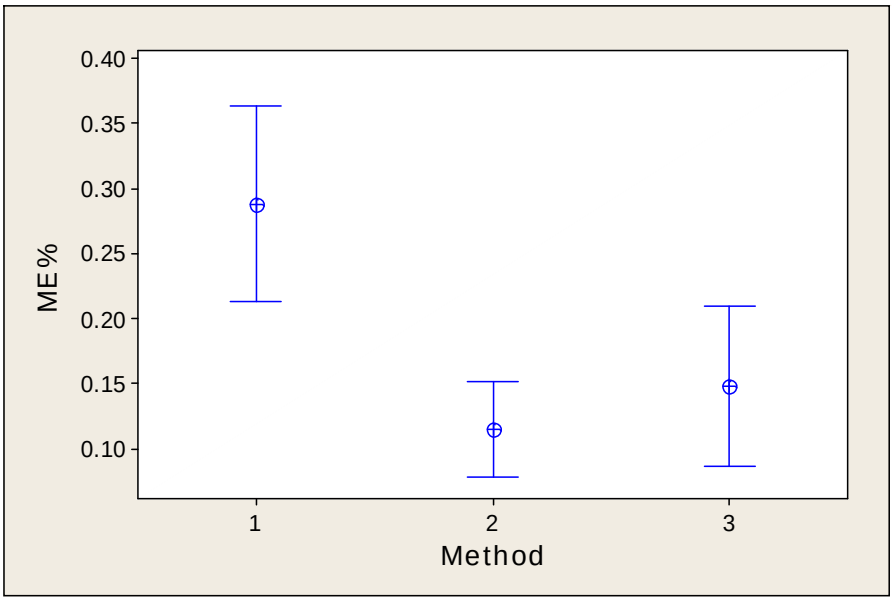


Figure 5.2: Measurement error (ME%) ± 95% Confidence Intervals for each method of measuring HT length. ME% was calculated using Haldane's (1955 in Lynch *et al*. 1997) correction for small sample sizes.

(b) Inter-operator variability in measuring HT lengths using Method 1

Three operators (E, P, J) measured the HT length from images of 10 voles from Raasay five times independently using Method 1 above. The coefficient of variation (CV%) and measurement error (ME%), were estimated for each vole for each operator (Figure 5.3).

On average, ME was 0.22% larger than the CV across all the data. The maximum ME% across all people was <0.82% but there was significant variation among operators (One-way ANOVA, $F_{2,27} = 11.26$, $P < 0.001$). Post-hoc Tukey's multiple comparisons show that the mean ME% for E was significantly different to J and P but there was no difference between J and P. J was most variable (mean ME% = 0.54, max ME% = 0.83), followed by P (mean ME% = 0.43, max ME% = 0.62) and E

(mean ME% = 0.23, max ME% = 0.33). Thus, Operator E took all measurements for subsequent analyses and because measurement error was very small, only one measurement was taken per photo.

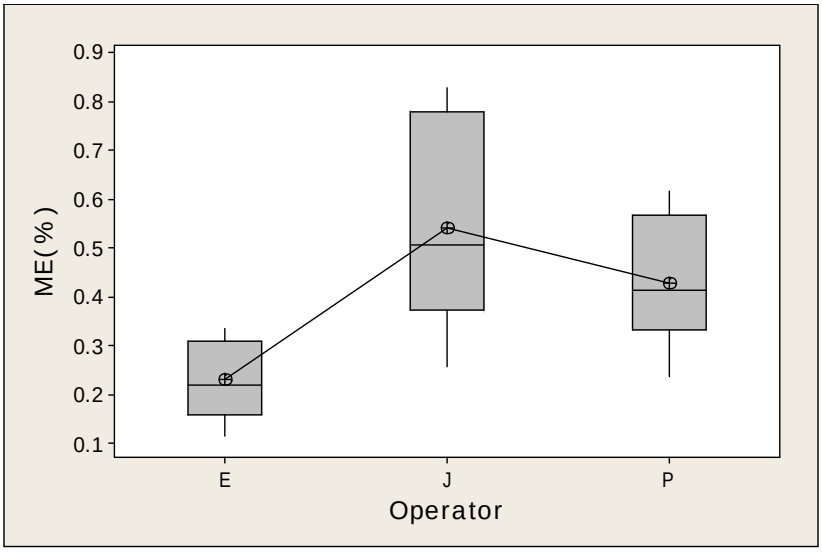


Figure 5.3: Inter-operator variability in HT measurements taken from 10 digital images of bank voles. Measurement error (ME%) was calculated using Haldane's (1955 in Lynch *et al*. 1997) correction for small sample sizes.

(c) A comparison of photograph and dissection body sizes

Traditionally, small mammal biologists take linear measurements, such as HT body length, from culled specimens, which can be much more easily manipulated than live animals. In order to test the reliability of measuring animals in the field, 23 bank vole corpses (obtained from various sources) were photographed (using the same protocol as for live animals) and then measured in the traditional manner, with calipers.

Whilst there were some discrepancies in the measurements obtained by the two different methods (Table 5.1), 11 digital image measurements were lower than the caliper measurements (range 0.8-4.4 mm), and 12 digital image measurements were larger (range 0.3-4.7 mm); the mean difference was not significantly different to zero

(one sample t-test, T = 0.02, P = 0.98). Therefore, overall there was no systematic difference between the two methods and no evidence that the method used in the field was any more variable than post mortem measurements.

	N	Mean	SD	CV (%)	ME (%)	Min	Max
Calipers	23	138.13	9.600	122.60	6.95	122.6	167.8
Digital Image	23	138.14	8.310	125.80	6.01	125.8	164.7

Table 5.1: Comparison of HT measurements (mm) taken from dead specimens using calipers and Method I (see Section 5.2.1a). Standard deviation (SD), coefficient of variation (CV) and measurement error (ME) are shown.

5.2.2. Main study

Animals were live-trapped using Longworth traps during the 2005-2007 field seasons. Data on body size (HT), weight, sex and breeding condition were collected from 201 individuals from four mainland and six island sites during spring (March, April, May; N = 135), autumn (September, October; N = 56) and summer (June, July; N = 10). Corbet (1964) suggests that size should be measured as the "maximum size attained by the individual" so that the measured characteristic is able to reflect the genetic factors affecting it. However, very few animals are likely to be trapped at their maximum body size, so in this study only adult individuals were selected for sampling and, because of measuring techniques and complications involved in body weight data, palpably pregnant females were excluded. To avoid losing valuable data and because of the small sample size, 'summer' animals were subsequently analysed with 'autumn' animals since they were considered to be more comparable in size and condition to this group than the overwintered, pre-breeding season animals captured in spring.

5.2.3. Statistical treatment of data

(a) Body size

Because all sites were not trapped in all seasons, body size data from 'spring' and 'autumn' animals were initially analysed independently. Data were first tested for normality using the Kolmogorov-Smirnov test. Differences in HT length and body weight between populations were tested using ANOVA with Tukey's multiple comparison as a *post hoc* test for normally distributed autumn data. Kruskal-Wallis with Dunn's multiple comparisons tests were used for non-normal data for 'spring' animals.

As well as body weight and structural body size, the relationship between the two, i.e. body condition, was also considered. Three methods have been commonly used to measure body condition of vertebrates (Wauters *et al*. 2007):

(i) estimating a condition index by calculating the ratio of body weight to a linear measurement of length, where animals with a higher ratio are considered to be in better condition than those with a lower ratio;

(ii) using the residuals of a regression of body weight on a measure of the length of individuals, where those with a positive residual value are in better condition than those with a negative residual value;

(iii) using length as a covariate in an analysis of body weight.

These methods are all potentially problematic when comparing different populations where there may be allometry in weight to length ratios. Regression of body weight on HT length for individual sites, for both spring and autumn data (see Fig. 5.6) clearly showed that regression slopes differed between populations, particularly for spring.

Thus, using the residuals from a regression analysis including all populations would not be appropriate. Using HT length as a covariate in an analysis of body weight encompasses the same problem because a single regression slope is also inherent in this analysis. Whilst condition index can also be criticised on a similar basis (Wauters and Dhondt 1995), when used in conjunction with regression analyses for individual populations, this method provides a simple and biologically informative way to compare changes in condition (or at least body shape) between seasons, sexes and animals in different breeding states within a population. The data were analysed by all three methods, and gave very similar results. I report the results for the condition index here.

Condition indices were compared between sites for 'spring' and 'autumn' separately and used to compare seasonal effects within populations (data from populations only trapped during spring or autumn were excluded from this analysis). General Linear Models were used to examine the effects of sex and breeding condition together with population on spring and autumn data independently. Two categories of breeding condition were used:

- 'breeding' - males had large or medium scrotal testes and females had perforate vaginas, were lactating or had previously produced offspring (pregnant animals were not included);
- 'non-breeding' - males had abdominal or small scrotal testes and females had imperforate vaginas.

(b) Tail length to HB ratios

Using HT length as a linear measure of body size assumes that the ratio HB length to tail length does not vary among individuals. Tail length and HB length are most commonly measured on culled specimens by measuring from tip of the tail, minus terminal hairs, to the top of the tail at the pelvic girdle, i.e. essentially the length of the caudal vertebrae (John Gurnell, pers. comm.). However, taking these measurements on live animals was not possible without risking injury to the animals. Taking measurements of HB size alone (without tail length) from photographs would potentially introduce additional measurement error because body fur length is likely to have an influence and is an unquantifiable variable. Differences in tail:HB proportions have been previously been described between mountain and lowland populations in Switzerland (Claude 1967 in Raczyński 1983), where mountain populations exhibited isometric growth of the tail and body, whereas lowland animals were characterised by allometric growth of the tail and body. Thus, in order to understand how tail length affected HT measurements, tail length was examined to see whether it significantly varied among populations.

Data on bank vole head and body and tail length (N = 171) were gathered from museum collections housed at the Natural History Museum, London and the Royal Museum of Scotland, Edinburgh (see Appendix Table 5.1 for the list of specimens included in this analysis). Data from 16 different populations from Britain (mainland and island populations) and Europe (mainland populations) with a minimum of five individuals were included in the analyses. It is important to note that these specimens were admitted to the collections at different times over the last century (1893-2000), were trapped during different seasons and were measured by several different

curators/collectors. However, because of small sample size, it was not possible to factor these variables into the analyses and the results must be treated with caution. A regression of tail length on HB length was performed, and tail length:HB length ratios were analysed between populations using one-way ANOVA.

(c) Analysis of HT data with correction for tail size

Museum data indicated that HT and body length ratios were variable both within and between populations (Section 5.3). Thus, using weight to HT length ratios to look at condition of animals is prone to error. Furthermore, if tail length is a poor predictor of HB length then using HT length as a measure of body size is also potentially problematic when comparing between sites. However, because it was not possible to gather accurate tail length data for individual specimens from digital images, and because museum data are not directly comparable with field data, a population correction for tail size was devised from limited dissection data to examine the effects of tail length on condition indices and body size comparisons.

A small number of bodies (N_{total} = 36) from six sites (Isle of Wight, Jersey, Morvern, Orielton (Pembrokeshire), Ramsey, Skomer) were used in the analysis. Tail and HB lengths were measured during dissection (measuring from the pelvic girdle as described above). These were used to generate mean tail:HB ratios for each population, which were subsequently tested with one-way ANOVA. To produce body size measurements correcting for tail size, the mean tail:HB ratios for each population were used to calculate tail length for each individual. Estimated tail length was then subtracted from HT length. The corrected body size data were tested for differences between populations for spring and autumn separately. Corrected body size data were

also used to calculate condition indices and these data were compared between sites for spring and autumn captures separately.

5.2.4. Comparison of body size measurements with published literature

Mean weights of male and female bank voles captured during spring and autumn from populations across Europe were gathered from the published literature and tabulated with data from this study. Populations were classified according to whether they were island (1) or mainland (2), and habitat type was grouped into (1) coniferous forest, (2) deciduous forest or (3) open habitat (e.g. heathland, alpine). Latitude, longitude and altitude data were taken from Google Earth. Climate data for literature sites were taken from various web sources, in particular ttp://www.tutiempo.net/en/Climate. Meteorological stations were selected as close to the study sites as possible. Climate records were for the year 1995, unless these records were incomplete, in which case another year was selected. Climate data included mean rainfall and mean temperature data for January and July. Temperature range was calculated as the difference between January and July mean temperatures. Conrad's Index of Continentality was calculated for each site (CIC, Conrad 1946 in Berry 2009). CIC combines annual temperature range and latitude to represent the fact that island temperatures are buffered by the sea.

$$CIC = \frac{1.7T}{\sin(L+10)} - 14$$

where T is the annual temperature range (here January to July temperatures) and L is latitude.

CIC values range from 2-14 for the British Isles to 100 for Verkoyansk in central Russia (Berry 2009). Although climate data for Skomer, Ramsay and Raasay were taken from meteorological stations on the nearby mainland, the index was calculated

to see if there was relationship between the index and vole body weight. Male:female weight ratios were calculated for each population to look for evidence of sexual size dimorphism.

Body weight data were split into 'spring' and 'autumn' and a Spearman's rank correlation matrix was produced for each group, comparing all tabulated variables. Since several of the site variables were correlated, climate variables (temperature and rainfall for January and July) along with altitude, latitude, longitude, habitat category and island/mainland category were analysed using Principal Components Analysis to see whether the resulting principal components would be more useful variables against which to compare body size data. Male and female weights and male:female weight ratios were compared between island/mainland categories and habitat categories using non-parametric Mann-Whitney *U* and Kruskall-Wallis tests.

5.3. Results

5.3.1. Body size

Body size, as measured by HT length (mm), was significantly different among sites in spring caught animals (Kruskal-Wallis; $H_8 = 103.67$, $P<0.001$) (Fig. 5.4a). Kruskal-Wallis multiple comparisons tests showed that Raasay, Jersey and Skomer voles were significantly larger than all other populations but not significantly different to each other. Body size from all other mainland and island populations, including Mull, were not significantly different. Skomer voles were not significantly different to mainland Pembrokeshire (Pembs) voles despite a 19.9 mm difference in median body length between the two populations (Skomer; N = 15, median = 160, 95% confidence interval = 155.9-161.2; mainland Pembrokeshire; N = 5, median = 140.1, 95% confidence

interval = 133.3-146.5). This lack of significant difference was almost certainly due to the small sample size of Pembrokeshire animals.

Between island comparisons of body weight data for spring showed a similar significant but less definitive pattern (Kruskal-Wallis; H_8 = 115.9 ($P<0.001$) (Fig. 5.4b). Jersey voles were significantly heavier than all other populations apart from Raasay. Raasay and Skomer voles were heavier than those from the Isle of Wight, Morvern and Surrey, and Raasay voles were also significantly heavier than voles from Ramsey. In addition, Mull voles were heavier than those from Morvern. Body weight data from Ramsey were heavily influenced by one particularly large individual.

There was a significant difference in HT length amongst populations captured in autumn (one-way ANOVA; $F_{6,59}$ = 9.36, $P<0.001$ (Fig. 5.4c). *Post hoc* Tukey's comparison tests showed that the four island subspecies (Jersey, Mull, Raasay and Skomer) were significantly larger than the two mainland populations, but only Raasay voles were significantly different to those on Ramsey Island. Raasay animals were also significantly larger than those from Jersey and Skomer but not Mull.

Autumn body weights also differed significantly amongst populations (one-way ANOVA; $F_{6,59}$ = 10.27, $P<0.001$) (Fig. 5.4d). Tukey's comparison tests revealed a slightly different relationship to that of HT length in that only voles from Raasay and Mull were significantly heavier than those from Pembrokeshire. Raasay voles were also significantly heavier than Skomer voles. All other populations were similar in weight, with the exception of mainland France animals, which were significantly lighter than voles from every other site barring Pembrokeshire.

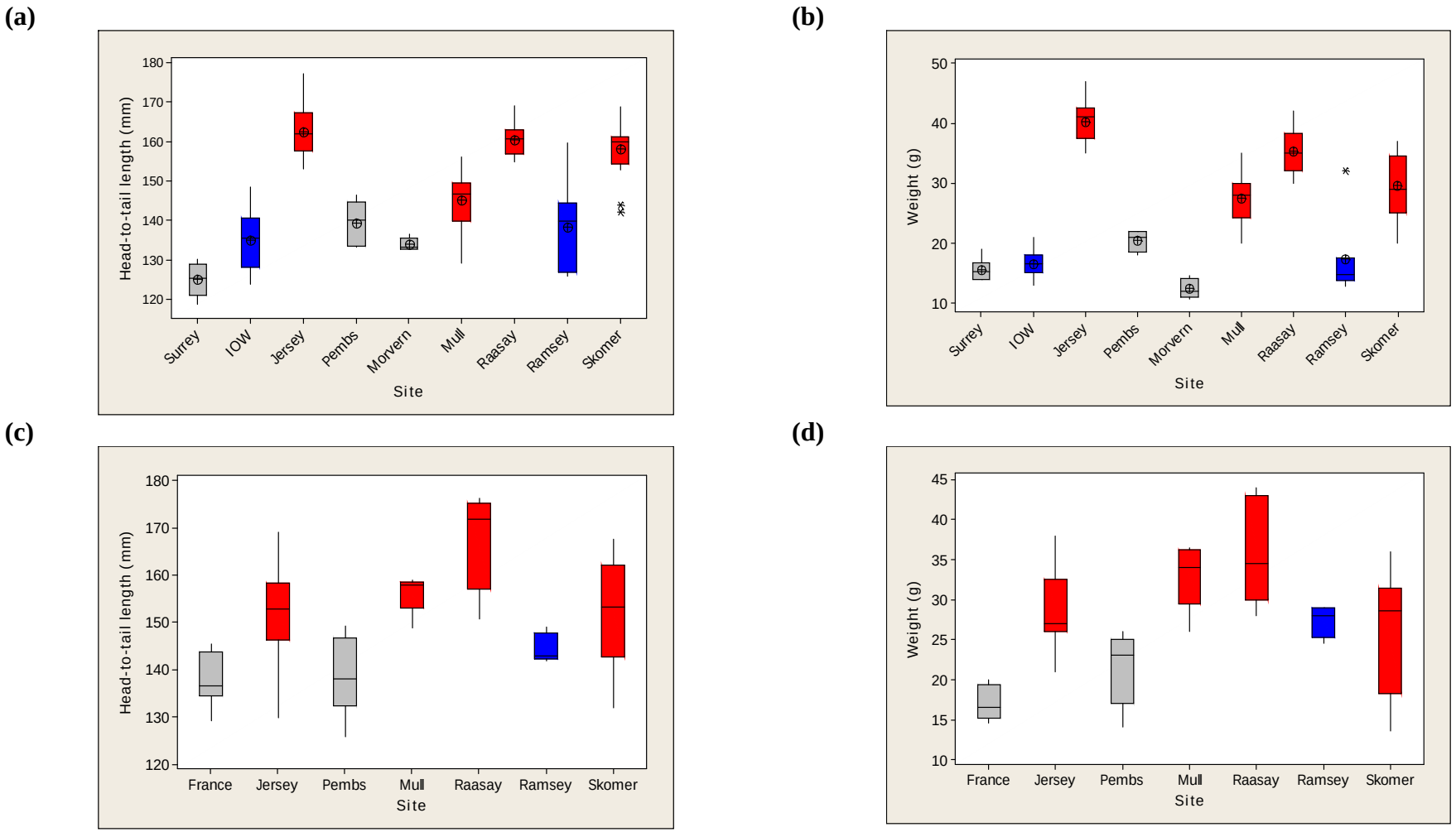


Figure 5.4: Boxplots of measurements taken from adult bank voles from island, mainland and islands with subspecies populations, shown in blue, grey and red respectively; (a) spring HT length (b) spring body weights (c) autumn HT length (d) autumn body weights.

5.3.2. Body condition/shape

Body condition, as measured by ratio of body weight (g) to HT length (mm), varied significantly amongst populations in both spring (one-way ANOVA; $F_{8,126} = 93.25$, $P<0.001$) and autumn (one-way ANOVA; $F_{6,59} = 8.50$, $P<0.001$) (Fig. 5.5). However, regression of weight on HT length showed that the relationship between these two variables also varied among populations (Fig. 5.6), and particularly for spring. For example, in the Isle of Wight population, increases in HT length tended to result in a very small corresponding increase in weight, where as on Raasay (spring season), increases in body size tended to be accompanied by much steeper increases in weight relative to other populations. The different regression slopes may indicate changes in allometry between some populations, meaning that whilst differences in condition index between populations with similar regression slopes (e.g Skomer and Ramsey in spring) may be interpreted as differences in body condition, condition indices in populations with vastly differing slopes (e.g. Raasay and Jersey in spring) may indicate a change in overall body shape as well as or instead of a difference in body condition.

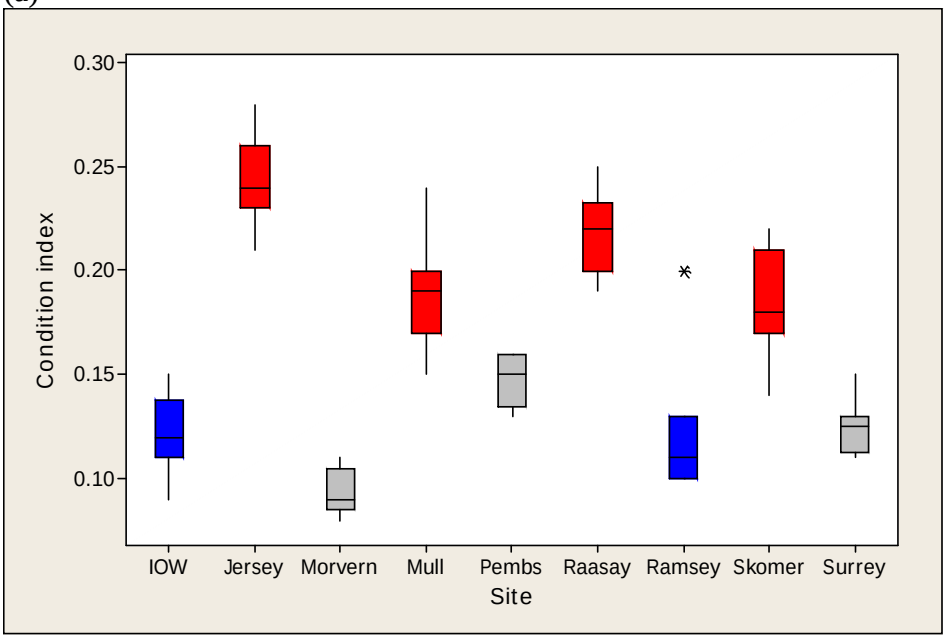
Spring condition indices differed significantly among sites ($F_{8,126} = 93.25$, $P<0.001$) *Post hoc* Tukey's comparisons showed that the named subspecies (Mull, Skomer, Jersey and Raasay) had significantly higher condition indices than all other populations (Fig. 5.6a). Jersey voles had significantly higher weight to HT length ratios than all other populations and Raasay voles had significantly higher ratios than all other populations barring Jersey. There was no difference in mean condition index between Mull and Skomer, and no difference in condition between populations from the Isle of Wight, Pembrokeshire, Ramsey and Surrey. Morvern voles had the lowest

mean condition index although this was not significantly different to that of voles from the Isle of Wight, Ramsey and Surrey. It is worth drawing attention to the fact that Ramsey voles had significantly lower condition indices than Skomer voles. Regression of weight on HT length produced almost identical slopes for these two populations indicating that there was a real difference in body condition of voles on these nearby islands.

Autumn condition indices also differed among sites ($F_{6,59}$ = 8.5, P<0.001) (Fig. 5.6b). Tukey's *post hoc* tests showed that French voles had a significantly lower mean condition index than all other populations barring mainland Pembrokeshire. There was no difference in condition indices between Skomer, Ramsey, Jersey and mainland Pembrokeshire animals, but Raasay and Mull voles had significantly larger condition indices than Skomer and Pembrokeshire voles. Regression of weight on HT length produced very similar slopes for Raasay, Jersey, Skomer and Pembrokeshire populations (Fig. 5.6), indicating that direct comparisons of body condition between these populations are probably valid.

Spring condition indices also differed significantly between the sexes and between breeding and non-breeding animals (GLM ANOVA; $F_{8,124}$ = 60.72, P<0.001; $F_{1,124}$ = 18.02, P<0.001; $F_{1,124}$ = 29.16, P<0.001 for population, sex and breeding condition respectively). Mean condition indices were higher for males than females in all populations (with the exception of Morvern, where no males were captured) and breeding animals of both sexes were in better condition than non-breeding animals. In

(a)



(b)

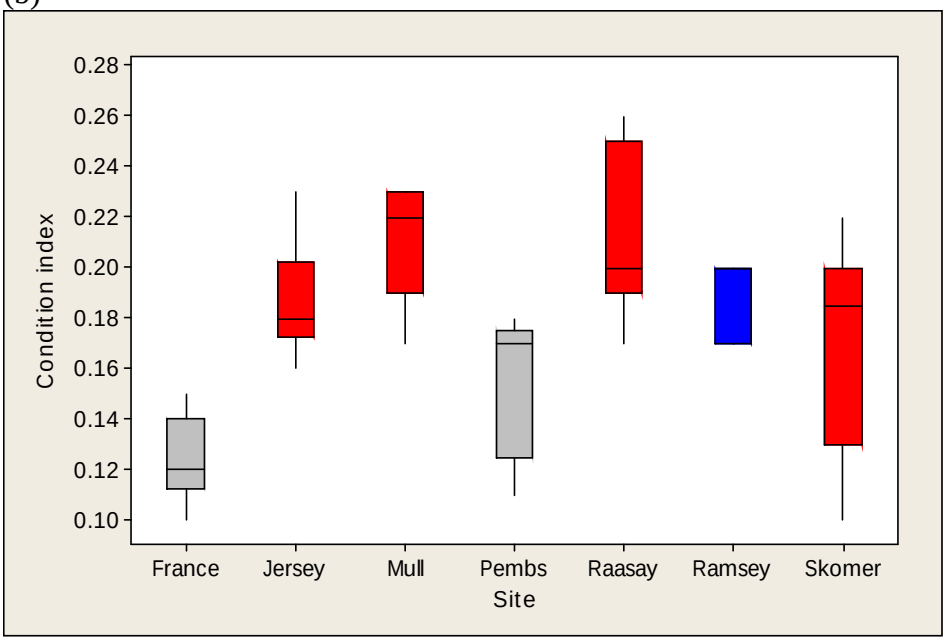
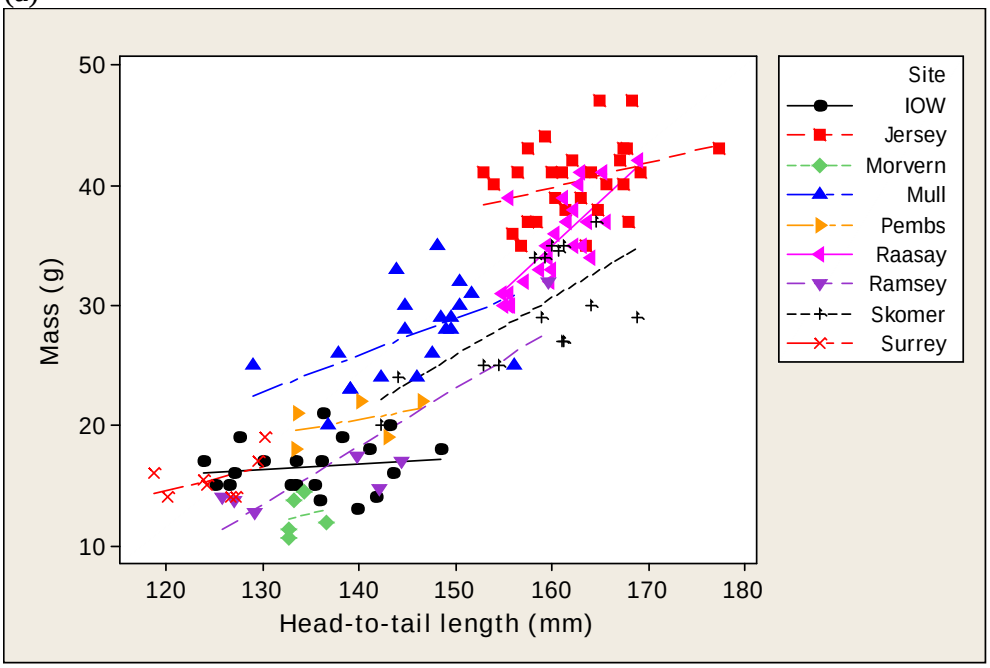


Figure 5.5: Condition index of bank voles captured during spring (a) and autumn (b) from mainland, island and island with subspecies populations (coloured grey, blue and red respectively). Condition index was calculated using ratio of weight (g) to HT length (mm).

(a)



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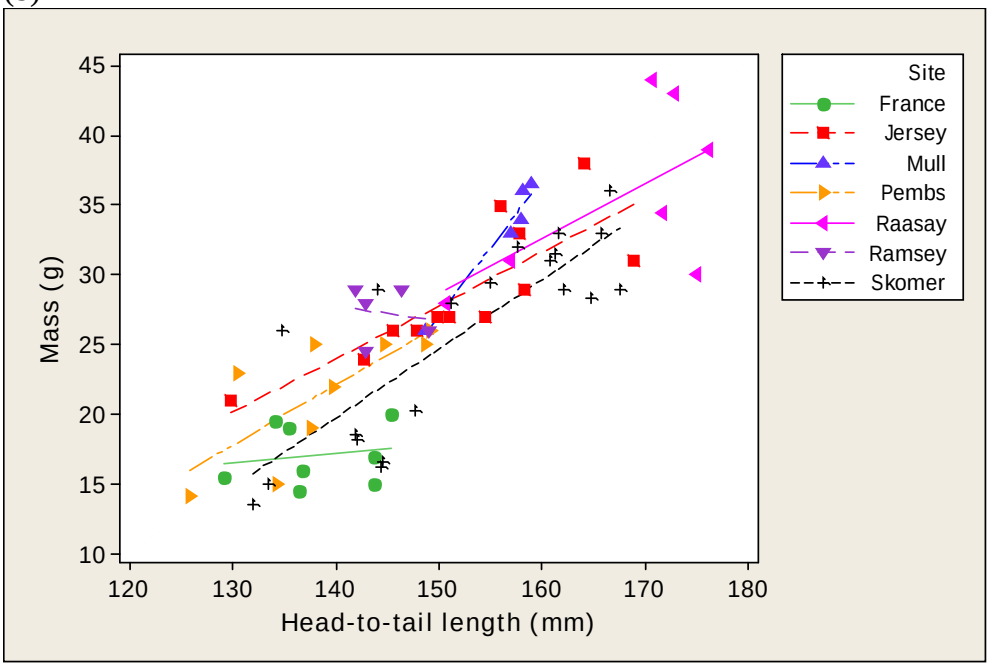


Figure 5.6: Plot of weight against HT length of bank voles captured during spring (a) and autumn (b) with regression lines for each population. Site codes; Isle of Wight (IOW), mainland Pembrokeshire (Pembs).

autumn-captured animals, there was no significant difference in condition indices between the two sexes but breeding condition did have an effect (GLM; $F_{6,57}$ = 8.36, P<0.001; $F_{1,57}$ = 3.53, P<0.065; $F_{1,57}$ = 16.73, P<0.001 for population, sex and breeding condition respectively). Again, breeding animals had higher condition indices than non-breeding animals and this was apparent across all populations (where both breeding categories of animals were caught) (Fig. 5.7).

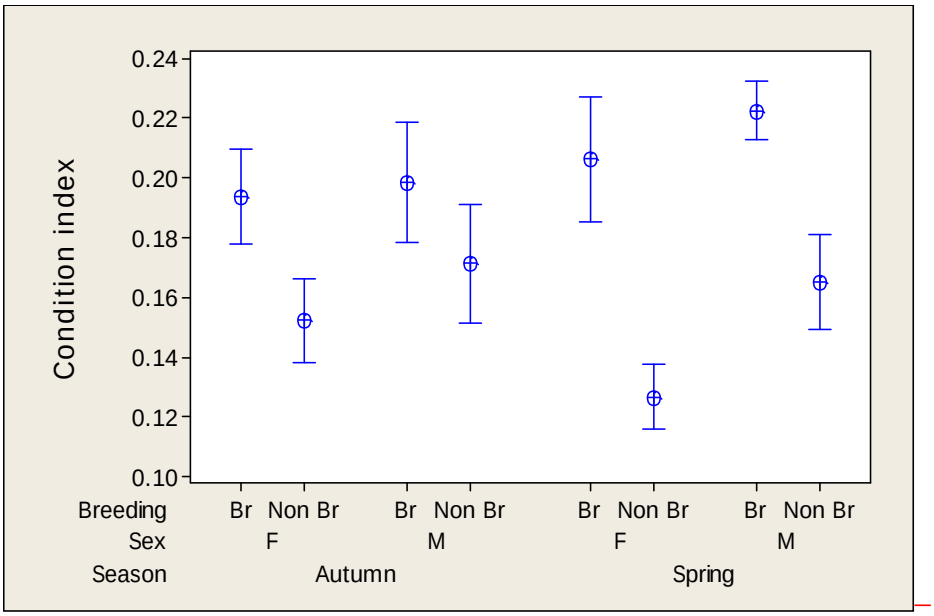


Figure 5.7: Mean condition index across all sites for breeding (Br) and non-breeding (Non Br) male (M) and female (F) bank voles captured in spring and autumn.

When condition indices were tested between populations with season as a factor, there was still a significant difference between populations and, whilst season alone did not have a significant effect, there was a significant interaction between site and season (GLM; $F_{5,148}$ = 27.93, P<0.001; $F_{1,148}$ = 0.52, P = 0.427; $F_{5,148}$ = 16.65, P<0.001 for population, season and interaction respectively). There was little difference in mean condition index between spring and autumn caught animals from Pembrokeshire and

Raasay, whereas Ramsey and Mull animals were generally in poorer condition in spring than in autumn, yet the converse was true for Raasay and Skomer animals (Fig. 5.8).

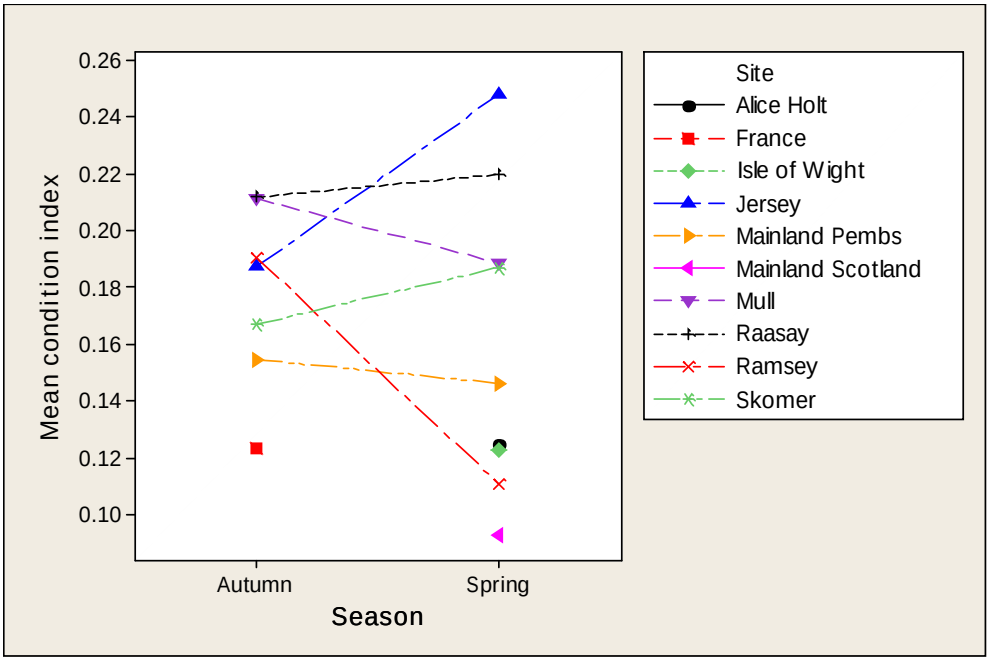


Figure 5.8: Interaction plot of mean condition index for bank vole populations captured in spring and autumn.

5.3.3. Relationship of tail length to HB length

Regression of HB length on tail length data collected from museums revealed that whilst there was a significant positive relationship between the two variables, tail length was a fairly poor indicator of body size (Head and body (mm) = 44.51 + 1.124 tail (mm), R^2 = 34.8%, $F_{1,169}$ = 90.14, $P < 0.001$) (Fig. 5.9). Furthermore, tail length to body size ratios differed significantly between populations (one-way ANOVA; $F_{15,155}$ = 8.34, $P < 0.001$). *Post hoc* Tukey's comparison tests showed; voles from Anglesey, Isle of White, Spain and France had significantly higher tail:HB length ratios than the Argyllshire, Jersey, Mull, Perthshire, Raasay and Suffolk populations; Surrey animals

had higher ratios than Perthshire, Jersey, Mull and Raasay animals; Mull animals had significantly lower ratios than voles from Skomer and Gwynedd (Fig 5.10).

Conversely, when tail:HB length ratios from dissection data were analysed, there was no significant difference between populations (one-way ANOVA; $F_{5,28} = 1.83$, $P = 0.139$). However, this may partially be due to small sample size; Isle of Wight animals (N = 3) did appear to have relatively high tail:HB ratios compared to other populations as suggested by the analysis of museum specimens (Fig. 5.11). It is also worth noting that there were fewer populations in this analysis than included in the museum data analysis. Nevertheless, Jersey animals (N = 9) displayed much greater variability in tail:HB ratios than revealed by museum data (min-max values; dissection animals 0.40 - 0.71, museum specimens 0.44 - 0.49).

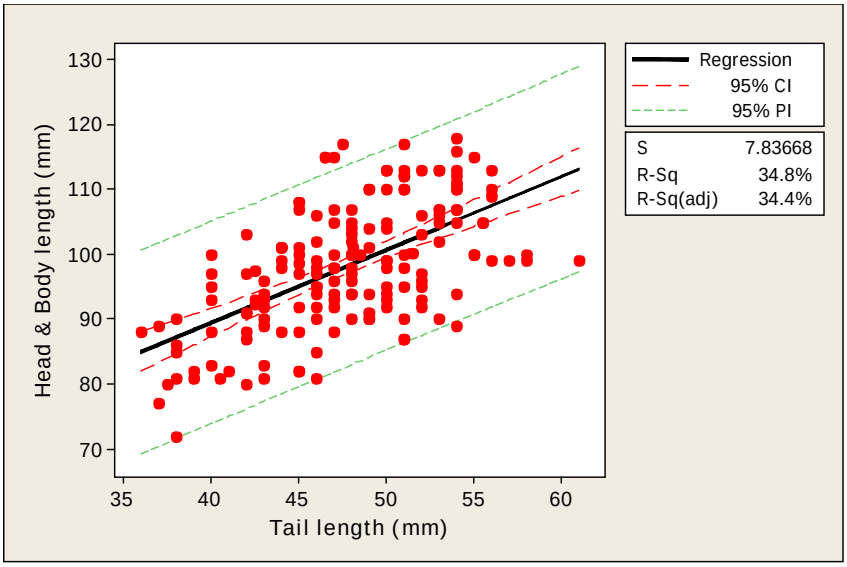


Figure 5.9: Regression of HB length of bank voles on tail length ($y = 44.51 + 1.124 x$). Data collected from museum specimens. N = 171; 95% confidence intervals (CI) and prediction intervals (PI) are shown.

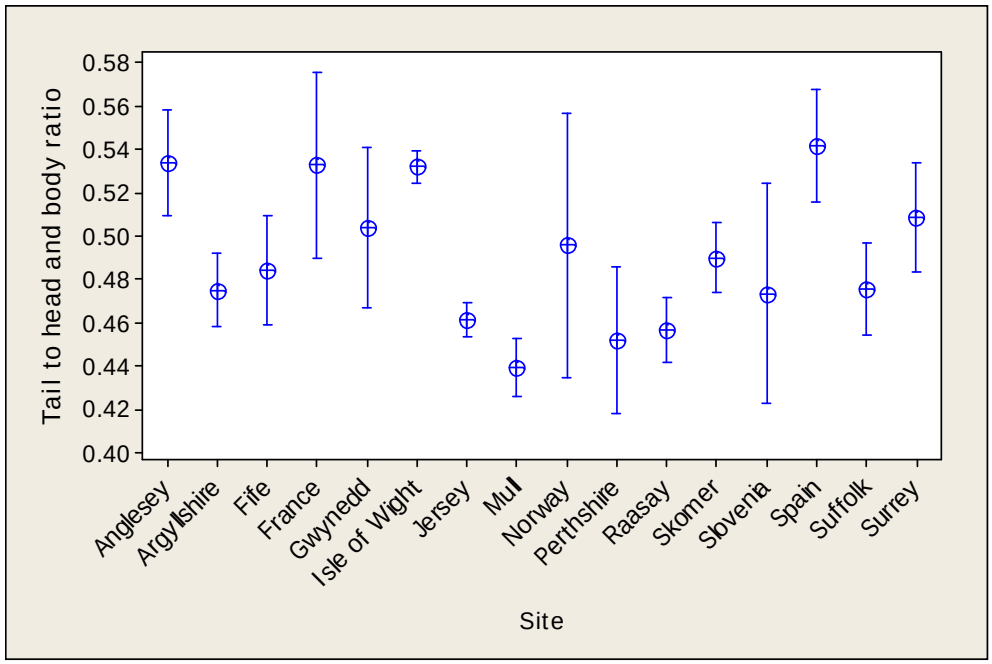


Figure 5.10: Mean tail length to HB ratios (mm) for bank voles from 16 sites with 95% confidence intervals. Data collated from museum specimens, N = 171.

When a subset of the body size data were reanalysed using corrected body size, there was still a significant difference between populations (Kruskall-Wallis multiple comparisons; $H_5 = 68.51$, $P<0.001$). Spring-captured Skomer and Jersey voles were found to be significantly larger than populations from mainland Scotland, Isle of Wight and Ramsey, and Skomer voles were also significantly larger than mainland Pembrokeshire animals. Analysis of corrected body size data for autumn showed a similar difference (one-way ANOVA; $F_{3,42} = 20.21$, $P<0.001$). Skomer voles were significantly larger than mainland Pembrokeshire and Ramsey voles. Jersey voles were significantly larger than mainland Pembrokeshire voles.

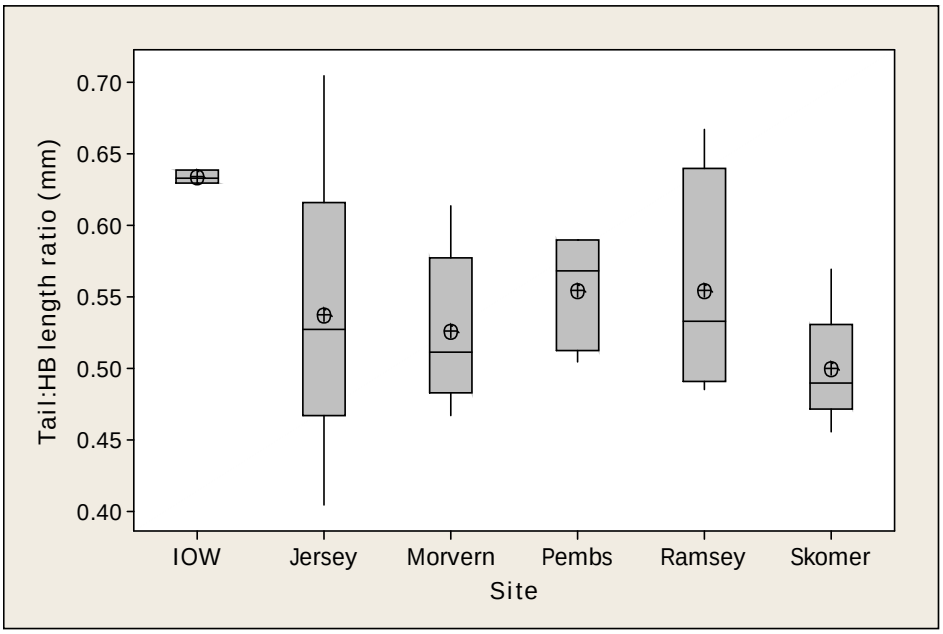


Figure 5.11: Boxplot of tail to HB length ratios (mm) for 36 dissected bank voles from six sites. Mean symbols are shown. Site codes; Isle of Wight (IOW), mainland Pembrokeshire (Pembs).

Condition indices using corrected body size data showed a very similar pattern to condition indices calculated using HT length data. There was a significant difference in condition between sites in spring captured animals (one-way ANOVA; $F_{5,75} = 86.31$, $P<0.001$). Jersey voles still had significantly higher condition indices than all other populations. Pembrokeshire, Isle of Wight and Skomer animals had significantly higher condition indices than Morvern animals and Skomer animals were also in significantly better condition than Ramsey animals. The main difference between the two analyses is that Skomer animals did not appear to be in better condition than mainland Pembrokeshire animals when tail size was accounted for and animals from the Isle of Wight appeared to be in relatively better condition than the previous analysis suggested (Fig. 5.12). When adjusted condition index data for autumn captured animals were analysed, there was a significant different between populations (one-way ANOVA; $F_{3,42} = 4.85$, $P = 0.005$). *Post hoc* tests showed this was because Jersey and Ramsey animals had higher condition indices than Skomer animals. When

tail size was not controlled for there was no difference in condition indices between these populations.

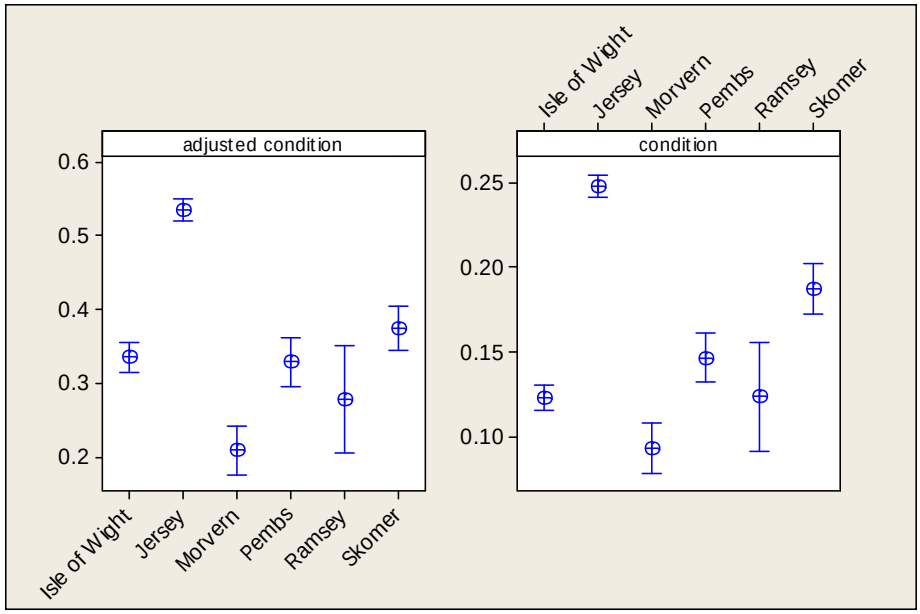


Figure 5.12: Mean condition index with 95% confidence intervals for bank voles captured in spring, calculated using; (i) adjusted condition – ratio of weight (g) to body size (mm) adjusted for tail length; (ii) condition – ratio of weight (g) to HT length (mm).

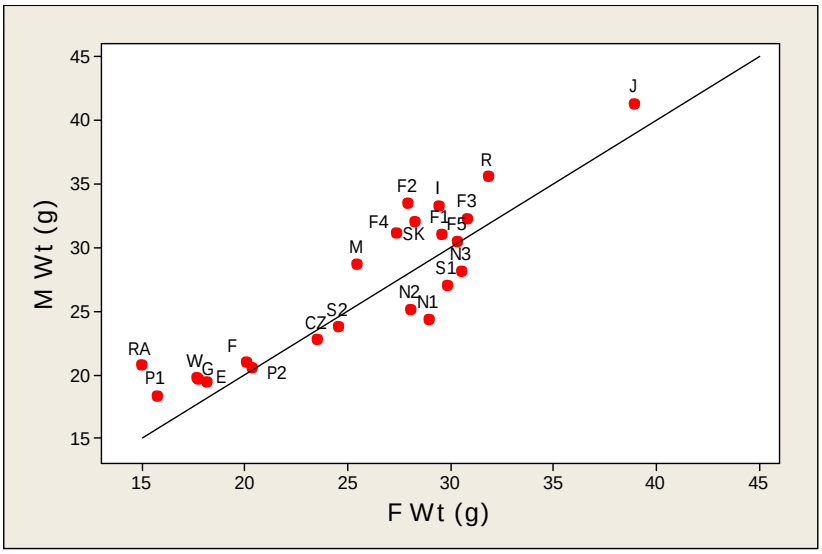
5.3.4. Body size, sexual dimorphism and the island rule in bank voles

Table 5.2 shows mean body weight data for European populations of bank voles gathered from the published literature, combined with data from this study. Although several of the site descriptive variables were significantly correlated (Appendix Table 5.2), the results of the Principal Components Analysis were relatively uninformative because the variation explained by the first few components was relatively low (Appendix Fig. 5.1). Thus, the PC scores were not deemed useful for further investigation with respect to body size data.

Table 5.2: Mean body weight data (g) for male and female bank voles in spring and autumn, collated from this study and the published literature. * denotes means of largest individuals captured from population.

Site	Code	Spring		Autumn		Source
		M	F	M	F	
County Durham, N. England	E1	21.0				Crawley 1970
Lauvitel Spruce, France	F1	31.1	29.5	25.6	24.7	Yoccoz and Mesnager 1998
Lauvitel Boulder, France	F2	33.5	27.9	23.8	24.8	Yoccoz and Mesnager 1998
St Germaine, France	F3	32.3	30.8	25.9	24.3	Yoccoz and Mesnager 1998
Prachou, France	F4	31.2	27.3			Yoccoz and Mesnager 1998
Entraigues, France	F5	30.5	30.3	24.8	25.0	Yoccoz and Mesnager 1998
Alice Holt Forest, Surrey, England	E	19.5	18.1	18.2	17.4	J. Gurnell, pers. comm.
Risberget, Norway	N1	24.4	28.9			Bondrup-Nielsen and Ims 1990 in Yoccoz and Mesnager 1998
Varaldskogen, Norway	N2	25.2	28.0			Bondrup-Nielsen and Ims 1990 in Yoccoz and Mesnager 1998
Kviteseid, Norway	N3	28.2	30.5			Wiger 1979 in Yoccoz and Mesnager 1998
Strömsund, Sweden	S1	27.1	29.8	18.2	17.6	Hansson 1192, Bondrup-Nielsen and Ims 1990 in Yoccoz and Mesnager 1998
Revinge, Sweden	S2	23.8	24.5	18.0	17.9	Hansson 1992 in Bondrup-Nielsen and Ims 1990 in Yoccoz and Mesnager 1998
Berlin, Germany	G	19.7	17.7			Stein 1956 in Yoccoz and Mesnager 1998
Hernstein, Austria	A			19.5	18.4	Radda 1968 in Yoccoz and Mesnager 1998
Dombes, France	F	21.0	20.0	18.1	18.0	Fayard 1974 in Yoccoz and Mesnager 1998
Wroclaw, Poland	P1	18.4	15.7	15.1	15.3	Haitlinger 1965 in Yoccoz and Mesnager 1998
Bialowieza, Poland	P2	20.6	20.3	14.3	14.4	Kubik 1965 in Yoccoz and Mesnager 1998
Monte Gargano, Italy	I	33.3	29.4			Hagen 1958 in Yoccoz and Mesnager 1998
Hodonin, Czech Republic	CZ	22.8	23.5			Zejda 1965 in Yoccoz and Mesnager 1998
Jersey	J	41.4	38.9	26.1	26.1	This study
Mull	M	28.7	25.4	22.1	23.9	This study
Raasay	R	35.6	31.8	28.5	26.4	This study
Mainland Pembs	W	19.8	17.6	15.9	16.0	This study
Skomer	SK	32.1	28.2	24.6	23.6	This study
Ramsey*	RA	20.8	14.9	25.3	28.7	This study

(a)



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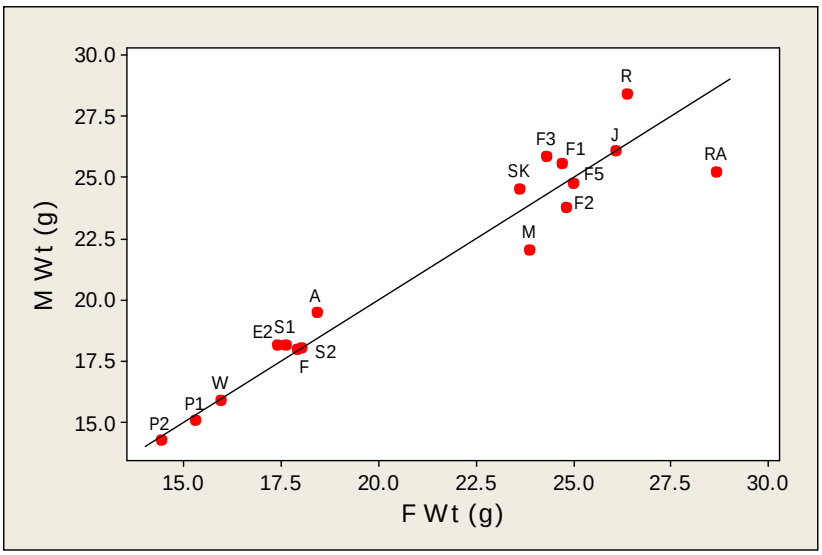

Figure 5.13: Relationship between mean male and female body weights in (a) spring and (b) autumn. Slope of line = 1. For site codes see Table 5.2

Male and female body weights were significantly positively correlated in both spring and autumn (r_s = 0.83, N = 23, P<0.0001; r_s = 0.91, N = 18, P<0.0001 respectively) and there was little evidence of consistent reverse sexual size dimorphism in any populations, except for perhaps those from Scandinavia (Fig. 5.13a). In fact, males in

the majority of populations tended to be heavier than females in spring (Fig. 5.13a) but this difference was negligible in autumn captured animals (Fig. 5.13b). Male:female weight ratios were not significantly different for island populations than mainland conspecifics in either season (U = 19, P = 0.055; U = 34, P = 0.721 for spring and autumn respectively). However, this analysis included the French alpine populations studied by Yoccoz and Mesnager (1998), which have previously been described as differing in male:female weight ratios from other mainland populations. When these populations were omitted, island voles were found to have significantly higher male:female weight ratios than mainland populations in spring (U = 54, P = 0.035) but not autumn (U = 23, P = 0.73).

Contrary to the predictions of Bergmann's Rule, there was no correlation between latitude and male or female body weights in either spring or autumn (Fig. 5.14). Nor was there any relationship between Conrad's Index of Continentality (CIC, Conrad 1946 in Berry 2009) and vole body weight.

Overall, there was no correlation between altitude and vole body weights for either season. However, when the island populations were removed, regression analyses showed altitude (Alt) was a good predictor of male body weight (MWt) for both spring and autumn (Spring; MWt = 21.58+0.008Alt, $F_{1,17}$ = 35.29, P<0.0001, R^2 = 68%, Autumn; MWt = 16.19+0.0063Alt. $F_{1,10}$ = 43.05). P<0.0001. R^2 = 81%) (Fig. 5.15). When island populations were omitted, altitude was also a good predictor for female body weight (FWt), although much more so in autumn than spring (FWt = 15.97+0.062Alt, $F_{1,10}$ = 47.41. P<0.0001, R^2 = 83%; FWt = 22.18+0.002Alt, $F_{1,16}$ = 7.64, P = 0.014, R^2 = 32% respectively).

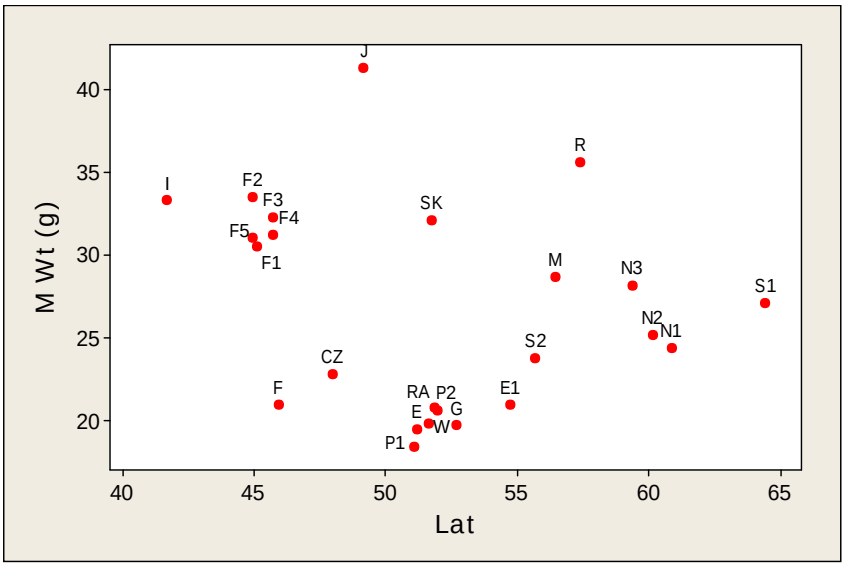

Figure 5.14: Relationship latitude and bank vole body weights in spring. For site codes see Table 5.2.

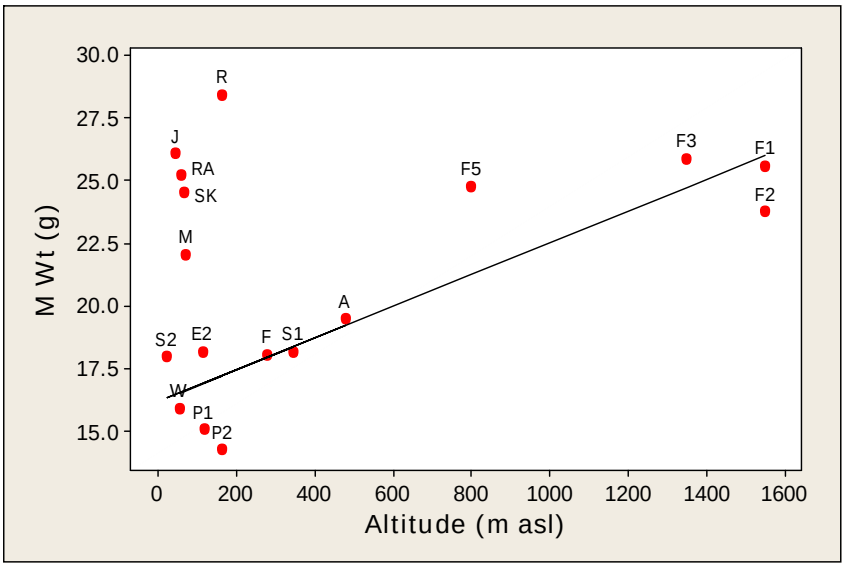

Figure 5.15: Relationship altitude and male bank vole body weights in autumn. Regression line shows the relationship when island populations were removed from the analysis (clustered in top left). For site codes see Table 5.2. MWt = 16.19+0.0063Alt. $F_{1,10}$ = 43.05. P<0.0001. R^2 = 81%.

Spring male:female weight ratios were significantly correlated with latitude, January temperatures and temperature range (r_s = -0.44, P = 0.04; r_s = 0.64, P<0.01; r_s = -0.49, P = 0.02 respectively; Appendix Table 5.2). However, no such correlations were found

for autumn data (P>0.05 in all cases). There was a significant positive correlation between male body weight and January rainfall in spring (r_s = 0.49, P = 0.02), and between male and female weights and January rainfall and in autumn (r_s = 0.66, p = 0.01; r_s = 0.63, P = 0.01 for males and females respectively).

Island voles were significantly heavier than mainland conspecifics in autumn (U = 9, P = 0.027; U = 8, P = 0.019 for males and females respectively) but not in spring (U = 23, P = 0.085; U = 35, P = 0.491 for males and females respectively). Whilst male body weights in spring were found to be significantly different between habitat categories (K_2 = 6.572, P = 0.037), the same pattern was not evident in female body weights or in autumn male body weights (P>0.05). *Post hoc* Dunn's multiple comparisons test showed that spring captured males in 'open' habitat were heavier than those in deciduous forest habitat.

5.4. Discussion

5.4.1. Body size as a comparative measure

Bank vole intra-population body size can vary significantly according to population phase (in populations that exhibit multiannual cycles), year, season, sex, age, breeding condition, parasite load, animal density and natural food availability (Aalto *et al.* 1993, Yoccoz and Mesnager 1998, Norrdahl and Korpimaki 2002, Table 5.3, Appendix Fig. 5.2). For example, maximum breeding male body weight in spring from six years of study at Alice Holt Forest, Surrey, varied over a range of 6 g, and mean body weights over a range of 3 g (J. Gurnell, unpub., Appendix Fig. 5.3).

Moreover, the weight of shrews, for example, varies greatly depending on the amount of time the animals are left in traps (Ochocinska and Taylor, 2003). Whilst body length is also likely to be affected by resource availability and individual health status in the long-term, body length does not fluctuate in the same way as weight - whilst animals can rapidly lose body fat, they do not tend to decrease in length. Thus, body length is perhaps overall a better trait to look at when studying the effects of long-term selection on populations rather than immediate environmental pressures, but is not frequently measured on live small mammals.

Overall, it is not clear as to which is the characteristic, phenotypic body size for the population to be used in comparative studies among populations. This is also a problem when data are retrieved from literature surveys, because it is likely that they are not truly comparable. Ideally, for systematic studies standardised sampling procedures should be adopted across all populations (Yoccoz and Mesnager 1998), but this is not feasible on a large scale. In most comparative studies, it appears that mean weights have been used for a group of animals as the measure of body size (e.g. Innes and Millar 1994, Lomolino 2005, Merei *et al.* 2005, 2006, Yoccoz and Mesnager 1998).

Sex	Breeding	N	Mean	CV %	Max	Q3	Med	IQR
Female	Non breeding	396	15.0	19	24.5	17.0	15.0	3.5
	Breeding	55	18.4	10	23.0	20.0	19.0	3.0
	Pregnant	51	23.2	11	30.0	25.0	23.0	3.5
Male	Non breeding	280	15.2	17	27.0	17.0	15.0	3.5
	Breeding	358	19.4	13	27.5	21.0	19.5	3.0
Breed M:F			1.05		1.20	1.05	1.03	

Table 5.3: Descriptive weight statistics from 1140 individuals captured at Alice Holt Forest, Surrey between May 1975 and July 1980, with individuals captured in different trapping months taken as independent (J. Gurnell, unpublished).

5.4.2. Measuring body size

Taking body size measurements from animals in the field using digital images is a novel approach that has not, to my knowledge, been used in small mammal studies before. Traditionally, body length measurements are taken from culled specimens but destructive sampling has become much less acceptable in recent years and may be an untenable in many situations. For example, conservation of rare populations may be a priority, or animals may need to be kept alive in order to gather other ecological data. In the case of this study, permission to access many of the field sites was undertaken on the basis that destructive sampling would not be carried out. I gathered length measurements from live animals in the field as well as weight data in order to investigate size relationships between island and nearby mainland populations of bank voles. Using a comparative approach and looking at environmental factors influencing each population, it was hoped that adaptive explanations for gigantism among island populations might be forthcoming.

Preliminary analyses revealed that it was possible to gather HT length data from live animals without substantial measurement error, and that there was no systematic bias in these data when compared to traditional measurements taken from dissected animals. However, subsequent analyses revealed that there might be a problem with including tail length within body size measurements. Analysis of museum and dissection data showed that tail length to body size ratios were highly variable (0.44-0.54) and therefore tail length is a poor predictor of HB length. Nevertheless, if tail size is variable within individuals but not systematically different between populations then overall, using head-to-tail length to compare body size between populations should still be a valid approach. However, analysis of tail:HB ratios from museum

specimens indicated that there were significant differences between populations included in this study. Most notably, voles from the Isle of Wight appeared to have much longer tails relative to HB length than other populations. Whilst this was not statistically supported by analysis of the small sample of dissected animals, the same trend was noticed; the three dissected specimens from this population all had relatively high tail:HB ratios. Museum data also indicated that Mull voles tended to have relatively shorter than average tails for their body size. However, due to a lack of freshly culled specimens from Mull, it was not possible to provide further support for this hypothesis with quantitative data (although anecdotal evidence from reviewing digital images would suggest that this may be the case). Conversely, analysis of dissected specimens from Jersey revealed a much greater range in tail:HB length ratios than suggested by museum data. The museum data in particular should be treated with some caution because data were collected by several people over the last century and it was not possible to ascertain whether the measurements were gathered using a standard procedure. In addition, tail:HB length ratios within populations may not remain static but vary over time. Corbet (1975) observed an example of rapid population level morphological change whilst studying Scottish bank voles; he documented a fairly radical change in tooth morphology that had occurred within a period of 15 years, from an isolated population at Loch Tay. Unfortunately, due to limited data availability, it was not possible to investigate whether tail lengths to body size ratios are variable between age classes within populations. Thus, it would be interesting to gather further data on tail length variation in bank voles to see whether there are any biologically meaningful patterns behind tail size evolution.

In this study, only adult animals were sampled as an attempt to represent the maximum body size attained by individuals in each population at the time of trapping. However, in hindsight it may have been more informative to take measurements from the entire range of age classes along with some estimate of tail size. Nevertheless, data collection in the field was limited by time constraints (all voles had to be released from traps within a reasonable period) and weather conditions, so photography of every individual captured was not feasible.

5.4.3. Body size in island and mainland bank voles

For the most part, the results of this study supported previous evidence (see Shore and Hare 2008) in that named subspecies were larger in terms of body length than voles from other islands or mainland populations. However, there were exceptions. Mull voles captured in spring did not differ significantly in length from the non-subspecies populations. This may partially be an effect of tail size because if Mull voles have significantly lower tail to head and body ratios than other populations, comparing HT length may underrepresent the true size difference between these voles and other populations. However, weight data also indicated no difference between Mull voles and other non-subspecies populations. Nevertheless, by autumn this relationship had changed and Mull voles, along with the other three named subspecies were significantly larger in terms of HT length than the two mainland populations. Body weight data revealed similar patterns to length data but differences between populations were less distinct for both spring and autumn data sets, emphasising the importance of using additional measurements to detect size variation.

Interestingly, Ramsey voles appeared to be of intermediate body size between mainland and subspecies populations. This island race has not previously been reported as 'enlarged' when compared to mainland animals. However, the mean weight of the small sample of spring animals was heavily influenced by one unusually large specimen. This animal was captured close to the volunteer wardens' quarters, where compostable food waste is routinely 'thrown to the wildlife'. Voles are commonly seen feeding on the waste (Greg Morgan, pers. comm.) thus animals captured from around this area may not be highly representative of the 'natural' population, particularly in terms of weight. Positive effects of supplemental food on growth rates and body weight are not consistent in all studies on bank voles (e.g. cf. Banach 1986 and Löfgren *et al.* 1996).

Because of small sample sizes (N_{total} = 10), data collected from Ramsey animals in June and Pembrokeshire animals in July were included in the 'autumn' data set for simplicity of analysis. It was felt that these animals were probably more comparable to autumn-captured animals than the freshly overwintered animals captured in spring. However, the disparity between seasons may have hidden any true size differences between these populations and other populations captured in autumn. Bank voles commonly start to put on weight at the beginning of breeding season during spring (Corbet, 1964, Gurnell unpub.) and then experience a loss of weight during winter months (Grodzínski 1985; Tanton 1969). In Polish populations, weight has been shown to reach its maximum during June and July (Haitlinger 1965 in Raczyński 1983). Thus, combining body weight data from summer animals may be misleading. Nevertheless, body length data is probably less susceptible to seasonal fluctuations

and these data still indicated an intermediate body size, somewhere between mainland and subspecies populations for Ramsey animals.

Reanalysing a subset of body length data with a crude correction for tail size actually made very little difference to between population relationships. However, perhaps this is not surprising because the correction for tail size was devised from mean tail:HB ratios, which did not differ significantly between populations. Nevertheless, if tail:HB ratios differ in the manner suggested by museum data, then subtracting tail size from overall body length should only enhance the effects of size differences between the populations in this study, rather than nullifying the relationship. This is because populations with the highest tail:HB ratios (e.g. France and Isle of Wight), and therefore the relatively longest tails, were already significantly smaller than the subspecies populations when tail size was not accounted for. Furthermore, populations with the lowest tail:HB ratios and therefore the shortest tails, Mull, Jersey and Raasay, were already significantly larger than other populations when tail size was not accounted for.

5.4.4. Body condition

Condition-dependent body size that reflects nutrient, fat and energy storage, which in turn reflects health status and potential reproductive success, is an important component of fitness (Moya-Laraño *et al.* 2008) and is usually measured non-invasively. Differing relationships between weight and HT length between populations may in some cases be explained by timing of trapping. For example, Jersey 'spring' animals were trapped in early May, where as mainland populations from Surrey and Morvern were trapped in early March. Seasonal fluctuations of body weight are well

documented in bank voles (Raczyński 1983, Appendix Fig. 5.2) and animals often exhibit a rapid increase in weight following the winter months (Corbet 1964, see Appendix Fig. 5.3). Thus, if weight increases at a faster rate than length it stands to reason that newly overwintered voles may show different weight:length ratios than those trapped two months later in the season. Unfortunately, due to man-power and equipment constraints, it was not possible to trap populations simultaneously. Whilst every attempt was made to trap animals in consecutive weeks, the number of field sites visited invariably meant that there was a significant time lag between the first and last populations trapped in 'spring' and 'autumn'. However, differing weight to length ratios cannot purely be explained by seasonal variability, because regression slopes were also erratically inconsistent between populations that were trapped in consecutive weeks. For example, populations on the islands of Mull and Raasay were trapped consecutively as were Skomer and mainland Pembrokeshire voles. If there is a change in allometry between populations then comparison of condition indices as a measure of body condition (in terms of fat reserves) is potentially problematic. Nevertheless, significantly different condition indices between populations still imply a biologically interesting difference in morphology, so to some extent, whether this is due to an overall shape change rather than a direct variability in fat reserves is immaterial.

The fact that subspecies populations appeared to be in better condition than mainland or other island populations may well be a result of the difference in tail:HB ratios. To examine the strength of this effect, crude estimates of tail length were produced for several populations and the data were reanalysed using a ratio of weight on body length corrected for tail size. Whilst the results of the condition analysis did not

change greatly when tail size was accounted for (but see comments above about caveats involved in estimating tail size), two out of four of the island subspecies populations had to be dropped from the analysis because of lack of comparable tail size data. Moreover, museum data suggested that the condition index in Mull animals were most likely to shift downwards when tail size was subtracted from overall body length. Of the two subspecies remaining in the analysis, only Jersey animals appeared to be in significantly better condition than all other island and mainland populations. For the consecutively trapped populations of Skomer and mainland Wales, there was no significant difference in condition in either spring or autumn and Jersey voles may well appear to be in better condition than voles from other populations because of the timing of trapping (see above). Thus, it would be entirely misleading to speculate about increased body condition in subspecies populations. However, these data do pose some poignant questions regarding body condition, food resources and potential fitness, and how these vary between populations. It would be interesting to simultaneously trap island and mainland populations (if a better estimate of tail size could be produced) to see whether larger voles start or end the year in better condition than their mainland and other island conspecifics. Another area that warrants more attention is the apparent difference in body condition between breeding and non-breeding animals. The data from this study imply that body condition may have a direct impact on individual fitness. However, this finding should be treated with caution because the methods employed failed to distinguish between increases in body weight due to greater fat reserves/muscle tissue and increases weight caused by the development of reproductive organs. It is likely that breeding male voles appeared in better condition than non-breeding males simply because body weight increases as testes develop. Nevertheless, reproductive organ development is likely to have much

less of an influence on the weight of (non-pregnant) females and thus the apparent relationship between fitness and condition may well hold true. Alternatively, the difference in condition between female breeding and non-breeding voles may be an artefact of an allometric shift in tail length to body length ratio between different age groups.

5.4.5. Body size, sexual dimorphism and the island rule in bank voles

When body weight data from this study were compared with previously published data from bank vole populations across Europe, there was no evidence for a latitudinal gradient of body size, as Bergmann's rule would suggest. Perhaps this finding is unsurprising because while there is substantial evidence that mammals as a class follow a Bergmannian trend in colder climates (Ashton *et al*. 2000, Rodríguez *et al*. 2006, 2008), when considered at species level, it has been shown that only around 65% of mammal species conform to this pattern (Meiri and Dayan 2003). Furthermore, mammals of smaller body size (<500 g) are significantly less likely to conform to Bergmann's Rule than larger species (Meiri and Dayan 2003), which seems somewhat counterintuitive to the central tenet that size evolution is related to heat conservation mechanisms (Ashton *et al*. 2000). However, it may be the case, at least in this study, that other body size trends (island effects and increases with altitude) simply masked any weaker latitudinal signal in the data. Alternatively, other selective pressures, such as the ability to escape predators, may inhibit the evolution of Bergmannian increases in body size in species like the bank vole.

When island species were excluded, there was a significant relationship between altitude and vole body size. However, this altitude-weight relationship could be

criticised on the basis that all of the body weight measurements for high altitude populations came from one study conducted in the French Alps. Therefore it would be interesting to see if this relationship held if data from other high altitude populations (e.g. Pyrenean voles) were included in the analysis. Nevertheless, the apparent convergent evolution of increased body size on islands and at altitude leads one to question whether the same selective pressures are operating in both habitats. It is possible that increased body size in alpine populations may result from heat conservation adaptations to the decreased temperatures inherent with habitation at high altitude. However, there was no demonstrable effect of temperature on body size when all populations were included in the analysis, nor was there any relationship between body size and Conrad's Index of Continentality (CIC, Conrad 1946 in Berry 2009), suggesting that temperature is not the sole cause of differential size evolution amongst bank voles. Parallel factors that may distinguish island and alpine habitats from other populations include a lack of weasels and microtine competitors (Yoccoz and Mesnager, 1998). These hypotheses are fully discussed in relation to island syndrome in Chapter 7.

Other interesting patterns that arose from analysis of the published literature data include the positive relationship between vole body weight and January rainfall. The reason for this correlation is unclear although it may possibly be related to vegetation quality. Banks *et al*. (2008) found that rainfall significantly affected vole population dynamics during a mink removal experiment on a Finnish archipelago; vole populations were unable to increase, despite the release from predation pressure, in years where summer rainfall was below average. They attributed this to the direct impacts of rainfall on vegetation productivity. Body size differentiation in subspecies

of pocket gophers (*Thomomys bottae*) is thought to be directly related to the nutritional quality of vegetation (Smith and Patton 1988). Furthermore, gophers inhabiting artificially irrigated alfalfa plots are significantly larger than those feeding on poorer quality vegetation in terms of cranial size and body weight (Patton and Brylski 1987). Simard *et al* (2008) showed that a decline in vegetation quality was responsible for decreased body weights and the rapid evolution of dwarfism in an introduced population of white-tailed deer (*Odocoileus virginianus*) on Anticosti Island, Quebec.

Islands are likely to experience higher precipitation than geographically similar mainland sites (Frafjord 2008) and thus in areas where rainfall is a limiting factor, vegetation may be more productive on islands providing animals with the nutrients required to facilitate an increase in body size. However, long-term climate data from field sites investigated during this study (Chapter 2) indicate that increased precipitation is not the key factor in determining body size amongst island populations of British bank voles. For example voles from mainland Pembrokeshire were significantly smaller than those on Jersey (summarised in Table 5.4) despite average rainfall being much greater at the mainland site (Chapter 2). For logistical reasons, the climate data analysed for sites from published studies were not specifically matched to the year that body weight studies were conducted, thus perhaps the relationship between precipitation and bank vole body size across the species range warrants further investigation.

Island voles (including populations without distinct subspecies) were significantly heavier than mainland conspecifics in autumn but not spring. This seasonal effect may be related to the differing age structure of the island populations. Previous studies have shown that, compared to mainland populations, a higher proportion of adults

alive in spring survive into autumn on the islands of Mull and Raasay (Delaney and Bishop 1960) and Jersey (Bishop and Delany 1963). These animals are undoubtedly heavier than young-of-the-year animals and thus higher survivorship may significantly increase the mean weight of autumn populations. Overwinter mortality of these animals would then perhaps explain the lack of difference in mean weights between insular and mainland populations in spring.

There was a significant difference between male weights within the three habitat types in spring but not autumn and there was no such difference in female weights. Male voles in 'open' habitat were significantly heavier than those in deciduous forests but not coniferous forests. The reason for this relationship is unclear but it could be influenced by the montane and island habitats largely falling into the 'open' habitat category. Sexual size dimorphism showed that males tend to be larger than females in spring but not autumn therefore this may explain the seasonal variation and the lack of relationship between female weights and habitat type. However, why there was no apparent size difference between voles in open habitat and coniferous forest is unknown. In addition, specific habitat details from different sites were not always available, and the distinction between coniferous and deciduous in this analysis must be treated with caution.

There was little evidence to support the existence of reverse sexual size dimorphism in bank voles as suggested by Bondrup-Nielson and Ims (1990 in Yoccoz and Mesnager 1998), apart from in the Scandinavian voles. Thus the assumption that females are larger than males in *Myodes* species requires further investigation. In the majority of populations, mean weights of males were greater than those of females in spring, but

this trend disappeared in autumn. This pattern is to be expected if increased body size in males is a result of sexual selection because animals captured in spring are likely to be sexually mature or maturing adults whereas autumn populations tend to be dominated by immature young of the year animals (Chapter 4).

Yuccoz and Mesnager (1998) suggested that male:female body size ratios were higher in alpine bank voles than other mainland populations. They hypothesised that this difference was due to increased survival rates, resulting in decreased reproductive rates which lead to increased competition amongst males thus selecting for increased body size. Increased survival and decreased reproductive rates are a commonly cited characteristic of insular small mammal populations (Adler and Levins 1994), therefore it follows that one may expect to see the same pattern of increased size dimorphism in such populations, if this hypothesis is correct. Indeed, when male:female size ratios were examined between island and mainland populations, and the alpine populations studied by Yuccoz and Mesnager (1998) were excluded from the analysis, island voles did have significantly higher ratios than mainland conspecifics. The relationship between body size and demography is returned to in Chapter 7 but these data highlight another interesting parallel between insular and montane populations.

Spring male:female weight ratios were negatively correlated with latitude and temperature range, and positively correlated with January temperatures. This pattern may be related to the maternal advantages associated with increased body size (Ralls 1976), which may become particularly important for small mammals in areas of higher latitude where lower winter temperatures and greater annual fluctuations in temperature are typical. For example, larger mothers may produce larger offspring that

may be metabolically advantageous in colder climates. This may explain why only Scandinavian voles showed reverse sexual size dimorphism.

5.5. Summary

1. Body size variation, in terms of length, weight and condition, were investigated between four mainland and six insular populations of bank voles in spring and autumn.
2. A summary of the mean body size measurements from these populations is given in Table 5.4.
3. A novel approach was used to gather head-to tail body length data from live animals in the field but subsequent analysis of museum specimens showed that there may be problem including tail length in body size measurements.
4. Body size data largely supported previous observations that voles of Raasay, Skomer and Jersey are larger than mainland and other island populations.
5. There was less distinction between Mull voles and mainland populations but this effect was seasonally dependent.
6. Voles from Ramsey Island were found to be slightly larger than mainland populations but sporadic sampling may have influenced this result and further data are needed for this island.
7. Body size and weight data revealed slightly different patterns, thus the appropriateness of different body size measures are discussed.
8. Body condition was found to vary between populations but this was most likely attributable to an allometric shift in shape rather than a difference in fat reserves.

9. Data from the literature were combined with data from this study and relationships between vole body weights and environmental variables were examined.
10. There was no relationship between latitude and body size but there was a significant relationship between altitude and size (when island populations were excluded).
11. Body size was positively correlated with January rainfall indicating that vegetation quality may have an effect. However, this is unlikely to be the primary cause of gigantism in island populations.
12. Males were found to be larger than females in most populations and this effect increased on islands, suggesting increased sexual selection.
13. Reverse sexual size dimorphism (females larger than males) reported by previous authors may only occur in more northerly populations.

Table 5.4: Summary of mean body size measurements gathered from nine British bank vole populations, and one French population. Site codes; Isle of Wight (IOW), mainland Pembrokeshire (Pembs).

Measure	Jersey	Skomer	Raasay	Mull	Pembs	France	IOW	Ramsey	Surrey	Morvern
Body weight spring (g)	40.3	29.7	35.3	27.5	20.4	*	16.5	17.4	15.6	12.5
Body weight autumn (g)	28.7	25.7	35.6	33.1	21.6	17.1	*	27.3	*	*
HT length spring (mm)	162.5	158.1	160.5	145.2	139.3		134.9	138.3	125.0	133.9
HT length autumn (mm)	152.2	152.0	167.8	156.1	138.7	138.1	*	144.5	*	*
Condition index spring	0.25	0.19	0.22	0.19	0.15	*	0.12	0.12	0.13	0.09
Condition index autumn	0.19	0.17	0.21	0.21	0.15	0.13	*	0.19	*	*
Tail:HB ratio from dissections (mm)	0.54	0.5	*	*	0.55	*	0.63	0.55	*	0.53
Corrected HT length spring (mm)	75.3	79.1	*	*	62.1	*	49.4	61.7	*	59.7
Corrected HT length autumn (mm)	70.5	76.0	*	*	61.8	*	*	64.4	*	*
Corrected condition index spring	0.54	0.37	*	*	0.33	*	0.34	0.28	*	0.21
Corrected condition index autumn	0.40	0.33	*	*	0.35	*	*	0.42	*	*

Chapter 6: Skull morphology and fluctuating asymmetry

6.1. Introduction

6.1.1. Geometric morphometrics

Traditionally, biologists have relied on descriptions and simple linear measurements of morphological differences to classify organisms into taxonomic groups (Adams *et al*. 2004). With the advent of the genetic revolution, this approach may seem to have lost its importance in modern biology. However, recent advances in geometric morphometric techniques have enabled biologists to quantify phenotypic differences in shape in a much more detailed manner than traditional methods allow (Lawing and Polly, in press). Thus, particularly in the field of phylogeography, geometric morphometric analyses are now being used in conjunction with molecular techniques to help identify and explain the causes of variation both within and between species (Barciová 2009).

Shape information for geometric morphometric analyses is most commonly gathered in the form of landmark coordinates, which are specific points on a given object that can be compared to biologically homologous points on other specimens (e.g. cranial suture junctions). However, in cases where biologically homologous points are difficult to define, there are several alternate methods that permit the analysis of curves and outlines (Barciová 2009). Geometric morphometric analyses have an advantage over traditional techniques because they produce a quantitative, size-independent measure of the dissimilarity between shapes and furthermore, the

geometric coordinates can be plotted to aid visualisation of existing differences (Lawing and Polly, in press).

Loy *et al*. (2001) used a combination of genetic and morphometric techniques to investigate the possibility of hybridisation between two closely related species of mole (*Talpa europea* and *T. romana*) in an area of sympatric occurrence. Whilst there was little evidence for free interbreeding between the two species, analysis of allozyme variation combined with geometric morphometric skull analysis revealed some degree of hybridization or backcrossing had occurred in the past.

Caumul and Polly (2005) studied the effects of multiple environmental variables in combination with mitochondrial DNA variation on shape variation of skulls, molars and mandibles in Eurasian marmot species. Despite the relatively strong influence of some environmental variables on cranial features, maximum-likelihood trees constructed from molar and skull data produced relatively congruent phylogenetic trees to those inferred from mtDNA sequence analysis, whilst mandible shape was far less reliable.

Geometric morphometric analyses have also been extended to analyse shape in a three-dimensional as well as two a dimensional-plane. Cardini and Thorington (2006) used 3D landmark coordinates to describe cranial variation between marmot species, finding that similarities were closely linked with subgeneric classification or geographic distribution. A similar approach was employed to describe clinal variation in cranial features of red colobus monkeys (*Procolobus*) (Cardini and Elton 2009).

Geometric morphometrics have unsurprisingly proven useful in other fields. For example, Haney *et al*. (2001) used this approach to explore the effects of paleoenvironmental conditions on valve shape variability in fossils of the brachiod genus *Sowerbyella*. Many other authors have used geometric morphometrics to investigate the effects of ontogenetic disturbance on the morphology of a variety of taxa occupying fragmented, isolated or insular habitats (e.g. mice: Hopton *et al.* 2009; birds: Lens *et al*. 2002; humans: Shaefer and Bookstein 2009 and shrews: White and Searle 2008b).

Previous studies of variation in skull morphology of British bank vole populations found Skomer voles to have distinctively shaped nasal bones, and Jersey voles have a distinctive anterior palatal foramina compared to other populations (Corbet 1964). However, (with the exception of skull size) no consistently different cranial traits have been observed between voles from the island populations of Mull and Raasay and those from mainland Scotland (Delany and Bishop 1960). Raasay, Skomer and Jersey voles all have complex 3rd upper molar, where the fourth inner ridge is well developed (Corbet 1964) but this varies in a different manner between populations and is not exclusive to island subspecies (Corbet 1975). I have employed geometric morphometrics in this study, specifically avoiding traits with already clearly described differences, to see whether any other morphological changes in our island subspecies that separate them from mainland populations. In particular, I test the following hypotheses:

1. Island vole subspecies are morphologically distinguishable in features other than body size;

2. Skull morphology does not support the subspecies classification of voles from Mull and Raasay;
3. Sex and age influence vole skull morphology;
4. There is regional variation in skull morphology;
5. Island vole populations without designated subspecies have skull morphology that is indistinct from mainland vole populations.

6.1.2. Fluctuating asymmetry

Within higher metazoans, conspicuous asymmetry in typically bilaterally symmetrical features is a common occurrence (Palmer 1996). Classic adaptive examples include sexually selected traits such as the enlarged claw exhibited by fiddler crab species (*Uca* spp.) and the horn-like tooth of narwhals (*Monodon monoceros*). Such traits can be divided into two general categories based on development; directional asymmetry and antisymmetry. Directional asymmetry (DA) describes an anatomical feature that is consistently larger on one side than on the other, and this holds true for the majority of individuals within a given population (e.g. narwhals), whilst antisymmetry (AS) occurs when a feature is consistently larger on one side than the other but the enlarged side is variable and unpredictable within a population (e.g. most fiddler crab species; Palmer 1996).

Whilst asymmetrical features may sometimes arise as adaptations (although this is not implicit with DA and AS derived features), a further type of asymmetry exists that is thought to result from perturbations during development. This is known as fluctuating asymmetry (FA). FA refers to the asymmetrical development of features that are

normally bilaterally symmetrical and this asymmetry occurs in a non-specific direction. FA is commonly used as a phenotypic indicator of developmental stability (Hopton *et al*. 2009), which is defined as "an organism's ability to develop toward an adaptive end-point despite perturbations during its ontogeny" (Brown *et al.* 2008). FA can occur when organisms are subjected to stress, such as disease, exposure to extreme temperatures or toxins (e.g. Oleksyk *et al*. 2004), overcrowding (Almeida *et al.* 2008) or lack of food (Moller 1990) during developmental stages. For this reason, FA in secondary sexual characteristics has been described as an "honest signal of individual quality" (Watson & Thornill 1994), and there is some evidence that FA may have an effect on mate choice, and consequently mating success, in some species of birds and insects (Moller 1992, 1993, Harvey and Walsh 1993).

Fluctuating asymmetrical development has been shown to occur in a variety of mammalian characteristics including, red deer (*Cervus elaphus*) antlers (Bartos and Bahbouh 2006), red squirrel (*Sciurus vulgaris*) hind feet (Wauters *et al.* 1996) and bank vole (*Myodes glareolus*) skulls and teeth (Marchand *et al*. 2003). However, there is some debate as to whether FA is a real biological phenomenon or simply a result of measurement error in many of these cases (Pomory 1997, Hingle *et al*. 1999). Furthermore, the validity of FA as a measure of stress has been subject to extensive criticism (Bjorksten *et al*. 2000).

It has been suggested that the effects of FA are likely to be more pronounced in small, isolated populations (such as those found on islands) than in larger populations where random mating is more likely to occur (Wauters *et al*. 1996, Suchentrunk *et al*. 1998). FA is predicted to increase with selection against heterozygotes and loss of genetic

diversity caused by inbreeding. In their study of red squirrel hind feet, Wauters *et al*. (1996) claimed to have found an association between FA and habitat fragmentation. However, the relationship was not a straightforward correlation between genetic diversity and FA. Whilst squirrels in fragmented woodlands exhibited a higher degree of FA than those inhabiting larger woodlands, the effect was much more pronounced in the 'poor quality' (i.e. smaller and non-breeding) individuals.

In a 'natural' experiment Schaefer *et al*. (2006) compared dental arch asymmetry between human inhabitants of a Croatian island in the Adriatic and those in the capital city, Zagreb. These authors found that FA was much higher in the island population and became further pronounced amongst the insular populace with increasing levels of inbreeding. Other studies have found a negative correlation between facial and body asymmetry and levels of attractiveness in humans (Brown *et al*. 2008) indicating that symmetry in such traits is an 'honest signal' of quality.

Gilligan *et al.* (2000) investigated the relationship between genetic diversity and FA of the sternopleural bristle numbers in *Drosophila melanogaster*. They were unable to find a clear link between these two variables and concluded that FA was a poor monitoring tool for trying to detect inbreeding and genetic diversity loss. Studies investigating links between habitat fragmentation and developmental differences in two South American frog species showed that changes in body size and body shape were correlated with habitat loss. However, FA measured on bone length ratios was evident in all populations and only radio-ulna length of one species showed a significant trend towards increasing FA with habitat fragmentation (Delgado-Acevedo and Restrepo 2008).

Hopton *et al*. (2009) studied FA in populations of two species of deer mice (*Peromyscus*) following the devastation of forest habitat after a tornado. They found opposing effects of disturbance on the two species; *P. leucopus* experienced an increase in FA in disturbed habitat compared to undisturbed areas, whereas the converse was true for *P. maniculatus.* These authors postulated that disparity was probably due to the differential habitat preference of the two species, with the former preferring forest habitat and the latter preferring open or herbaceous habitat. However, contrary to predictions, food availability and body condition were higher for both species in disturbed areas, indicating that FA in *P. leucopus* was driven by other environmental stressors when the species was forcibly switched to less preferable habitat.

Marchand *et al*. (2003) compared the skull morphology and dentition in bank vole populations inhabiting a number of areas, with varying levels of disturbance. This study revealed that animals living in fragmented hedgerows showed higher levels of FA than those in more connected habitats. In addition to geometric morphometric analyses, Marchand *et al*. (2003) used multiple linear measurements from dorsal views of bank vole skulls and from the first lower molar to assess levels of FA. However, they found that only two out of 16 of these characteristics reflected the expected pattern of increased FA in individuals from populations residing in fragmented habitats. Using teeth as a marker for FA is particularly problematic because the protective enamel is susceptible to wear, and potentially there may be some asymmetry to this wear that reflects environmental rather than developmental effects. In addition, in small features such as teeth, even minor changes in alignment will

greatly amplify measurement error. Thus, in this study, I have avoided using linear measurements to investigate FA.

Geometric morphometric analyses have also been employed to study levels of FA in island versus mainland populations. White and Searle (2008b) measured FA in populations of common shrews (*Sorex araneus*) inhabiting 13 small islands off the western coast of Scotland, as well as six populations from mainland Britain. Some level of FA was found in all populations and there was a significant inverse correlation between FA and genetic diversity at the population level, but not at the individual level. However, this correlation was largely driven by one island, and the relationship became non significant when that population was removed from the analysis.

Animals living on islands, and particularly small islands, may be subject to a different set of stressors, or more extreme stressors, to their counterparts living on the mainland. For example, island communities may be subject to severe natural disturbances such as extreme weather, as well as biotic and human disturbances. Low genetic diversity resulting from small founding populations, inbreeding or the amplified effects of genetic drift may cause genetic stress in insular populations. Incomplete colonisation of islands by flora and fauna may lead to depauperate habitat (Adler and Levins 1994). Thus food and predation may pose different problems for island animals. Furthermore, occupation of less preferable habitat has been shown to increase levels of FA in some species of small mammal (Hopton *et al*. 2009). Island animals may also be subject to greater intraspecific and or interspecific competition if population densities reach high levels (see Chapter 4).

A case in point is Skomer Island, where in the absence of woodland or hedgerow habitat, voles are found closely associated with patches of bracken. Bracken is routinely controlled in some areas by 'bashing' and historically, bracken cover was probably minimal because large parts of the island were farmed or grazed by cattle. In this severely modified habitat, voles must exist on a different diet to that of mainland counterparts (Healing 1984). Whilst there are no ground predators, Skomer voles may be subjected to increased disturbance by other prevalent species on the island such as shearwaters, rabbits and mice. Since such stressors may increase the frequency and size of disturbances to developmental processes, and thus elevate FA, I have explored FA in island bank voles. In particular I address the following hypotheses:

1. FA in island voles is greater than in mainland voles;
2. FA is greater in island vole subspecies than other island or mainland populations.

6.2. Methods

6.2.1. Samples

Skulls of 73 individuals from eight island and seven mainland populations were examined; one clearly anomalous specimen (Museum No. 65.285, Table 6.2) was subsequently removed from the analyses because of the difficulty of accurately placing landmarks during the digitisation process. Specimens were predominantly from collections housed at the Natural History Museum (London), augmented with donations from various other sources. Small sample sizes were a result of many of the

skulls from the collection being unsuitable for inclusion in this study due to post mortem damage. It was not possible to obtain fresh specimens because permissions to access many of the field sites were granted on the basis that destructive sampling would not take place. Thus, it was not possible to collect morphometric and genetic data from the same animals.

6.2.2. Photography and digitisation of images

In order to determine the most informative landmarks, a pilot study undertaken on 10 skulls collected from five island and mainland locations. Photographs were taken of dorsal, lateral and ventral views of skulls; the skulls were oriented and photographed five times to minimise the effects of misalignment. Unfortunately due to the fragility of the skulls it was not possible to use exactly the same individuals for all three orientations. Preliminary analyses suggested that the dorsal views were more informative and less prone to error in placing landmarks than either the ventral or lateral views (Appendix Figs. 6.1-6.4). Thus the main study was restricted to dorsal views only.

The intention was to utilize landmarks described by Marchand *et al.* (2003) but many of these were indistinct, ambiguous or absent on particular skulls due to post mortem damage. Selected landmarks included suture junctions along the nasal-occipital central axis, along with a number of bilateral features so that the data could also be used to look for the presence of fluctuating asymmetry. Landmarks chosen are shown in Table 6.1 and Figure 6.1. Digitisation of photographs was carried out using the software tpsDig2 (Rohlf 2005). For two individuals where images were unsuitable and skulls were unavailable to re-photograph, one of the four remaining images was chosen at

random and the digitised coordinates duplicated to maintain consistency within other parts of the analysis.

6.2.3. Statistical analysis

Replicates for all individuals were aligned using Generalised Procrustes Analysis with the software tpsRelw (Rohlf 2003). This analysis rotates, scales and translates landmark coordinates for each specimen to minimise the distance between corresponding landmarks on other specimens. Procrustes distance (PD) is essentially a measure of the total difference between all pairs of landmarks following this superimposition. Thus, PD is size-independent measure of the dissimilarity between two shapes, with a PD of 0 corresponding to two identical shapes. These aligned coordinates were used to perform a principal components analysis (PCA), utilising 'relative warps' in tpsRelw (Rohlf 2003). This analysis groups skulls of similar shape close to each other in principal component space. Principal component coordinates were averaged across the five replicates for each individual before plotting. To further examine patterns of morphological differentiation, PD was calculated for all pairwise comparisons between all replicate images and divided into three categories: (i) within individual, N=720; (ii) within non-self individuals within population, N=4050; (iii) between populations, N=59850.

In order to examine the effect of age on skull morphology, head-to-tail body size was used as a surrogate measure for age. Animals were placed into 'small', 'medium' and 'large' categories based on pooled data on body weight, breeding status and time of year caught. There were two groups of animals: (i) mainland and non-subspecies

island populations (ii) islands with subspecies of known larger body size (Appendix Table. 6.1).

6.2.4. Randomisations

To test for structure within the results of the principal components analysis, a series of randomisations were carried out following a procedure adapted from that described in Rossiter *et al.* (2005). Specifically, I was looking for differences in PD between; (i) island and mainland populations (ii) island, mainland and islands with subspecies populations (iii) English, Scottish, Welsh and Channel Island combined with French populations. In these analyses, populations were randomly assigned to the sub-groups of interest and the mean pairwise distance between populations within each category was calculated. This process was repeated 100,000 times, generating for each intragroup comparison (e.g. island-island, mainland-mainland) a null distribution to which the calculated values in the actual data set could be compared, using a Z-test. This approach has the advantage of preserving any asymmetries within the data, such as unequal sample size, along with other specific attributes of the data set.

6.2.5. Fluctuating Asymmetry

To measure FA the mean aligned scores for each individual were taken and coordinates relating to the right half of the skull, including the central line landmarks, were reflected in the Y-axis to produce a mirror image which could be superimposed on the left half of the skull. The Procrustes fit of right side against left side for each skull was then calculated. In this instance, a PD of 0 would indicate perfect skull symmetry, with greater values corresponding to increasing asymmetry. FA values

were compared between (i) populations, (ii) island and mainland populations, (iii) island, mainland and islands with subspecies populations. Following methods described by White and Searle (2008b) a corrected version of FA (here designated FA_{corr}), removing the effect of within population directional asymmetry, was also calculated for each individual.

To assess whether the observed estimates of FA arose simply because of errors in skull orientation or digitisation of landmarks, PD was calculated within each set of replicates for all pairwise comparisons of, first, left sides, and second, right sides. Since in each case identical objects were being compared, any lack of congruence can only arise as error in one of the two ways outlined above. The resulting distances were then randomly sub-sampled 10,000 times and the "population" mean FA calculated, generating a null distribution of the same dimensions as the actual data set to which the observed FA values could be compared.

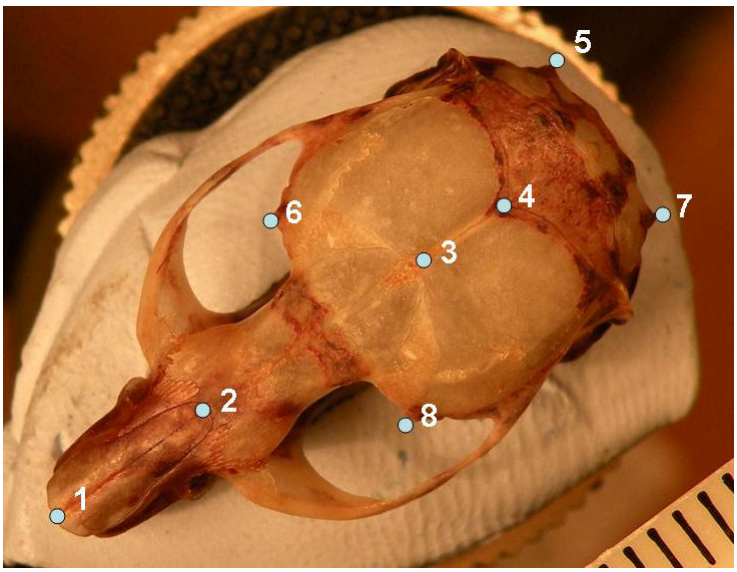

Figure 6.1: Position of landmarks used in the shape analysis of the dorsal view of bank vole skulls.

Table 6.1: Description of landmarks used in the shape analysis of the dorsal view of bank vole skulls.

Landmark	Description
1	Tip of nasal bones
2	Junction of nasals and frontal bone
3	Junction of coronal and sagittal sutures
4	Junction of lambdoidal and sagittal sutures
5	Tip of right process of nuchal crest
6	Tip of right supraorbital process
7	Tip of left process of nuchal crest
8	Tip of left supraorbital process

Table 6.2: List of specimens included in the morphometric analyses, origin of specimens and population abbreviations used in this chapter are shown along with sex and head-to-tail body size measurements (H-T) where known. Source indicates current location of specimens. Asterisk denotes approximate longitude/latitude.

Population	Location	Latitude/ Longitude	Museum No.	Sex	H-T (mm)	Source
Island subspecies						
JER	Jersey	49°12'N, 2°07'W	JDIS3	m	149.9	Donated
			JDIS4	f	152.4	Donated
			JDIS5	m	156.5	Donated
			60.2028	f	159	NHM
			60.1991	m	163	NHM
			60.1997	m	164	NHM
			60.2027	f	170	NHM
			60.2026	f	172	NHM
MUL	Mull	56°27'N, 5°52'W	65.285	f	123	NHM
			65.289	f	128	NHM
			65.293	f	133	NHM
			65.292	m	133	NHM
			65.290	f	135	NHM
			66.4609			NHM
RAA	Raasay	57°23'N, 6°02'W	65.306	m	139	NHM
			65.296	f	145	NHM
			65.313	m	145	NHM
			65.308	f	148	NHM
			65.310	f	152	NHM
			87.901			NHM
SKO	Skomer	51°44'N, 5°17'W	S53M	m	122.6	Donated
			SSK	f	141	Donated
			67.652	f	153	NHM
			3.7.4.7	f	155	NHM
			3.7.4.4	m	161	NHM
			3.7.4.6	f	164	NHM

Islands						
ANG	Anglesey	53°16’N, 4°21’W	65.578	F	138	NHM
			65.574	m	144	NHM
BUT	Bute	55°49’N, 5°04’W	73.36			NHM
			73.37			NHM
			47.738			NHM
IOW	Isle of Wight	50°40’N, 1°17’W	60.307	f	133	NHM
			60.298	m	135	NHM
			IOW23	f	139	Donated
			60.304	m	143	NHM
			60.301	m	143	NHM
			11.1.3.224	f	149	NHM
RAM	Ramsey	51°51’N, 5°20’W	RAM3	f	135.4	Donated
			RAM1	m	138.2	Donated
Mainland						
MOR	Morven, Argyll	56°26’N, 5°26’W	MM6	f	131.3	Donated
			MM4	f	132.2	Donated
MWA	Gwynedd, Snowdonia	52°55’N, 3°59’W*	11.1.3.254	f	137	NHM
			11.1.3.231	m	144	NHM
			11.1.3.256	f	148	NHM
	Loughasne, Carmarthen	51°51’N, 4°18’W*	11.1.3.259	f		NHM
			11.1.3.260	f		NHM
STM	St. Malo	48°38’N, 2°01’W	72.1993			NHM
			72.1995			NHM
			72.1998			NHM
			72.1999			NHM
			72.2009			NHM
SUF	Rougham, Suffolk	52°13’N, 0°48’E	60.2077	f	143	NHM
			60.2075	f	146	NHM
			60.2061	m	150	NHM
			60.2072	f	158	NHM
			60.2060	m	162	NHM
SUN	Strontian, Sunart	56°41’N, 5°34’W	65.175	m	132	NHM
			65.177	m	137	NHM
			65.187	f	139	NHM
			65.173	m	155	NHM
			65.183	f	159	NHM
SUR	Wimbledon Surrey	51°25’N, 0°12’W	30.3.14.23	f	119	NHM
			30.3.14.17	m	127	NHM
			30.3.14.26	f	131	NHM
			30.3.14.22	f	147	NHM
			30.3.14.15			NHM
TAY	Loch Tay, Perthshire	56°30’N, 4°9’W	65.28	f	124	NHM
			65.18	m	145	NHM
			65.14	m	145	NHM
			65.13	f	150	NHM
			65.20			NHM

6.3. Results

6.3.1. Geometric morphometric analyses

Table 6.3 shows the relative contribution each landmark made towards the PC scores produced by the morphometric analysis. These data clearly show that shifts in landmarks 3 and 4 are mainly responsible for deformations away from the consensus configuration calculated by the Generalised Procrustes Analysis. In the PCA, the first two principal components were able to explain 55% of the variance in the data set (Table 6.4). When plotted, these were the only components that showed interpretable clustering of specimens. Thus, data for the third and fourth principal components are not shown.

Table 6.3: Relative contribution of individual landmarks to principal components analysis (calculated from covariance matrix)

Landmark	% contribution
1	0.96
2	6.05
3	40.09
4	36.51
5	2.73
6	5.19
7	2.89
8	5.58

Table 6.4: Explanatory power of individual principal components produced from geometric morphometric analyses of bank vole skulls, represented as a percentage and cumulative percentage.

Principal component	% variance explained	Cumulative % variance explained
1	35.93	35.93
2	18.79	54.72
3	14.75	69.47
4	9.96	79.43
5	6.37	85.80
6	4.87	90.67
7	4.01	94.67
8	2.14	96.81
9	1.28	98.09
10	0.73	98.82
11	0.69	99.51
12	0.49	100.00

Figure 6.2 shows the change in position of landmarks away from the consensus alignment along both axes. On the left of the *x* axis, landmark 4 (the junction between the lambdoidal and sagittal sutures) moves anteriorly, while landmarks 6 and 8 (the supraorbital processes) are slightly displaced in a posterior-medial direction. On the right of the *x* axis, deviations from the consensus are reversed but less pronounced. In addition, landmark 1 (the tip of nasals) moves very slightly anteriorly. At extreme negative values of *y,* skulls are relatively narrower, with medial displacements of all four of the landmarks not located on the midline (5, 6, 7 and 8). There is also posterior movement of landmarks 3 (the junction between the coronal and sagittal sutures) and 4. At the other extreme of axis 2, the opposite pattern is seen.

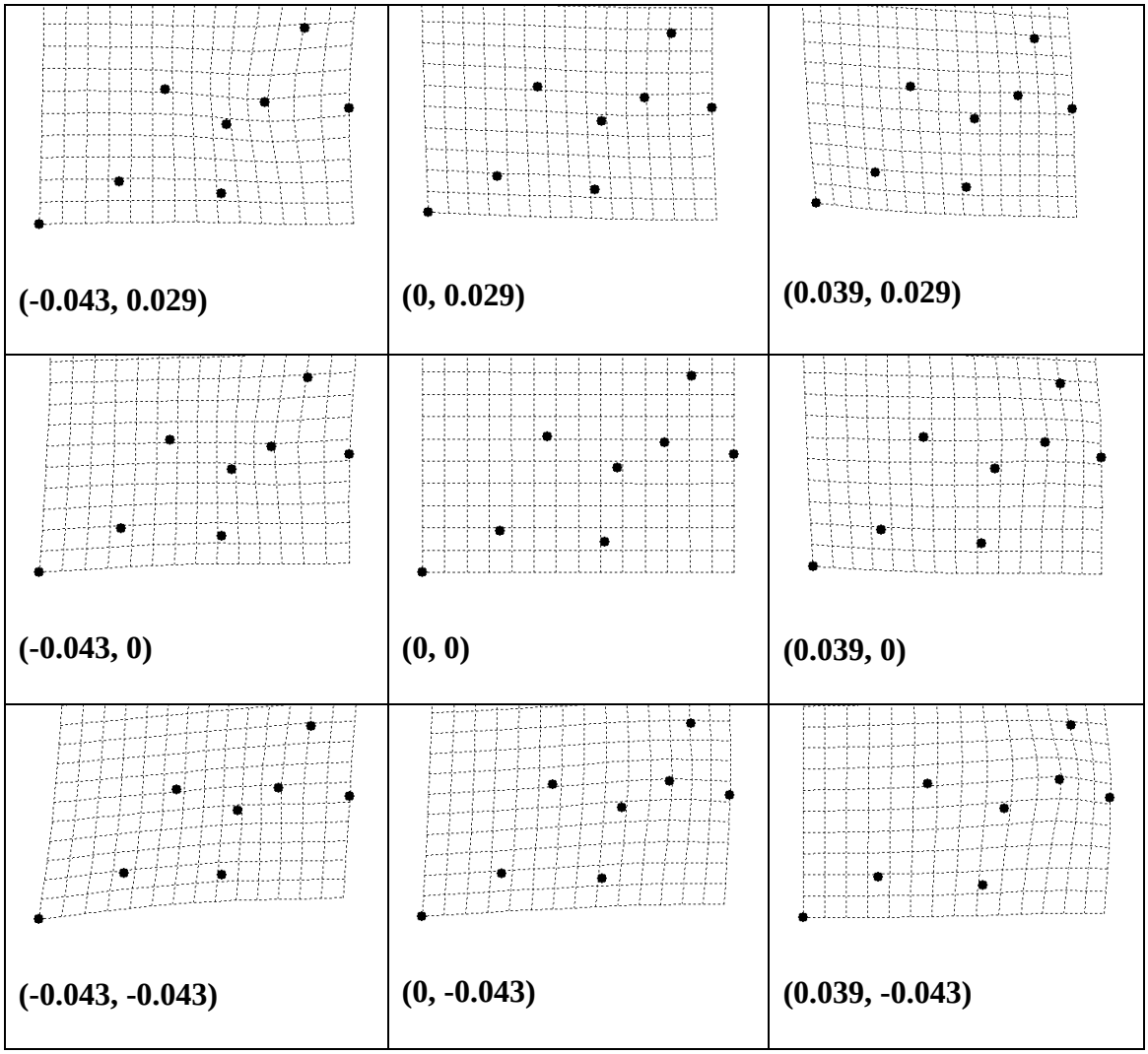

Figure 6.2: Spline plots showing the consensus configuration (0, 0) and deformation of landmarks (black dots - see Fig. 6.1) from this configuration produced by geometric morphometric analyses of dorsal views of 72 *Myodes glareolus* skulls. Coordinate values (*x*,*y*) along the PC axes (as in Fig. 6.3) are given in brackets beneath plots. Each spline is shown in a position reflecting these coordinates. Thus deformations along PC axis 1 are in rows and deformations along PC axis 2 in columns.

Only two populations showed any tendency to form distinct clusters. Skomer voles were partially separated from the other populations by both PC1 and PC2, whereas Jersey voles occupied the extreme end of variation predominantly along axis 2 (Figs 6.3 and 6.4). Thus, Skomer voles tend to be characterised by skulls that are relatively wider with a shortened sagittal suture and a corresponding lengthening of the occipital

bone and condensing of the frontal bone. Jersey voles tend to have skulls that are relatively narrower, with a comparatively condensed occipital bone and a corresponding lengthening of the frontal bone. Arguably, Raasay and Anglesey voles were also located on the periphery of the PCA plot (Fig. 6.3). Skulls showed no consistent differences in patterns of morphology according to either sex or age class (data not shown).

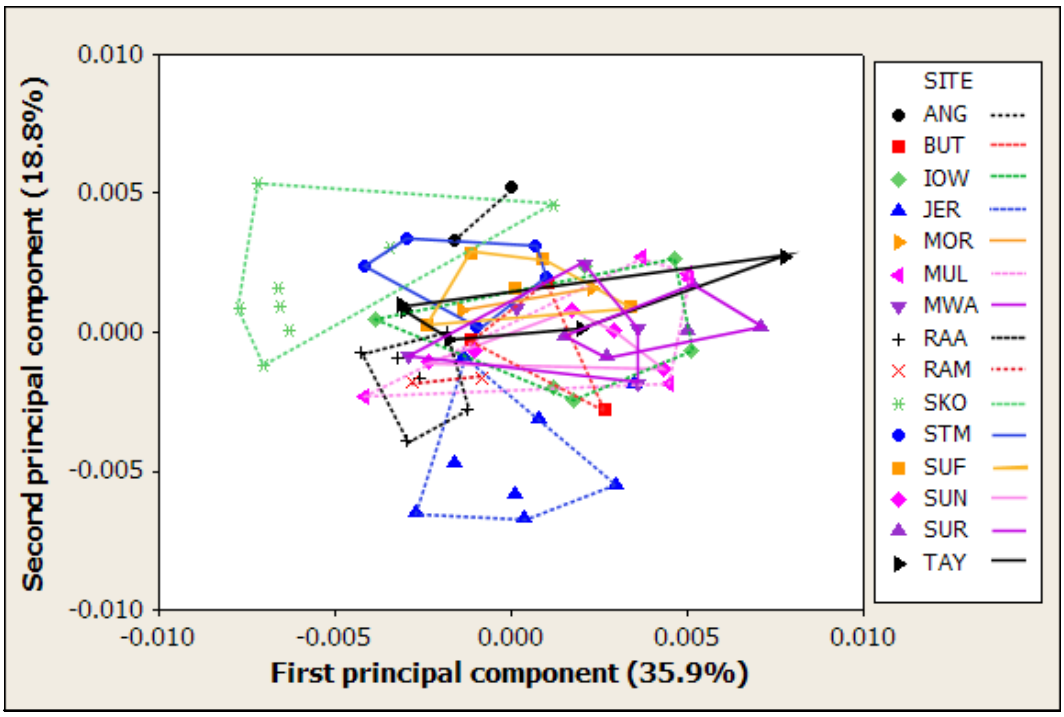

Figure 6.3: Scatterplot of first and second principal component scores from geometric morphometric analyses performed for dorsal views of 72 *Myodes glareolus* skulls. Percentage variance explained by both axes and convex polygons for each population are shown. For an explanation of population abbreviations see Table 6.2.

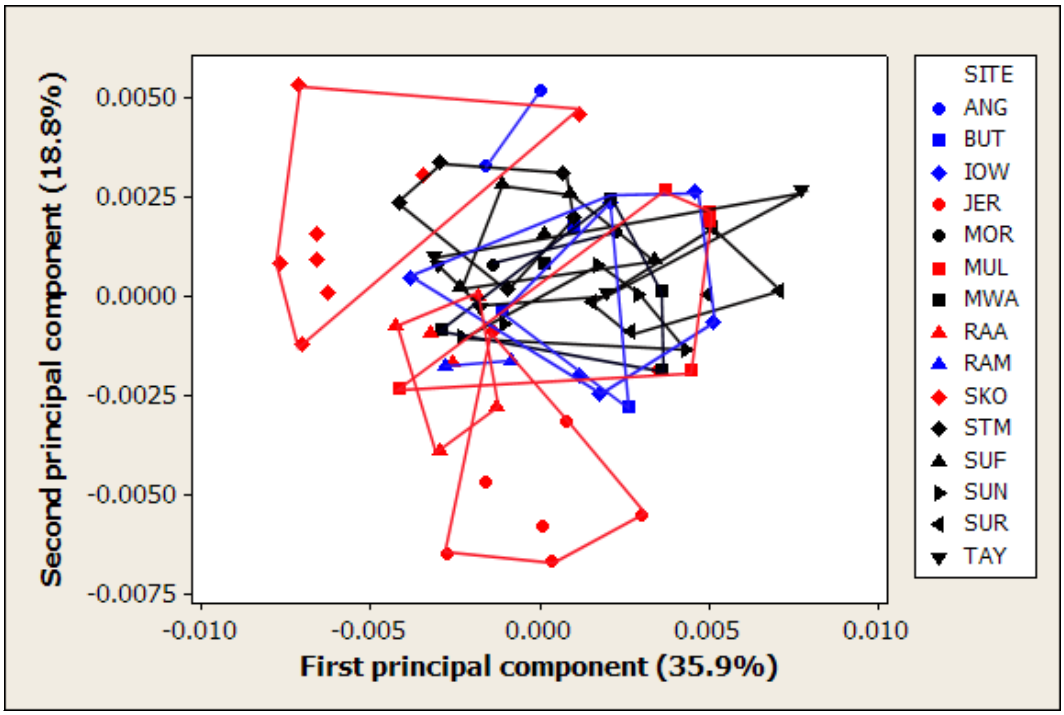

Figure 6.4: Scatterplot of first and second principal component scores from geometric morphometric analyses performed for dorsal views of 72 *Myodes glareolus* skulls. Convex polygons for mainland populations, island populations and islands with subspecies populations are shown in black, blue and red respectively.

Pairwise comparisons between replicates of all skulls showed significantly better fit between replicates from the same individual than between different individuals from the same population, and significantly better fit between different individuals from the same population than between individuals from different populations (Kruskal-Wallis test: H=2882.58, DF=2, P<0.001; Fig. 6.5).

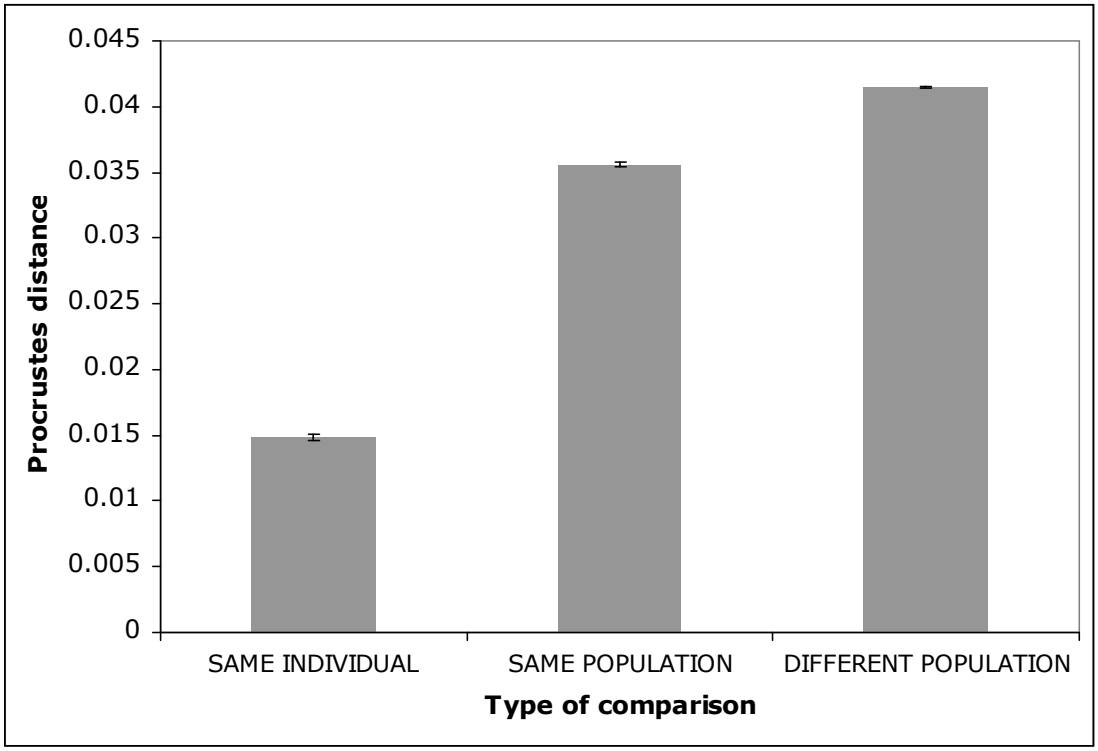

Figure 6.5: Pairwise comparisons between all replicate images measured as mean Procrustes Distances (± 1 se) produced for three categories: (i) within individuals, N=720; (ii) within non-self individuals within population, N=4050; (iii) between populations, N=59850.

6.3.2. Randomisations

To test whether subgroups (e.g. mainland and island populations) were randomly distributed within principal component space, real PC coordinate values were compared to randomly generated null distributions with a series of Z-tests. Skulls showed no consistent differences in patterns of morphology according to geographical distribution ($P>0.05$ in all cases). However, in both the island-mainland and island-mainland-island subspecies analyses, PD between mainland populations were lower than expected by chance (randomisation tests: $P=0.039$ in each case).

6.3.3. Fluctuating Asymmetry

To measure asymmetry within individuals, Procrustes fit for right against left half of skull was calculated using the average aligned coordinates for each individual. These fits were tested with a series of one-way ANOVAs and clearly showed there was no evidence to support a difference in levels of FA between (i) populations ($F_{14,57}$=0.92, P=0.54), (ii) island populations and mainland populations ($F_{1,70}$= 0.05, P=0.83), and (iii) island, mainland and islands with known subspecies populations ($F_{2,69}$=0.12, P=0.89) (Fig. 6.6). This pattern did not change when FA_{corr} was used: (i) populations ($F_{14,57}$=1.58, P=0.11), (ii) islands populations and mainland populations ($F_{1,70}$= 0.20, P=0.66), and (iii) island, mainland and islands with known subspecies populations ($F_{2,69}$=0.59, P=0.56).

Randomisations revealed that the observed values of FA were significantly greater than those arising purely as a result of errors in skull orientation or digitisation of landmarks (randomisations: P<0.0001).

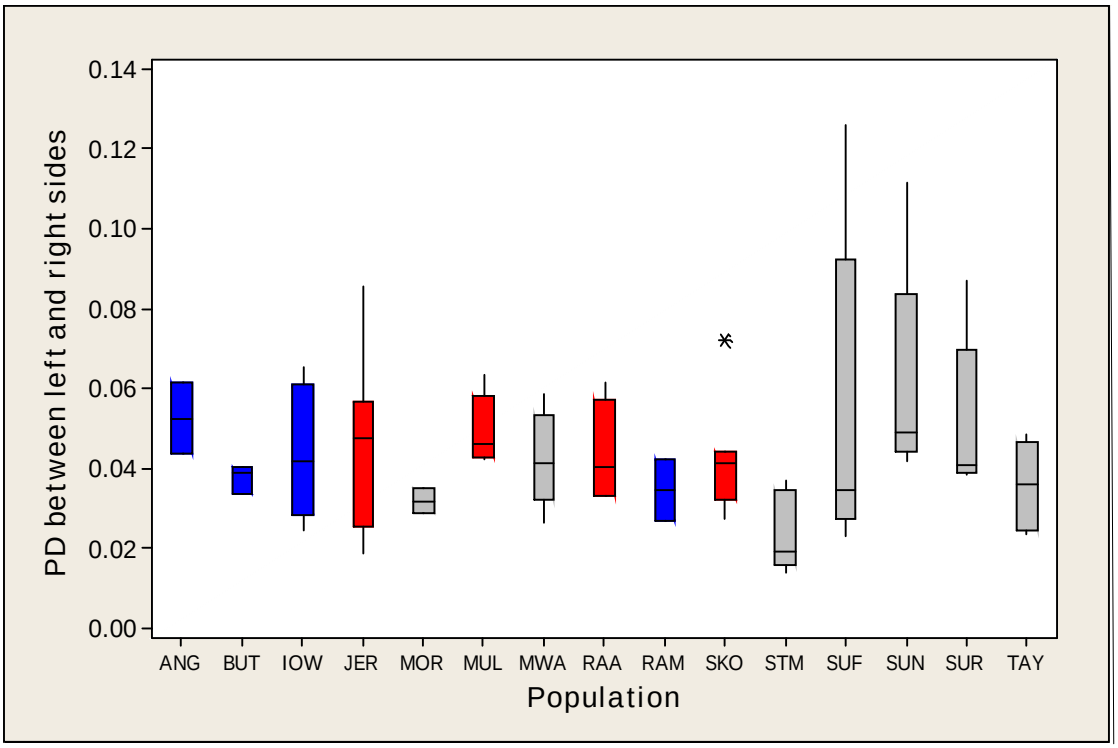

Figure 6.6: Boxplots showing levels of fluctuating asymmetry (FA) for island, mainland and islands with subspecies populations shown in blue, grey and red respectively. FA was measured by calculating Procrustes distance (PD) between right sides of skulls superimposed onto and left sides of skulls. Asterisk denotes an outlier.

6.4. Discussion

6.4.1. Geometric morphometric analyses

Geometric morphometric analyses of bank vole skulls elucidated and confirmed patterns previously described by Corbet (1964) based on an entirely different set of characteristics. Chief among these patterns are the clear differentiation of the Jersey and Skomer populations, and the general homogeneity within mainland populations. It is striking that, although in this study fewer landmarks were used than in some others (see for instance: Marchand *et al*. 2003, Wojcik *et al.* 2006, White & Searle 2008b), the results still show such clear patterns. The sensitivity of these landmarks to

detecting morphological differences between individuals and between populations was further confirmed by results of the pairwise comparisons analysis (Fig. 6.5), where PD was found to be much smaller between replicates for the same individuals than between different individuals from same population, and than between individuals from different populations. This analysis also clearly showed that error between replicates for the same individuals was small, indicting that effects of misalignment and discrepancies in the digitisation process were negligible in comparison to the overall effect.

Whilst Jersey and Skomer voles proved to be the only populations that showed clearly distinctive skull morphology, Raasay voles arguably showed tendencies towards skull shape changes that were not apparent in the mainland populations (Fig. 6.3). This somewhat supports the findings of Delany and Bishop (1960), who compared the skulls of Scottish mainland populations to those from Raasay and Mull. They described the frontal and parietal bones of Raasay voles as “frequently, but not invariably, more arched” than those of other Scottish populations. Thus, this tendency may have partially separated out the Raasay voles in the PCA of this study. Perhaps more regrettably, there were only two suitable specimens available for analysis from the island of Anglesey. Both individuals were located at the positive extreme of PC axis 2 and it would have been interesting to see if this population were discernable as a group if more individuals were added to the analysis. Thus, whilst these data show skull morphology does not support the subspecies classification of voles from Mull and Raasay, further data are needed refute the theory that morphological cranial differences are exclusive to island populations with named subspecies. Skulls from Mull specimens were indistinguishable from those of mainland populations,

concurring with previous descriptions of variation in British bank voles (Delany and Bishop 1960, Corbet 1964). Unlike the other named subspecies, Mull voles do not exhibit a complex third upper molar. However, this trait is highly variable between populations and is not exclusive to named subspecies. Corbet (1975) found evidence of an abnormally complex third molar in an isolated population of voles trapped in the mid-1950s at Loch Tay, Perthshire. Interestingly, when the population was re-examined in 1972, this condition had reverted to normal. Furthermore, this trait is clearly not linked to any of the morphological variation which separated the island voles in this study because the aberrant Loch Tay skulls collected by Corbet were indistinguishable from other mainland populations in the PCA (Fig. 6.3).

Most of the morphological variation that occurred was due to anterior/posterior shifts in the position of the sagittal suture and the relative lengthening/condensing of the frontal and occipital bones. There was also some deformation in the relative width of the skulls. However, the functional significance of these morphological variations, if any, was unclear. Caumul and Polly (2005) used path analysis to investigate the effects of climate, diet, vegetation, body size and mitochondrial DNA sequence divergence on cranial morphology in Eurasian *Marmota* species. They found that mtDNA phylogenetic distance was only able to explain 15% of variation in skull shape, whilst diet accounted for 25% of variation. However, this approach is likely to be less informative for populations at the subspecific level, particularly when there is little geographic distance between populations and variables such as dietary preference are less distinct.

Results of the randomisation analysis showed there was no clear geographical gradient to these morphological changes (i.e. voles from Scottish islands did not have a tendency to more closely resemble those from nearby mainland populations than, for example, those from southern England or France). Randomisations showed that mainland populations resembled each other more closely than expected by chance, but this was not associated with a corresponding dissimilarity between island populations or between island populations previously designated as subspecies (Jersey, Skomer, Mull and Raasay). However, this was almost certainly influenced by Mull (and to some degree, Raasay) animals being similar in morphology to both mainland and other island populations. This is consistent with a homogenous mainland population acting as a source for separate island radiations. However, it may also simply reflect an inevitable consequence of the greater differences in morphology between islands.

Preliminary analyses of dorsal, lateral and ventral views of skulls showed that dorsal views were the most useful in distinguishing between populations, partially because of consistency in placing landmarks. Several studies of rodent cranial morphology have demonstrated a disparity in the information gathered from different views of the skull (Barciová 2009). For example, in the South American caviomorph rodent *Trichomys aperoides,* the most informative feature in terms of geographic variation was mandible shape, followed by lateral and ventral views of skulls, whilst dorsal views were much less informative (Duarte *et al*. 2000, dos Reis *et al.* 2002a,b). Although mandible shape has proven much less enlightening in the separation of other species (e.g. Barćiová and Macholán, 2006) this feature may be worth investigating in British bank voles to see whether any additional morphometric variation exists.

6.4.2. Fluctuating asymmetry

Neither of the two pairs of lateral landmarks (5,6 and 7,8; Fig. 6.1) used to estimate FA contributed a great deal to the PCA (Table 6.3, Fig. 6.3). It thus might appear - because landmarks along the midline can necessarily be aligned perfectly (since they are in effect being aligned with their own mirror images) - that this measure of FA lacks sensitivity and relies entirely on landmarks that do not in fact vary a great deal. However, since the affine transformations inherent in calculating PD (scaling, rotation, translation) leave the shape unaltered, using total PD incorporates not only asymmetry in the bilateral landmarks, but also any asymmetries along the midline.

Measures of PD showed some indication of FA within individuals, but this was not significantly different between populations. Moreover, contrary to predictions, neither island populations as a whole, nor island populations with named subspecies, were found to have greater levels of FA than mainland populations. Randomisation tests showed that the effects of misalignment and digitisation error were negligible in comparison to measures of asymmetry. Therefore, these results either indicate that the island populations investigated in this study were not subjected to increased developmental stresses in comparison to the larger mainland populations, or that FA, in this instance, is a poor biological indicator of developmental stress.

Neither were any effects observed when using FA_{corr} instead of FA, as described by (White and Searle 2008b). This measure of FA eliminates the effects of directional asymmetry (when a character is consistently larger on one side, within a given population) by subtracting the R-L population mean for each coordinate from the corresponding coordinates for each individual in that population. However, the use of

this correction is problematic, especially when population sizes are small, partly because the population mean is not independent of the individual values and partly because of the disproportionate influence of outliers in small sample sizes. Furthermore, by studying asymmetry of human jaw bones in an island and corresponding mainland population, Schaefer *et al.* (2006) were able to demonstrate that directional asymmetry as well as FA appeared with both environmental stress and genetic stress (caused by inbreeding). Thus, under some circumstances, directional asymmetry may also be an indicator of developmental stress and perhaps should not be immediately discounted from such analyses.

One of the major drawbacks of this study was the reliance on museum collections for specimens, which invariably led to the inclusion of samples that had been collected up to 100 years previously. This poses a particular problem when studying FA because historical collections may not display characteristics representative of the current focal population. However, culling of animals for non-essential scientific research is commonly considered unacceptable when dealing with small populations or those of particular conservation interest. Hopton *et al*. (2009) provided a unique solution to this dilemma by taking images of live animals using an X-ray machine and then returning them to the field. Whilst this approach may prove promising for future research, it would undoubtedly be problematic for studying island populations because of animal/equipment transportation issues. Furthermore, the anaesthesia of animals required for this technique is a procedure regulated in the UK by the Home Office and thus it may be difficult to obtain a license for such research.

In comparison with the results presented here, Marchand *et al*. (2003) were able to detect a significant decrease in FA in populations living in non-fragmented habitats compared with fragmented habitats and attributed this to increased opportunities for dispersion associated with living in a continuous habitat and the consequential maintenance of genetic variability.

White and Searle (2008b) used geometric morphometric analysis to investigate the relationship between FA and genetic diversity in common shrews (*Sorex araneus*). These authors looked at asymmetry in mandibles from 13 island and six mainland populations. They detected FA in both the size and shape of mandibles in all populations, and found a significant inverse correlation between both measures of FA and population genetic diversity. However, one particular island population was strongly driving these correlations, which were non-significant when this population was removed. Shrews from the smallest island in their study, Sanda, had higher levels of FA than all the other populations and substantially reduced levels of genetic diversity. These authors concluded that genetic variability has little, if any, bearing on FA and that these effects are only likely to be biologically informative in very small populations. Thus, it is worth noting that the island of Sanda is 127 ha and this is less than half the size of the smallest island from which specimens were collected from this study (Skomer; 292 ha). If White and Searle's supposition is correct, it is unsurprising that this study was unable to detect significant differences in FA between populations. Furthermore, inconsistencies between the findings of this study and that conducted by Marchand *et al*. (2003) may simply be a result of differences in subject population size.

6.5. Summary

1. This study investigated cranial variation between island and mainland populations of British bank voles using geometric morphometric analyses.
2. The landmark coordinates from these analyses were subsequently used to examine levels of fluctuating asymmetry (FA) within vole populations, specifically asking whether FA was elevated in island voles.
3. Only Skomer and Jersey voles showed distinctive skull morphology when compared to other populations, although voles from Raasay and Anglesey showed tendencies towards skull shape changes that were not apparent in the mainland populations.
4. Skull morphology does not support the subspecies classification of voles from Mull and Raasay.
5. Further data are needed refute the theory that morphological cranial differences are exclusive to island populations with named subspecies.
6. There was no geographical trend to this variation and sex and age did not influence cranial morphology.
7. Some level of fluctuating asymmetry was detected in all populations but there was no significant difference in levels of FA between island and mainland populations.
8. These results either indicate that the focal island populations were not subjected to increased developmental stress, or that FA is a poor biological indicator of developmental stress in these populations.

Chapter 7: General discussion

At the start of the thesis I described the general features of island syndrome and the theories that seek to explain its occurrence. In this concluding chapter, I first review the evidence for existing differences between island and mainland populations of bank voles, both from this study and previous work, and then discuss the possible causes of island syndrome. Finally, I summarise the areas of research I believe should be further investigated in light of the findings from this study.

7.1. Evidence for the existence of island syndrome in bank voles

Throughout the course of this study, morphological, demographic and genetic differences between island and mainland populations of voles have been examined. Whilst I was able to demonstrate population differences in both body size and skull morphology, I was unable to demonstrate the existence of other classical island syndrome traits: increased densities, more stable densities or a change in reproductive behaviour. Nor was I able to demonstrate any differences in developmental stress between island and mainland populations as measured by levels of fluctuating asymmetry. Nevertheless, the findings of the demographic studies are largely contradictory to previous research and thus the existence of these traits in British bank voles cannot be dismissed. In particular, island vole populations can clearly reach excessive densities in comparison to mainland populations and the breeding season of some populations in certain years appears to be reduced. However, contrary to previous studies on Skomer Island (Jewel 1966, Healing 1984), I would argue that there was no evidence of increased stability in this population or indeed, any of the

other island populations, relative to mainland populations that exhibit annual as opposed to multiannual cycles.

By examining the phylogeographic history of these populations, inferred through mitochondrial DNA analysis, this study was able to demonstrate that any common observable differences found in the four island populations with designated subspecies were not the direct result of shared evolutionary history. Moreover, the two populations that shared the most recent evolutionary history were Skomer and Ramsey. Since the Skomer population shows characteristics of island syndrome but the Ramsey population does not, this intimates that traits associated with island syndrome have evolved independently on all islands. This might suggest that such traits have evolved as adaptive responses to selective pressures on the islands; whether these are similar or different selection pressures on the different islands forms part of the debate. Specific island characteristics may have evolved quickly; morphological evolution, for example, is greatly accelerated amongst island mammals and can be up to 3.1 times faster (Millien 2006). However, the role of founder effects and genetic drift in shaping morphological differences between island populations is unclear. Founder effects provide a mechanism for rapid changes in gene frequencies as animals and plants are introduced into new environments (Frankam 1998) but are impossible to study without prior knowledge of the source population. Thus it is difficult to assess the impacts that such genetic forces may have had on the focal populations of this study.

7.2. Predator-release hypothesis

Many of the theories that seek to explain island syndrome highlight the absence of competitors and predators. In Chapter 2 I summarised presence and absence data for species most likely to affect bank vole population dynamics, with regards to all of the island populations in Britain. In terms of small mammals, weasels (as the smallest predators) are the most likely culprits to influence body size. Sundell and Norrdahl (2002) showed there was a negative relationship between the body size of *Microtus* species and the abundance of weasels in Finland. Furthermore these authors were able to demonstrate with laboratory experiments that smaller voles are able to fit down escape holes inaccessible to even the smallest weasels. Thus, there is some merit to the theory that predation pressure may influence the maximum size of small mammals on the mainland.

Weasels constitute a significant cause of mortality in mainland populations of bank voles and can take up between 20% and 40% of the standing crop (McDonald and King 2008). Thus their absence is likely to result in increased survival of island voles, if other predators do not take up the proverbial 'slack'. As well as affecting body size, increased survival may result in other manifestations of island syndrome such as elevated densities, which in turn may select for decreased reproductive output and changes in aggression (Adler and Levins 1994). Interestingly, weasels were also absent from the alpine populations of voles studies by Yoccoz and Mesnager (1998), and these voles also appear to be both significantly larger than other mainland populations and experience increased survival. However, weasels are absent from several of the British islands where bank vole populations do not show increased body size. Moreover, weasels appear to have been present on Raasay historically

(McDonald and King 2008), although whether they ever formed well-established populations on this island is unclear. Nevertheless, it seems that the absence of weasels alone is not sufficient to cause gigantism in small mammals.

Larger mammalian predators (e.g. foxes and stoats), along with avian predators, could also exert an influence on the body size of small mammals by affecting survival. Unfortunately I was unable to quantify the differences in predation pressure between the island populations during this study. Thus, the following argument must instead largely rely on rudimentary comparisons between the presence and absence data of predatory species.

There were no clear patterns between species presence and absence that would seem to explain the occurrence of larger voles on Skomer, Jersey, Mull and Raasay. Foxes are absent from most islands and stoats are present (or have historically been present) on three out of the four islands with enlarged voles. There was also no consistent pattern between the presence and absence of avian species. Nevertheless, release from predation pressure is likely to manifest itself in increased survival and data from previous studies (Delany and Bishop 1960, Bishop and Delany 1963) have indicated that survival is higher some of these islands than the mainland. However, to substantiate the 'predator-release' hypothesis as a sole cause of differential body size evolution and that of other syndrome traits, one would have to demonstrate that voles on other islands (e.g. Arran, Scalpay and Ramsey) do not have higher survival rates than mainland conspecifics. Whilst this was unfortunately beyond the scope of this study, there is some further evidence to suggest decreased predation pressure is not the solitary cause of island syndrome.

If decreased predation pressure alone influenced body size, one would expect all small mammals present on islands to exhibit gigantism concurrently with bank voles. However, comparisons between mean body weight data from this study and those in the published literature suggests that neither wood mice nor field voles were larger than mainland conspecifics in any of the focal populations. On the other hand, even small increases in maximum body mass (e.g. 2-3 g) would reflect a significant overall increase (e.g. 5-10%) in the body size of a small mammal. Thus, simply comparing mean body weights of insular mice and voles with the published literature may not a sensitive enough methodological approach to detect tendencies towards insular gigantism in these species. This may also cause a problem during large-scale meta-analyses of the 'island rule' (e.g. Meiri *et al*. 2008), where smaller differences in body mass (which may be proportionally large changes in body mass) are overlooked because of statistical 'noise' in the data. Nevertheless, with all caveats aside, it seems reasonable to conclude that whilst the absence of predators may partially contribute to the evolution of island syndrome traits such as increased body size, increased survival and increased densities, there must also be other significant contributing forces at work.

7.3. Competitive release/niche expansion hypothesis

If competitive release were the sole cause of island syndrome in bank voles, one would expect the absence of field voles to have the strongest effect on island populations. This is because field voles, unlike wood mice, are behaviourally dominant over bank voles and have been shown to influence female fecundity, maturation and survival (see Shore and Hare 2008). It is true that field voles are absent from the three islands with the largest bank voles (Jersey, Raasay and Skomer) and

present on most of the other islands. However, it is also apparent that field voles are absent from Ramsey Island and present on Mull, thus this argument does not really stand on its own as a cause of all manifestations of island syndrome in bank voles. Furthermore, if competitive release were the sole cause of body size differentiation in bank voles, one might expect mainland woodland populations to be larger than those in habitats where field voles are present. As far as I am aware, this is not the case, although gene flow may operate against this occurring. However, that is not to say that competitive release has no bearing on insular vole populations. It was clear from peripheral trapping exercises (i.e. not on grids established for the purpose of population studies) that bank voles on the islands of Raasay and Jersey were relatively abundant in grassland habitats. In a comparable mainland site, one would expect low densities of bank voles but high densities of field voles. Thus it seems there is some merit to the idea that bank voles are able to expand their niche in the absence of field voles and this may account for the occurrence of elevated densities and other linked traits. Conversely, the habitat on Skomer most preferred by voles is largely unsuitable for *Microtus agrestis*. Bank voles on this island achieve their highest densities in areas where the vegetation is dominated by tall bracken with an understory of bluebells and sorrel and are found at relatively low densities in areas dominated by grass species (see Chapter 2), which is where one would expect to find *M. agrestis* if this species were to occur on the island. Thus this provides little support for the competitive release/niche expansion hypothesis as a cause of elevated densities, increased body size and other manifestations of island syndrome in the Skomer population.

One of the most interesting comparisons to come from this study was the difference between Ramsey and Skomer voles. Both islands are of similar size, have similar

climates, similar faunal assemblages in terms of competitors and predators and mitochondrial DNA analysis revealed a close evolutionary history between the two populations. Yet Skomer voles appear to show most manifestations of island syndrome whilst Ramsey voles do not. One notable difference between the two populations is that until ten years ago, brown rats (*Rattus norvegicus*) were present on Ramsey Island (see Chapter 2). Whilst rats would not normally constitute a significant competitor of bank voles (due to their largely commensal lifestyle), rats on Ramsey were predating upon ground nesting birds such as manx shearwaters (*Puffinus puffinus*). Since voles on both Skomer and Ramsey appear to frequently use shearwater burrows, the rats on Ramsey may well have constituted a significant competitive effect thereby preventing the evolution of island syndrome in this population. It would be interesting to monitor this population now that rats have been eradicated from this island to see whether this has any perceivable affect on the morphology, demography or behaviour of the voles.

7.4. Food resource availability hypothesis

The survival of individuals in a given population is obviously intrinsically linked to food resource availability in that without adequate food supplies, individuals and possibly populations will not persist. However, the extent to which food availability governs maximum individual body size and maximum population densities is less clear. For example, in enclosure experiments the results of supplementary feeding have been equivocal (Ostfeld 1994). Thus it seems increased food resource availability may not necessarily lead to manifestations of island syndrome in an experimental situation. Increased food availability could theoretically result in increased growth of individuals and therefore increase the mean body size of populations inhabiting resource rich habitats. If this were the case, one would also expect body condition of

animals to be better on islands in resource rich habitats. Whilst this study attempted to look at body condition, methodological complications made it difficult to come to any conclusions; this subject is clearly a candidate for further research (see Section 7.9).

Increased food availability is also likely to lead to increased densities occurring on islands. However there is currently no evidence to suggest that increased densities of bank voles occur on the islands of Raasay and Mull. This suggests either that food resource availability is not greater on these islands, or that other factors (such as competition and predation) inhibit increased densities. Either way, food resource availability alone is unlikely to determine traits such as body size and densities on islands but must be considered to play a significant role. A case in point is the interesting utilisation of food resources by Skomer voles. Previous research has suggested that bracken and bluebells form a major part of the diet (Healing 1984). Given that these food items are rarely consumed by mainland voles, it is fascinating that a specialisation on alternative food resources can result in the greatly increased and relatively stable densities reported by Healing (1984). In truth, more research is needed into the influence of diet quality and food availability in island populations before the extent of their effects can truly be recognised. It may well be fruitful to compare the diet of the Skomer and Ramsey voles to see whether this can account for any of the observed differences between the two populations.

7.5. Effects of climate and geography on body size

This study found no evidence to support a latitudinal gradient in body size as predicted by Bergmann's Rule. This suggests that there are more important factors that govern size evolution in bank vole populations, than the perceived metabolic advantage of increasing body size in colder climates. Interestingly, by comparing climate data with body size measurements from the published literature a significant positive correlation between rainfall and vole body size was detected. Since rainfall is most likely to affect vegetation quality it was surmised that there might be a relationship between food quality and body size of bank voles. However, whilst this relationship may hold as a general pattern across the range of this species, the impact of rainfall in determining size variation amongst British vole populations is thought to be negligible. In Chapter 2 I summarised climate data for each of the field sites; it is evident that there is little relationship between the occurrence of island syndrome in voles and any of the climatic variables. However, since these variables are strongly related to latitude, perhaps this is unsurprising.

7.6. Density compensation versus excess density compensation

Although this study was unable to demonstrate the occurrence of increased densities in island bank voles, using data from previous studies I was able to roughly estimate the maximum rodent biomass achieved on islands and the maximum biomass probably reached in a productive mainland habitat. By comparing these estimates, it seemed apparent that overall, peak biomass on islands probably does not exceed that of an equivalent mainland habitat during peak field vole years, thus providing support for the density compensation hypothesis. However, in the case of Skomer Island this is probably not a valid assumption, since the voles on this island do not appear to reach

elevated densities in habitat suitable for field voles. Moreover, given that field voles exhibit multiannual density cycles (Lambin 2008), and that data from Healing (1984) suggests high densities of voles can be sustained for long periods (>10 years) on Skomer Island, this suggests that overall biomass is probably greater on Skomer than an equivalent mainland site. This indicates that both density compensation and excess density compensation can occur in island bank vole populations and this is probably largely dependent on food (and space) resource availability within a given habitat. Furthermore, the occurrence of gigantism in insular bank voles is not inextricably linked to increased densities, as is evident from the vole populations on the islands of Raasay and Mull. Thus, this brings into question the role of interspecific competition in selecting for increased body size on islands.

7.7. Fence effect hypothesis versus internal population regulation

The fence effect hypothesis (Krebs *et al*. 1969) predicts that, under favourable conditions (i.e. low predation rates, sufficient food resources), island populations will quickly increase in density and, because dispersal is restricted, decimate the available food supply and then crash. Thus, if this hypothesis holds true, one would expect to see periodic crashes following periods of excessive densities in island populations (i.e. multiannual cycles). However, perhaps this model is only applicable to 'isolated' populations much smaller in size than those considered during the course of this study. Whilst it is clear from this study and previous studies that vole densities on the islands of Skomer and Jersey may be subject to large between year fluctuations, I found no evidence to support the existence of multiannual cycles in any of the focal populations. Furthermore, long-term data suggests that Skomer voles can exist at remarkably high densities for sustained periods of time (Healing 1984), which is contrary to the central

tenet of the ‘fence effect’ hypothesis. It is notable that the two populations previously reported to have shortened breeding seasons are also the two populations reported to reach extremely high densities. The fact that neither increased densities nor shortened breeding seasons were recorded during this study suggests that modifications in reproductive behaviour may well be a density related mechanism (*sensu* Gliwicz 1980). However, there is a need for further research into densities and breeding seasons on islands, to see how closely these two factors interact.

7.8. The life history approach

None of the aforementioned hypotheses are able to adequately explain the occurrence of island syndrome in bank voles when used in isolation. Thus, it is should perhaps be concluded that the causes of island syndrome are multifactorial and a combination of these hypotheses are needed to truly explain this phenomenon. This approach was adopted by Palkovacs (2003). He suggested that trade-offs between life history traits combined with the differing influence of mortality rates and food resource availability on islands might best explain differential size evolution on islands. For example, under conditions of high mortality risk the best reproductive strategy is to expend less energy in growth and mature early. However, since there is a significant association between body size and fecundity, under conditions of low mortality risk there would be greater pay-offs for investing in growth first and reproduction later. Thus this hypothesis predicts that when predation rates are low, we should see increased body size, alongside increased age at maturity, increased fecundity (although possibly just larger offspring) and increased densities. Conversely if resource limitation predominates this would select for decreased growth rates, decreased adult body size, increased age at maturity and decreased population density. Palkovacs (2003) used this model to

explain why small animals tend to grow bigger on islands whilst large animals tend to decrease in size.

Although there is great merit in this multifactorial approach, in that, for example, the voles on Raasay and Mull show increased body size, increased survival and apparently few females breed in the year of birth, this hypothesis still fails to explain why increased densities are not apparent in these populations. Furthermore, delayed maturation also seems to be a characteristic of voles on the Scottish mainland, where breeding seasons are shorter than in southern England, so few young-of-the-year get a chance to breed in their year of birth. Thus, delayed maturation in the Scottish island populations may be largely governed by climatic conditions and not a consequence of insularity.

Unfortunately, data on survival and maturation rates are lacking from island populations such as Arran and Ramsey. Voles on these islands do not appear to exhibit gigantism yet risk of mortality is predicted to be low in these populations because ground predators are absent. This may suggest that resource limitation dominates on these islands. Obviously more detailed studies need to be conducted on these 'non-island syndrome' populations to determine whether traits such as delayed maturation and increased survival are apparent. However, it would also be very interesting to examine the levels of gene flow between Ramsey and the mainland populations versus Skomer and the mainland population to see whether varying levels of isolation could in part explain the divergence between these two insular populations.

It is clear from this study that island syndrome does exist in some populations of British bank voles, but that the manifestations of this syndrome vary from population to population. In addition, some traits such as reproductive behaviour and increased densities may vary with time. Rather than one all-encompassing theory, a suite of different factors could be used variously to explain the occurrence or absence of island syndrome on each particular island. These include predation, competition, resource availability and genetic effects. Taken together with the knowledge that the island rule and the island syndrome occur in a range of other animal groups from carnivores (e.g. foxes (Goltsman *et al.* 2005), birds (Clegg and Owens 2002) and lizards (Wikelski 2005), the idea that there is a single overriding factor responsible for island syndrome seems unlikely. However, it is beguiling to think that, at least with respect to small mammals, an understanding of what affects body size will lead to explanations for the other features of island syndrome.

7.9. Future research

In light of the findings of this thesis, there are several areas that warrant further research. For example, the role of founder effects in shaping the body size of individuals on islands remains untested. Thus the question remains, do founding populations with relatively large body size automatically give rise to populations with large body size? This is obviously a hypothesis that would be best tested by long-term introduction experiments, although performing such experiments on a sufficient scale and for a sufficient time period may be difficult to achieve.

The role of food resource limitation in shaping morphological and demographic differences on islands also remains to be investigated, and research into the body

condition of insular populations could prove a useful approach to help tackle this hypothesis. Stable isotope analysis could be an invaluable approach to studying diet in populations where culling of animals is to be avoided. Preliminary studies conducted on Skomer vegetation (sadly abandoned during the course of this study) suggested that stable isotope analysis of faecal pellets might be a good way to study vole diet in a non-invasive manner. Geometric morphometric analysis of mandibles may also prove useful in determining morphological consequences of differences in food resource utilisation between island populations.

There is a need for further investigation into the consequences of the elevated densities experienced by some insular populations. These include the effects on reproductive behaviour, levels of aggression and effects on dispersal (e.g. does a 'social fence' system operate under conditions of high density?). Investigations between 'personality' and life history trade-offs have also become rather popular in the literature recently and how these theories relate to island populations would be fascinating to test.

Finally, I think there is a need for direct comparative studies involving island populations that experience island syndrome and those that do not. I suggest that for bank voles, comparisons between Ramsey, Skomer and the adjacent mainland would be ideal. Such a study could encompass population genetic comparisons (using microsatellites) with studies on survival rates, reproductive behaviour and food resource utilisation, and thus may help to disentangle the relative influence of each of these factors in creating the 'island syndrome'.

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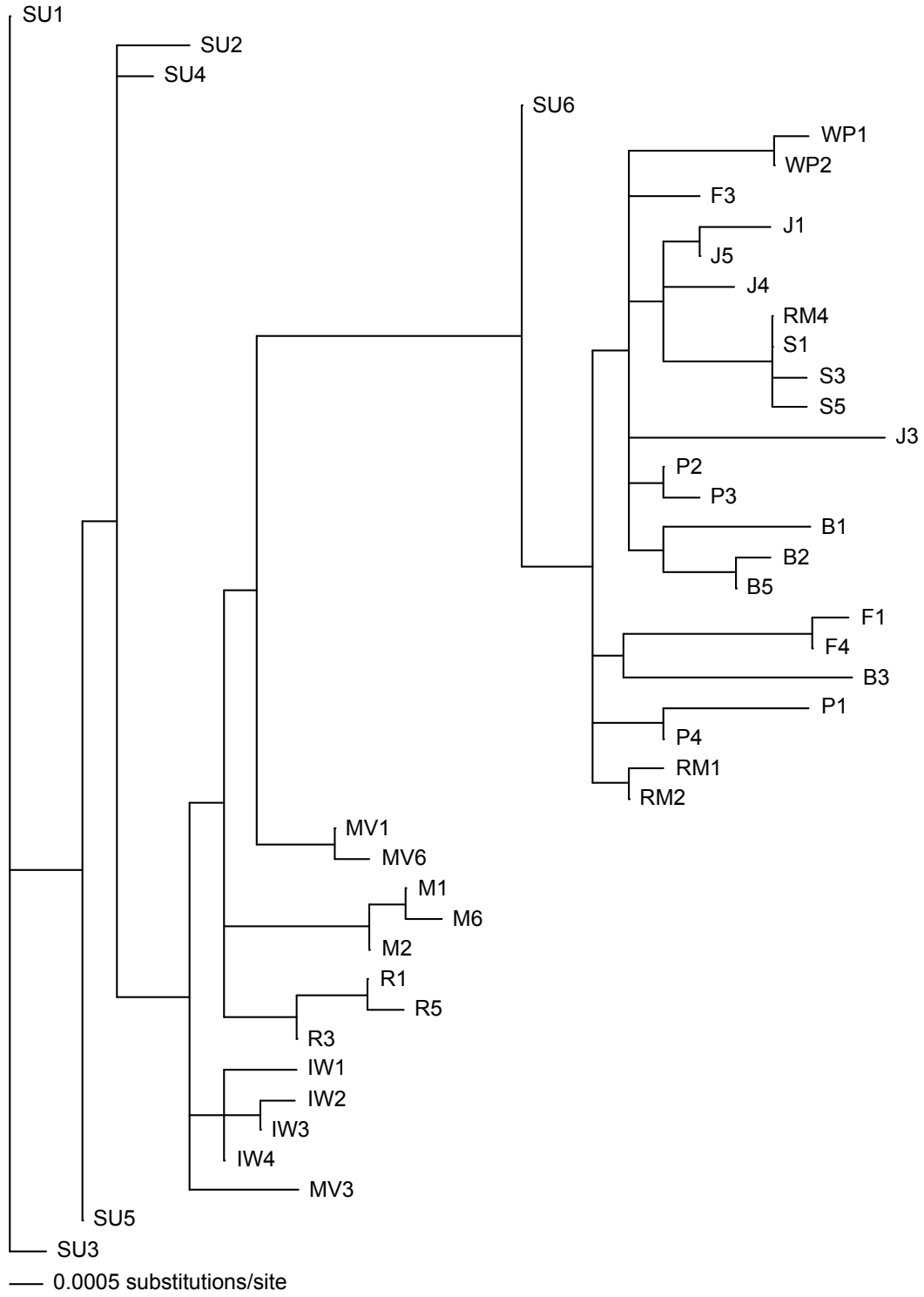
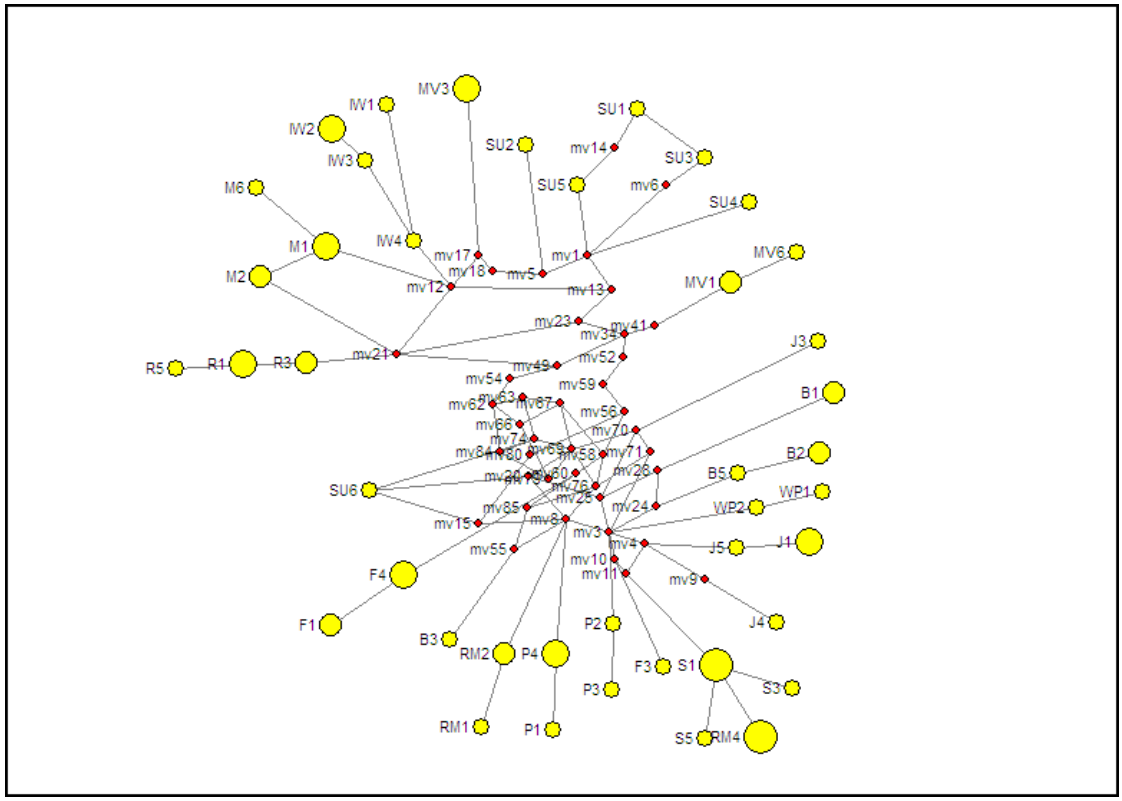
Appendix

Appendix Table 3.1: Geographic sampling locations and accession numbers for *Myodes glareolus* haplotypes downloaded from the GenBank database. Codes correspond to labels in Figure 3.5. 'ns' indicates data were not shown in any phylogenetic trees. Sequences with an accession number preceded by 'A' are from Deffontaine *et al*. (2005). Sequences with an accession number preceded by 'DQ' are from Kotlik *et al*. (2006).

Location	Code	GenBank Accession No.
Britain, Kielder	KIELDE	AJ639662
Britain, Derbyshire	DERBS	AJ639666
Britain, Gwent	GWENT	AJ639665
Britain, Gwent	GWENT2	AJ867971
Britain, Cambridgeshire	CAMBS	AJ867970
Britain, Essex	ESSEX	AJ867968
Belgium	BELG2	AJ639661
Austria	AUS1A	AJ639685
	AUS1D	AJ639689
	AUS1E	AJ639690
	AUS1F	AJ639696
	AUS3	AJ639693
Hungary	HUNG1A	AJ867954
	HUNG1B	AJ867955
	HUNG2	AJ639707
Romania	RO7A	AJ867951
	RO7B	AJ867961
Lituania	LIT2	AJ639686
Montenegro	MONT	AJ639706
Slovakia	SK1	AJ867948
	SK2	AJ867949
	SK4	AJ867964
Slovenia	SLO	AJ867953
Germany	GERM2	AJ867978
	GERM1	AF159401
France	FRA3A	AJ639682
	FRA3B	AJ639683
Finland	ns	AY185796
Italy	ns	AJ639692
Spain	ns	AJ639672
Macedonia	ns	AJ639660
Russia	ns	AF429794
Carpathian lineage	ns	DQ472264
	ns	DQ472289
Basal lineage	ns	DQ472304
	ns	DQ472340
Western lineage	ns	DQ472293
	ns	DQ472272



Appendix Figure 3.1: Maximum likelihood tree rooted with a default outgroup, showing the phylogenetic relationship between 42 bank vole mtDNA haplotypes (for concatenated cyt *b* and d-loop data). Haplotype abbreviations are from Table 3.2. The phylogram was generated in PAUP* using a HKY85 + G + I model of sequence evolution (see text) with molecular clock not enforced, tree score = 3247.2.



Appendix Figure 3.2: Median-joining network tree of bank vole mtDNA haplotypes (for concatenated cyt *b* and d-loop sequence data), when the value of epsilon is set at 10. For sample abbreviations see Table 3.1.

Appendix Table 4.1: Summary descriptive statistics for range (MCP) results (m^2). CV = coefficient of variation (%), Med = median, IQR = interquartile range.

		Adult Males					Adult Females					Young Males					Young Females				
Site	Month	N	Mean	CV	Med	IQR	N	Mean	CV	Med	IQR	N	Mean	CV	Med	IQR	N	Mean	CV	Med	IQR
Orielton	Nov-05	6	108	107	37	216	-	-	-	-	-	17	34	65	34	44	16	47	122	31	46
	Apr-06	10	77	121	61	99	1	24	-	24	-	-	-	-	-	-	5	40	83	29	46
	Jun-06	4	239	138	84	510	6	55	204	6	88	1	59	*	59	-	-	-	-	-	-
	Aug-06	3	91	84	59	143	4	31	90	24	50	-	-	-	-	-	1	11	-	11	-
	Oct-06	1	256	-	256	-	1	6	-	6	-	-	-	-	-	-	5	40	76	29	50
	Apr-07	5	18	116	6	35	5	73	103	59	136	-	-	-	-	-	1	1	-	1	-
	May-07	10	75	141	32	122	11	30	95	24	28	-	-	-	-	-	1	36	-	36	-
	Jul-07	6	10	105	6	21	9	49	165	11	65	1	16	-	16	-	2	14	26	14	-
	Oct-07	1	71	-	71	-	5	37	72	29	51	13	16	88	11	8	17	19	89	11	26
Grid T	Oct-05	8	162	183	27	165	3	45	121	29	105	12	86	160	11	159	22	170	189	54	174
	Mar-06	2	31	0	31	-	3	24	74	16	33	-	-	-	-	-	-	-	-	-	-
	Jun-06	12	270	110	120	504	14	217	114	127	282	-	-	-	-	-	-	-	-	-	-
	Oct-06	5	32	55	26	31	3	220	20	199	80	24	79	172	31	79	17	114	190	26	78
Grids E1	Oct-05	11	89	166	37	78	6	98	149	37	147	10	63	205	20	31	11	81	188	24	45
	Mar-06	9	51	210	20	34	6	115	210	12	176	-	-	-	-	-	-	-	-	-	-
	Jun-06	7	198	231	23	59	4	29	48	30	26	-	-	-	-	-	-	-	-	-	-
	Aug-06	16	255	123	157	353	13	122	139	41	156	6	159	122	91	181	3	15	56	11	15
	Nov-06	2	217	119	217	-	3	22	67	16	28	7	68	59	67	65	10	131	190	19	129
Grids E2	May-07	6	175	134	101	219	8	25	91	19	38	1	1	-	1	-	-	-	-	-	-
	Jul-07	14	33	124	16	28	25	28	192	11	21	4	6	96	6	10	2	6	118	6	-
	Oct-07	5	33	112	9	67	4	74	161	16	183	24	21	110	11	20	27	28	133	11	15

Appendix Table 4.2: Results of ANOVA of MCP data. P = period, S = sex, A = age, Ad = adult, F = F statistic, P = probability, - = unbalanced, N = not significant.

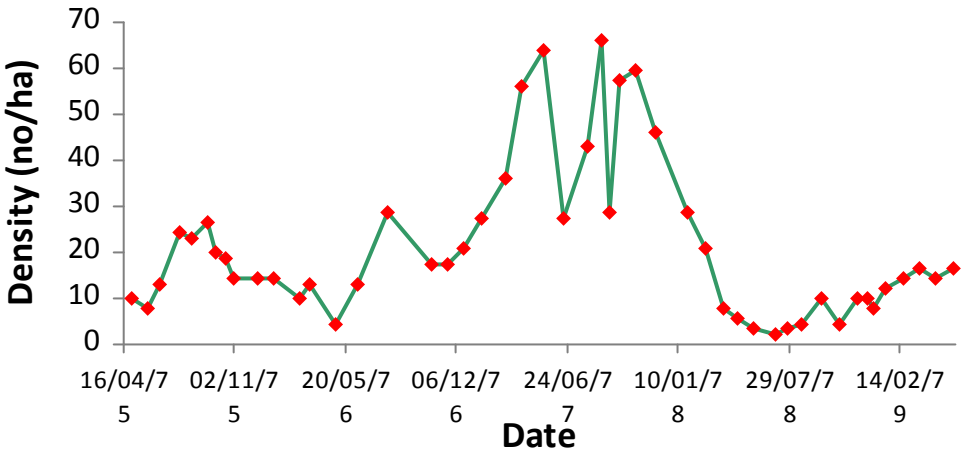
SiPe	Anova	TA	TS	AS	T	A	S	TS-Ad	T-Ad	S-Ad
Orielton	F	-	$F_{8.150}$=2.03	N	$F_{8,159}$=2.09	N	N	-	N	N
	P		0.023		0.04					
Grid T	F	-	N	N	$F_{3,121}$=3.48	$F_{1,123}$=4.21	N	N	N	N
	P				0.018	0.042				
Grids E1	F	-	N	N	$F_{4,119}$=3.01	N	N	N	$F_{4,72}$=2.65 0.04	N
	P				0.021					
Grids E2	F	N	N	N	N	$F_{1,118}$=7.02	N	$F_{2,56}$=3.77	N	$F_{1,60}$=4.29
	P					0.009		0.029		0.043

Appendix Table 4.3: Summary descriptive statistics for indices of grid activity (D) results (m). CV = coefficient of variation (%), Med = median, IQR = interquartile range.

		Adult Males					Adult Females					Young Males					Young Females				
Site	Month	N	Mean	CV	Med	IQR	N	Mean	CV	Med	IQR	N	Mean	CV	Med	IQR	N	Mean	CV	Med	IQR
Orielton	Nov-05	6	10	68	8	12	-	-	-	-	-	17	9	83	6	4	16	9	65	8	6
	Apr-06	10	13	85	11	12	1	5	-	5	-	-	-	-	-	-	5	9	30	8	5
	Jun-06	4	37	75	38	53	6	6	89	5	8	1	11	-	11	-	-	-	-	-	-
	Aug-06	3	18	56	18	19	4	13	52	13	13	-	-	-	-	-	1	7	-	7	-
	Oct-06	1	19	-	19	-	1	5	-	5	-	-	-	-	-	-	5	13	85	9	16
	Apr-07	5	16	127	5	33	5	10	84	7	11	-	-	-	-	-	1	0	-	0	-
	May-07	10	12	66	10	14	11	9	72	7	8	-	-	-	-	-	1	25	-	25	-
	Jul-07	6	6	122	4	10	9	9	86	7	7	1	11	-	11	-	2	7	73	7	-
	Oct-07	1	10	-	10	-	5	9	20	9	4	13	8	39	7	4	17	10	97	7	6
Grid T	Oct-05	8	16	54	15	16	3	6	88	8	10	12	14	85	9	20	22	22	85	16	19
	Mar-06	2	18	55	18	-	3	15	49	11	13	-	-	-	-	-	-	-	-	-	-
	Jun-06	12	18	54	19	18	14	23	49	24	17	-	-	-	-	-	-	-	-	-	-
	Oct-06	5	17	125	5	32	3	21	31	19	13	24	17	53	17	14	17	17	73	15	21
Grids E1	Oct-05	11	14	35	14	9	6	12	82	10	17	10	10	70	11	12	11	11	138	5	13
	Mar-06	9	9	125	7	16	6	14	167	6	22	-	-	-	-	-	-	-	-	-	-
	Jun-06	7	15	83	16	16	4	10	43	11	8	-	-	-	-	-	-	-	-	-	-
	Aug-06	16	20	77	16	18	13	12	67	8	12	6	24	37	25	15	3	11	53	10	12
	Nov-06	2	11	37	11	-	3	9	23	9	4	7	24	85	17	41	10	14	74	10	18
Grids E2	May-07	6	23	49	21	20	8	19	103	13	25	-	-	-	-	-	-	-	-	-	-
	Jul-07	14	12	67	11	10	25	7	72	5	4	1	0	-	0	-	2	4	141	4	-
	Oct-07	5	7	37	6	4	4	10	41	9	7	4	4	119	4	9	27	8	80	7	6

Appendix Table 4.4: Results of ANOVA of D data. T = month, S = sex, A = age, Ad = adult, F = F statistic, P = probability, - = unbalanced, N = not significant.

Site	Anova	TA	TS	AS	T	A	S	TS-Ad	T-Ad	S-Ad
Orielton	F	N	N	N	N	N	N	-	N	N
	P									
Grid T	F	-	N	N	N	N	N	N	N	N
	P									
Grids E1	F	-	N	N	$F_{4,119}$=3.32	N	N	N	N	N
	P				0.013					
Grids E2	F	$F_{2,114}$=4.83	N	N	N	$F_{1,118}$=6.47	N	N	$F_{2,59}$=3.87	N
	P	0.01				0.012			0.026	



Appendix Figure 4.1: Fluctuations in bank vole density (ha^{-1}) during a 10-year live-trapping study conducted at Alice Holt Forest, Surrey (Gurnell, unpublished).

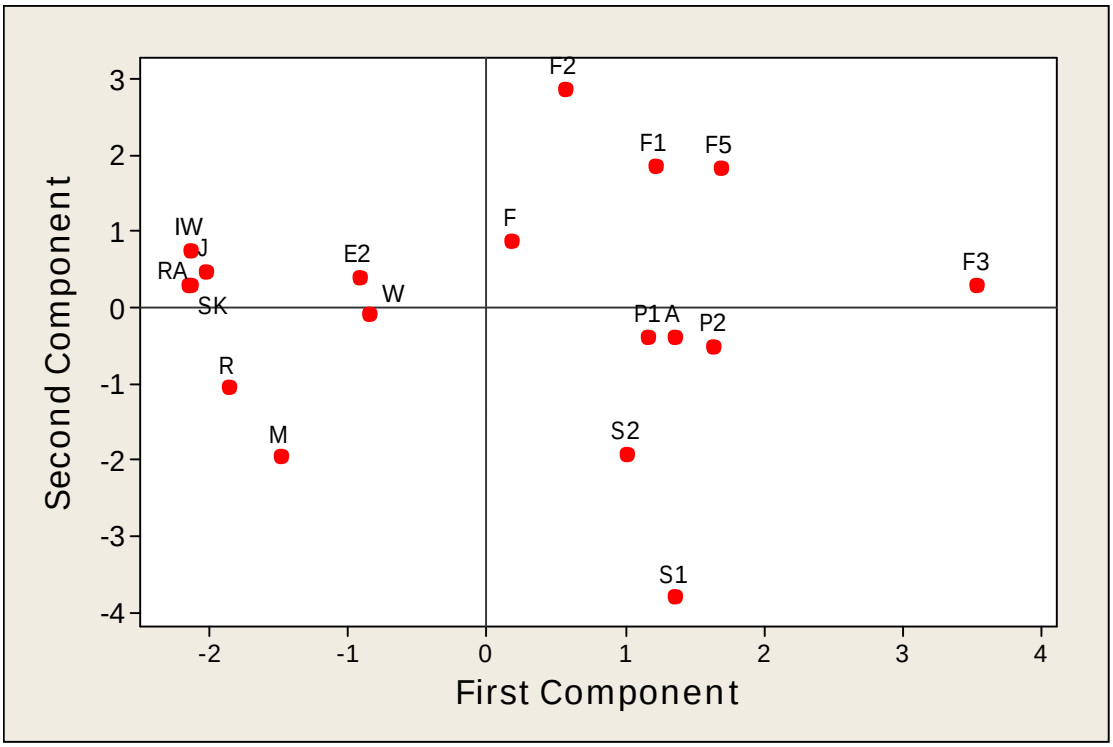
Appendix Table 5.1: List of museum specimens included in the tail to head and body length ratio analysis. Specimens from the Royal Museum of Scotland did not have individual numbers therefore the batch number is given.

Site	Museum numbers					
Natural History Museum, London						
Anglesey	65.576	65.577	65.578	65.579	65.570	65.571
	65.574	65.575	65.573			
Slovenia	47.1033	47.1034	47.1029	47.1030	47.1031	
Suffolk	60.2072	60.2073	60.2075	60.2077	60.2071b	60.2043
	60.2049	60.2060	60.2071	60.2047		
Surrey	30.3.14.2	30.3.14.21	30.3.14.22	30.3.14.23	30.3.14.24	30.3.14.25
	30.3.12.18	30.3.14.16	30.3.14.17	30.3.14.26		
France	63.132	64.287	64.288	64.29	64.328	19999.275
	63.1312	63.1313	64.298	64.305	63.131	
Jersey	60.202	60.2021	60.2022	60.2025	60.2026	60.2027
	60.203	60.204	60.1991	60.1992	60.1993	60.1994
	60.1998	60.2019	60.2032	60.2028	60.1997	
Mull	65.285	65.288	65.289	65.29	65.293	69.292
	69.296	65.286	69.293	69.295	65.292b	69.294
Norway	26.11.21.5.	5.8.5.2.	7.7.7.f	26.11.21.3.	26.11.21.4.	5.8.5.1.
	7.7.7.m					
Raasay	65.296	65.299	65.3	65.31	65.297	65.302
	65.305	65.306	65.307	65.308	65.309	65.311
	65.304	65.312				
Argyllshire	65.174	65.183	65.187	65.19	65.198	65.172
	65.175	65.176	65.177	65.173		
Perthshire	65.13	65.24	65.28	65.4	65.14	65.18
	65.31					
Skomer	16.67.652	70.1564	1.3.7.4.6	3.3.7.4.7	70.1563	70.1565
	70.1568	70.1569	70.157	70.1571	10.3..7.4.4	4.3.7.4.1
	11.3.7.4.5	70.1567	8.3.7.4.2			
Spain	64.829	64.83	64.823	64.825	64.828	64..824
Gwynedd	11.1.3.255	11.1.3.256	2962	2963	11.1.3.231	2981
	11.1.3.263	11.1.3.284				
Isle of	11.1.3.224	60.306	60.307	60.298	60.3	60.301
Wight	60.303	60.304	60.305	60.302		
Royal Museum of Scotland, Edinburgh						
Fife	12 individuals from Batch Number 1907 222					

Appendix Table 5.2: Spearman's Rank Correlation matrix between body weight data gathered from this study and the published literature, and various environmental variables. Above diagonal – correlation coefficient, below – P value. Values in bold are significant. Variable codes; Alt – altitude, Lat – latitude, Lon – Longitude, JanT – mean January temperatures, JulT – mean July temperature, TRange – difference between JanT and JulT, CCI – Conrad's Index of Continentality (see text), M Wt – mean male weight, F Wt – mean female weight, M:F – male to female weight ratio.

Correlation matrix (Spearman):

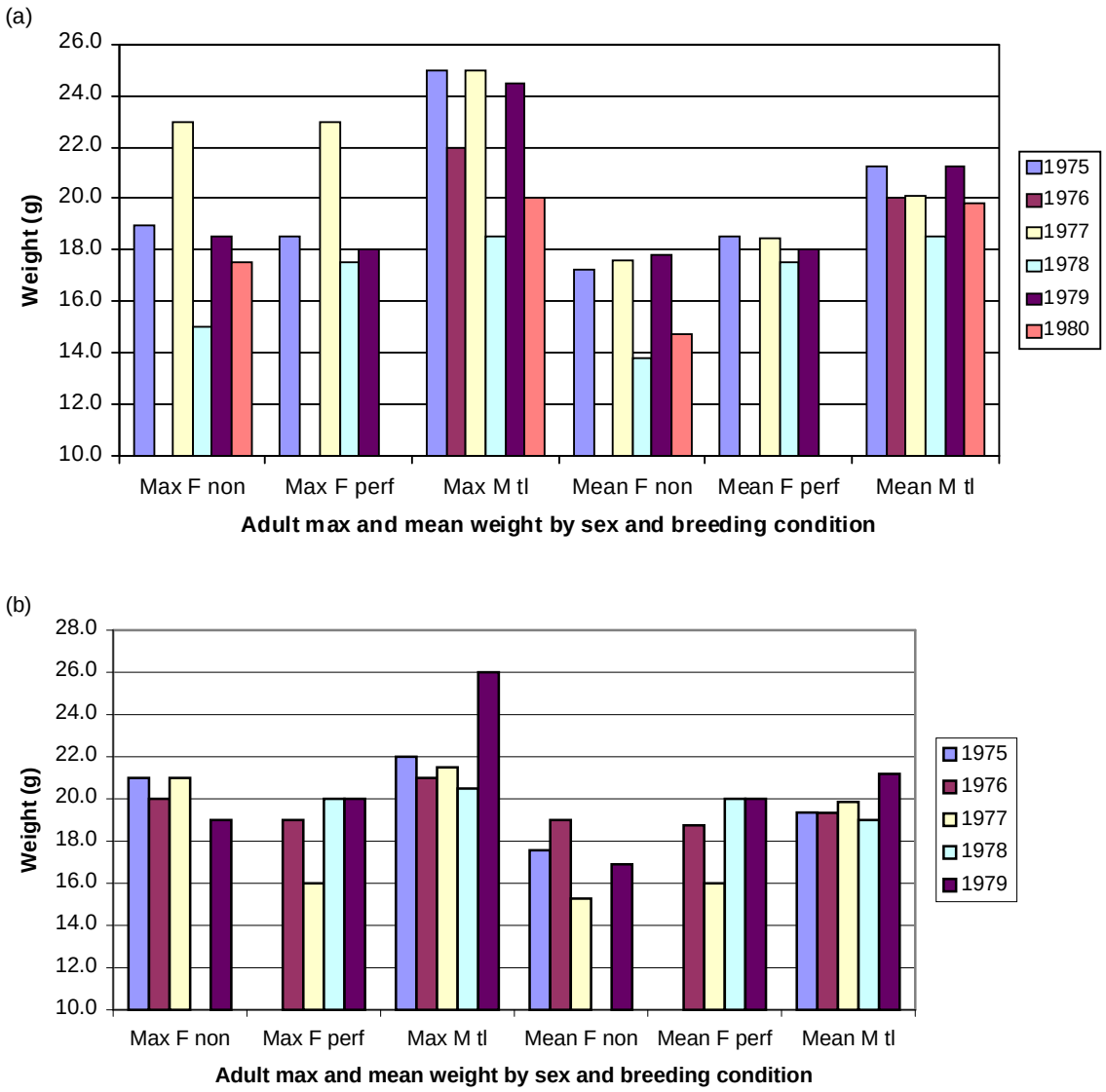
Variables	Alt	Lat	Long	JanT	JulT	JanR	JulR	TRange	CCI	M Wt (g)	F Wt (g)	M:F
Alt (m asl)		**-0.45**	**0.42**	**-0.44**	0.16	**0.42**	0.05	0.26	0.38	0.40	0.38	-0.12
Lat	**0.03**		-0.01	-0.30	**-0.50**	-0.37	0.41	0.07	-0.30	-0.29	-0.02	**-0.44**
Long	**0.05**	0.95		**-0.69**	0.23	-0.39	**0.51**	**0.72**	**0.77**	0.00	0.07	-0.23
JanT	**0.04**	0.16	**0.00**		0.10	0.16	**-0.48**	**-0.73**	**-0.64**	0.09	-0.17	**0.64**
JulT	0.46	**0.02**	0.28	0.64		-0.24	-0.21	**0.49**	**0.56**	-0.18	-0.19	0.06
JanR	**0.05**	0.08	0.07	0.45	0.27		-0.07	-0.36	-0.22	**0.49**	0.32	0.32
JulR	0.81	0.05	**0.01**	**0.02**	0.34	0.74		**0.43**	0.29	-0.09	0.03	-0.09
TRange	0.23	0.75	**0.00**	**0.00**	**0.02**	0.09	**0.04**		**0.87**	-0.22	0.00	**-0.49**
CCI	0.07	0.16	**0.00**	**0.00**	**0.01**	0.31	0.18	**0.00**		-0.17	-0.08	-0.27
M Wt (g)	0.06	0.18	0.99	0.67	0.42	**0.02**	0.68	0.32	0.45		**0.83**	0.10
F Wt (g)	0.07	0.95	0.76	0.44	0.39	0.14	0.87	0.99	0.72	**0.00**		-0.37
M:F	0.58	**0.04**	0.28	**0.00**	0.78	0.14	0.69	**0.02**	0.22	0.65	0.08	



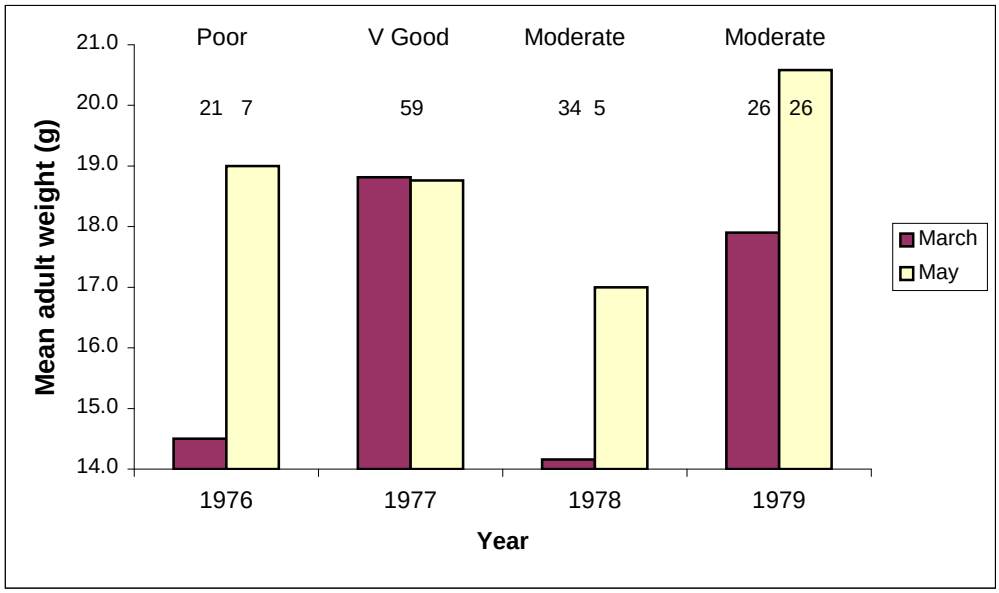
Eigenvalue	3.0108	2.4004	1.3804	0.9916	0.4352	0.3308	0.2326	0.1613
Proportion	0.335	0.267	0.153	0.110	0.048	0.037	0.026	0.018
Cumulative	0.335	0.601	0.755	0.865	0.913	0.950	0.976	0.994

Variable	PC1	PC2	PC3
Altitude (m asl)	0.344	0.351	-0.179
Lat	-0.147	-0.585	-0.009
Long	0.445	0.019	-0.349
JanT	-0.471	0.275	0.091
JulT	0.246	0.366	0.443
JanR	-0.198	0.409	-0.365
JulR	0.248	-0.179	-0.571
Hab	-0.224	0.349	-0.271
Island?	0.478	0.058	0.330

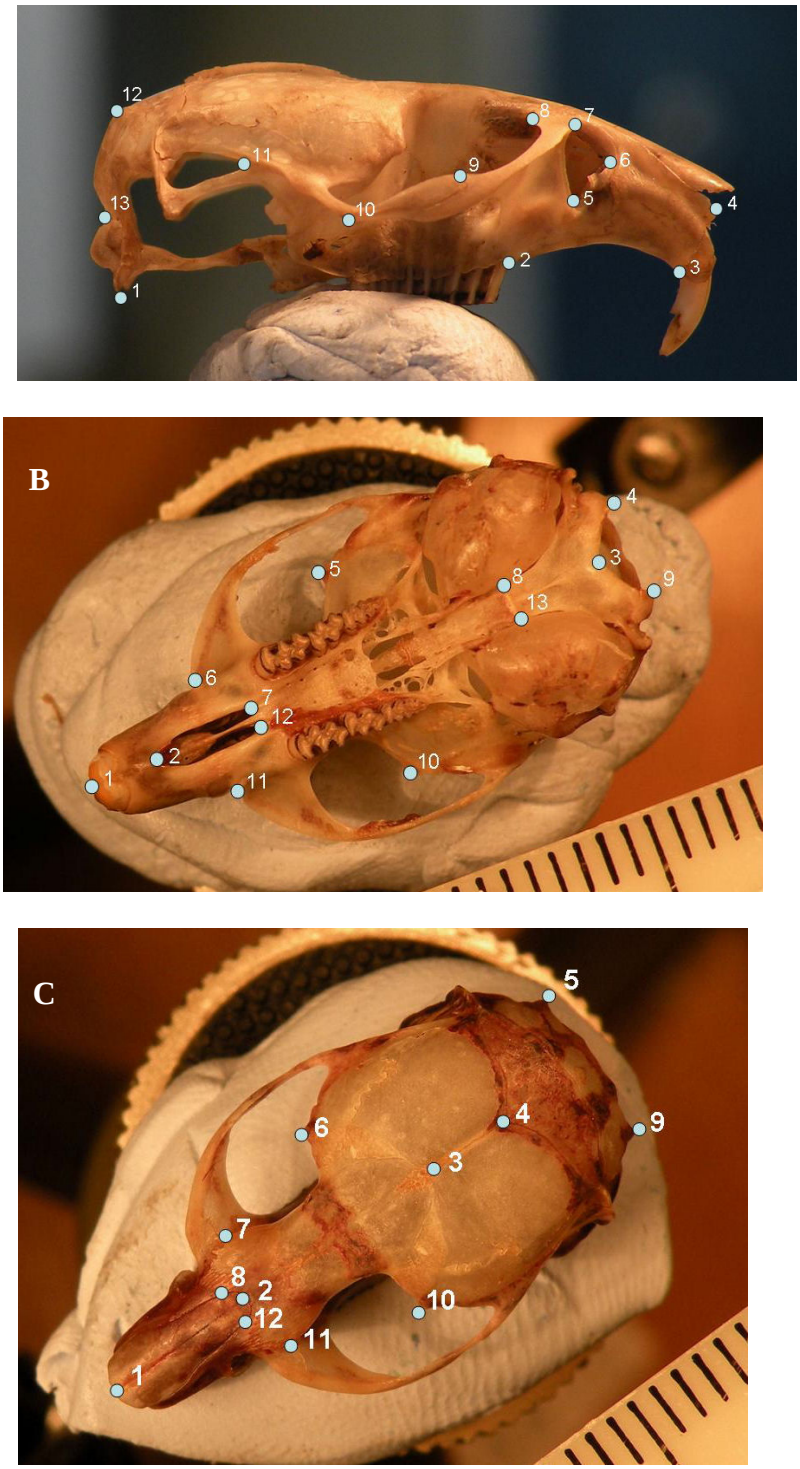
Appendix Figure 5.1: Results of a principal components analysis of climate variables from various European sites. These data were analysed to see whether the resulting principal components would be more useful variables against which to compare bank vole body size data. Eigenvalues and principal component scores are given.



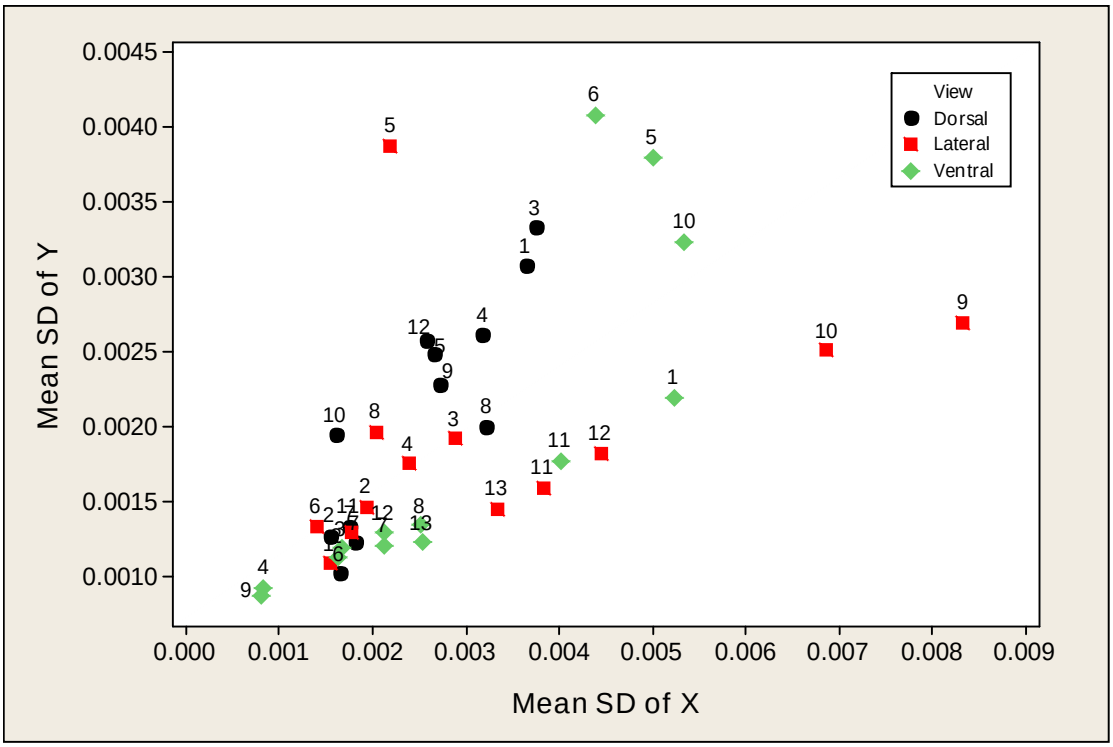
Appendix Figure 5.2: Maximum and mean weights for (a) spring (April/May) and (b) Autumn (September/October) adult bank voles at Alice Holt Forest, Surrey between 1975 and 1980. F = female, M = male, non = non-breeding, perf = perforate, tl = large testes (breeding) (J. Gurnell unpub).



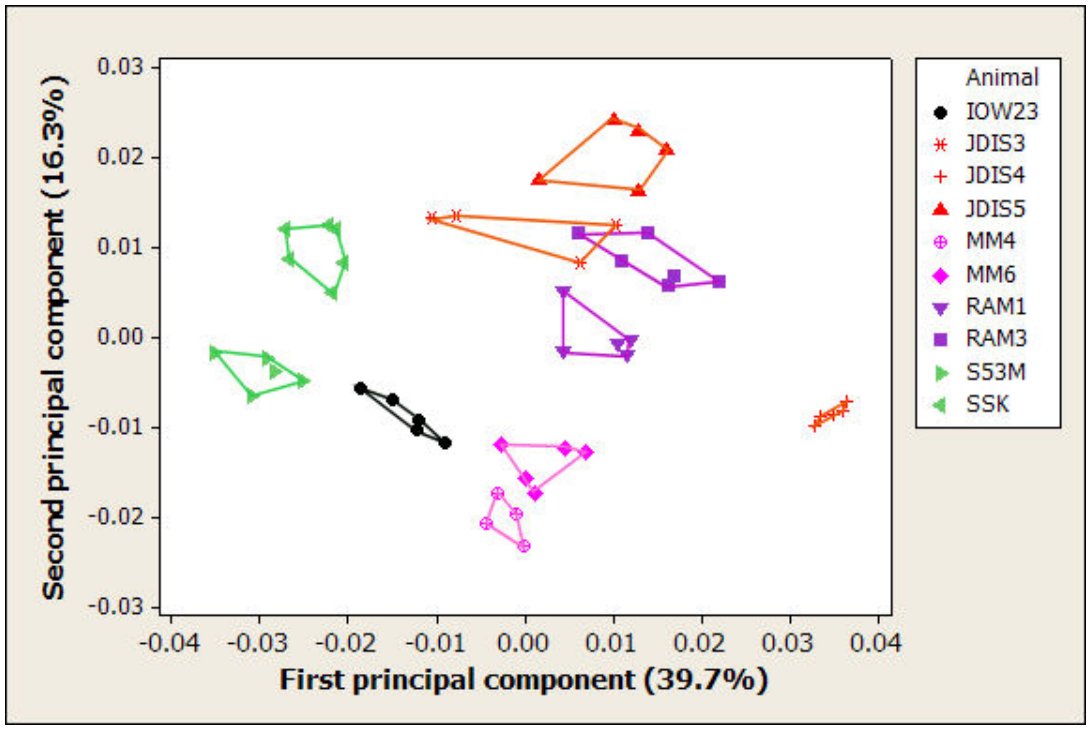
Appendix Figure 5.3: Change in male mean weight between March and May in four years at Alice Holt Forest, Surrey. Poor, V. Good, and Moderate refer to the previous autumns tree seed crop. Samples sizes are above each bar (J. Gurnell unpub.).



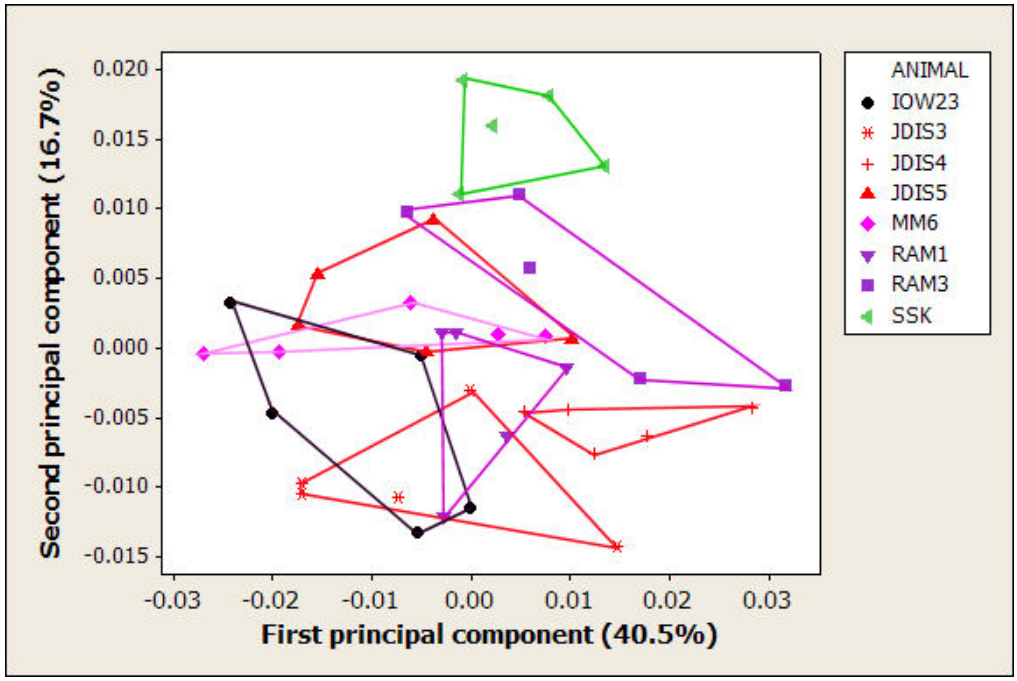
Appendix Figure 6.1: Landmarks used for preliminary morphometric analyses. A – lateral view, B - ventral view, C - dorsal view.



Appendix Figure 6.2: Mean of within individual standard deviations (SD) of aligned X and Y coordinates for landmarks (numbers) on dorsal, ventral and lateral views of *Myodes glareolus* skulls.



Appendix Figure 6.3: Scatterplot of first and second principal component scores from geometric morphometric analyses performed for lateral views of 10 *Myodes glareolus* skulls, 5 replicates of each. Number of landmarks = 12. Percentage variance explained by both axes and convex polygons of replicates for each individual are shown. Specimens from Isle of Wight, Jersey, Morven, Ramsey and Skomer are shown with black, red, pink, purple and green polygons and symbols respectively.



Appendix Figure 6.4: Scatterplot of first and second principal component scores from geometric morphometric analyses performed for ventral views of 8 *Myodes glareolus* skulls, 5 replicates of each (two problematic individuals were removed prior to the analysis). Percentage variance explained by both axes and convex polygons of replicates for each individual are shown. Specimens from Isle of Wight, Jersey, Morven, Ramsey and Skomer are shown with black, red, pink, purple and green polygons and symbols respectively.

Appendix Table 6.1: Size classes of individuals based on head-tail body measurements (mm) used to look for patterns of skull variation amongst different age groups in geometric morphometric analysis. SUB category consists of insular populations of known increased body size and NORM category contains all other island and mainland populations.

	SUB	NORM
Small	<145	<135
Medium	145-55	135-144
Large	>155	>145