Conservation and ecology of the

hazel dormouse, *Muscardinus avellanarius*

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: …….

Abstract

Conservation biologists require information on the distribution, ecology, behaviour and genetic diversity of endangered species in order to identify threatened populations, determine which mechanisms are driving populations closer to extinction, and design appropriate mitigating solutions. The hazel dormouse, *Muscardinus avellanarius*, is declining across much of its northern range. Dormice are detrimentally affected by habitat degradation, loss and fragmentation. Despite extensive studies and conservation work on hazel dormice, there remain many gaps in our understanding. This thesis aims to fill some of those gaps.

Hazel dormice are elusive, and therefore difficult to monitor in the wild. I demonstrate the utility of novel monitoring techniques for the rapid determination of dormouse presence, and provide algorithms for the objective verification of species identity from small mammal footprints. I design and utilise genetic microsatellite markers to investigate molecular ecology in this species. In one of the first studies of hazel dormouse population genetics, I describe high levels of population differentiation and genetic isolation across the southwest UK range. I find a powerful signal of reduction in genetic diversity, and an increase in differentiation between core and peripheral populations. I consider rival hypotheses for the mechanisms driving this population genetic pattern, and place the results in the context of conservation strategies for UK dormice. Further, I use molecular data to investigate the prevalence of multiple paternity in wild dormouse populations. Results contradict a recent estimate of very high rates of polyandry, but remain high at 50%. I investigate the effect of food availability on the hibernation behaviour of dormice. My findings, which demonstrate dormice are variable and flexible in their response to winter diet, increases our understanding of the trade-offs dormice must make in order to survive winter periods.

I hope that the research undertaken for this thesis will add to the understanding and conservation of an iconic British mammal, ultimately contributing to the persistence of this species.

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Acknowledgements

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Author's declaration

All chapters presented in this thesis were written by C.A. Mills under the guidance and supervision of D.J. Hodgson and B.J. Godley. All molecular analyses were conducted at the NERC Biomolecular Analysis Facility, University of Sheffield, under the supervision of T. Burke and D.A. Dawson, with guidance from A. Krupa, G.J. Horsburgh and F. Martins. Fieldwork was conducted by C.A. Mills, with the dedicated support of numerous licensed volunteers. Captive studies were undertaken at Paignton Zoo, under the guidance of A. Plowman and J. Chapman. C.A. Mills received funding from the People's Trust for Endangered Species for research on dormouse population genetics. Specific contributions to chapters are detailed below:

Chapter 2

C.A. Mills designed the footprint tracking equipment, conducted all fieldwork, collected reference samples, analysed the data and wrote the chapter. J. Chapman assisted with reference sample collection at Paignton Zoo. Work at Paignton Zoo was under the supervision of A. Plowman and J. Chapman. D.J. Hodgson provided guidance on data analysis. D. Groves provided comments on an earlier draft.

Chapter 3

C.A. Mills conducted fieldwork, collected reference samples, organised volunteer workshops, analysed the data and wrote the chapter. J. Chapman assisted with reference sample collection at Paignton Zoo. Work at Paignton Zoo was under the supervision of A. Plowman and J. Chapman.. D.J. Hodgson provided guidance on data analysis.

Chapter 4

C.A. Mills conducted fieldwork, collected samples, carried out the majority of genetic analyses, analysed all the data and wrote the chapter. A range of licensed

volunteers assisted with the collection of dormouse samples. G.J. Horsburgh cloned the microsatellite library. D. Dawson and A. Krupa provided guidance and assistance with primer design and molecular analyses. D. Dawson provided useful comments on the chapter manuscript.

Chapter 5

C.A. Mills conducted fieldwork, collected samples, carried out the majority of genetic analyses, analysed all the data and wrote the chapter. A range of licensed volunteers assisted with the collection of dormouse samples. A. Krupa, F. Martins and G.J. Horsburgh provided guidance on, and assistance with, the processing of samples in the laboratory. F. Martins, D. Dawson and D.J. Hodgson provided useful advice for data analysis.

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C.A. Mills conducted fieldwork, collected samples, carried out the majority of genetic analyses, analysed all the data and wrote the chapter. A range of licensed volunteers assisted with the collection of dormouse samples. A. Krupa, F. Martins and G.J. Horsburgh provided guidance on, and assistance with, the processing of samples in the laboratory. A. Santure and K. Phillips provided useful advice for data analysis.

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The Hazel dormouse *Muscardinus avellanarius*

"When one subtracts from life infancy (which is vegetation), sleep, eating and swilling, buttoning and unbuttoning - how much remains of downright existence? The summer of a dormouse."

Lord Byron

Chapter 1: General introduction

The following thesis is a study of the hazel dormouse, *Muscardinus avellanarius* Linnaeus, 1758, a rodent that has been identified of conservation concern, and as such receives legal protection. As a rare, iconic, and very endearing, resident of native UK woodland, the hazel dormouse receives much interest from professional ecologists and amateur naturalists alike. However, before embarking upon a description of hazel dormouse ecology and its key threats, it is appropriate to address some broader concepts surrounding conservation biology. Specifically: what is conservation; how can the concern in biodiversity preservation be justified, and how do conservationists go about this momentous task? A focus on UK mammals and dormice begins on page 23. A brief introduction to the life history of hazel dormice, details on their status, the threats they face and current conservation practices will then follow, focussing primarily on the UK. Finally, an outline of how the research within this thesis will further the knowledge and conservation of the hazel dormouse will be presented.

What is conservation?

Conservation biology is considered a relatively new scientific discipline (McNeely 2010, Soulé 1985). However, the roots of its underlying principles originate from early human culture, through traditional systems developed to avoid overexploitation of the ecosystems on which people depend, such as centuries-old sustainable fishing practices and plant harvesting by indigenous peoples (Johannes 1978, Turner *et al.* 2000). Many religions and spiritualities foster a respect for the natural environment and organisms within it (Yachkaschi & Yachkaschi 2012). Therefore it is difficult to pinpoint the origin of the paradigm of self-constraint in wildlife exploitation *per se*.

However, as human populations have expanded, urbanisation swelled and the reliance on technology ever increased, it is argued that many people have become alienated from the “natural world”, whilst simultaneously increasing pressure on the environment (Leopold 2004, Maxwell 2003, Pimm *et al.* 1995, Turner *et al.* 2004). It was with an awareness of the major impacts these anthropogenic actions were having,

and the realisation that humans may cause the degradation of habitats and mass extinctions of species, that modern day conservation biology has its foundations (McNeely 2010, Soulé 1985, Soulé & Wilcox 1980). Conservation biology is concerned with the scientific research of genetics, species, communities and ecosystems that are threatened by anthropogenic activities, in order to benefit and facilitate the persistence of biodiversity (Soulé 1985, Sutherland *et al.* 2009). As such, conservation is a goal-orientated, interdisciplinary field, necessarily linked with the social sciences, economic, politics and philosophy (Soulé 1985). How to balance the conflicting positions of pure science, policy and societal values continues to generate uncertainty within the discipline (Brussard *et al.* 2007, Noss 2007, Robinson 2006).

Why conserve?

Despite the general acceptance that biodiversity conservation is of great importance, there is little agreement on exactly why this is the case (Norton 2000). There is no single, simple answer to this question and conservationists have proposed various justifications for conservation (Ehrlich & Ehrlich 1992, Hector *et al.* 2001).

Most obviously, anthropocentric arguments for nature conservation highlight the instrumental value of biodiversity to humans. This is realised through: the provision of resources such as food, materials and medicine; cultural benefits such as recreation, scientific interest and aesthetics; and ecosystem services, for example the regulation of water, atmosphere and climate, erosion control and nutrient cycling (Hector *et al.* 2001, Justus *et al.* 2009, Millennium Ecosystem Assessment 2003). Such uses can be designated an economical worth which are valued by society (Costanza *et al.* 1997). Indeed, the sustainable exploitation of natural resources for present and future human benefit often features highly in political agendas (e.g. United Nations Environment Programme 1992).

However, not all species, habitats and ecosystems are beneficial to humans, and some are even malevolent (McCauley 2006). Therefore further justification of biodiversity preservation *per se*, beyond resource use and ecosystem services, is required. One alternative argument often employed is the concept that species

richness is integral to ecosystem functioning (e.g. Hector *et al*. 2001, Loreau *et al*. 2001, Tilman *et al*. 2001). The components of biodiversity have been likened to the parts of an aeroplane, whereby the removal of such parts leads to increasing instability (Nielson 1995). There is some debate over the extent to which this analogy holds true in complex ecosystems. For instance, a review by Schwartz *et al*. (2000) could not identify strong evidence of ecosystem functioning depending on high levels of biodiversity across many studies. Other authors point to the redundancy hypothesis, whereby certain species may be more important than others within a community. Therefore many species, especially rare ones, may be lost with no serious consequences to ecosystem functioning (Gitay *et al*. 1996). Contrastingly, other authors have presented evidence to demonstrate that less common species can provide significant contributions to ecosystem functioning (Lyons *et al*. 2005). Additionally, Naeem (1998) argues that species redundancy is critical to reliable ecosystem function. Such redundancy may provide insurance against environmental fluctuations, which may be of particular importance in a world with a changing climate (Loreau *et al*. 2001).

An additional argument is that many species may have unknown, as yet unrealised, benefits and that we should conserve biodiversity for future use (Faith *et al*. 2010). Linked to this is the precautionary approach, which advocates conservation due to general scientific uncertainty regarding ecosystem functioning, although parties can become suspicious of this justification if overused (Cooney 2004). It remains to be seen if these arguments are strong enough to influence political and economical drivers, which are often very myopic to long-term and uncertain factors (Ehrenfeld 1976). Consequently, the instrumental argument for conservation is weak for many species which have no obvious use or easily quantifiable, value or role in ecosystem functioning.

Due to such failings, it has been argued that the instrumental standpoint is precarious, and it is not always shrewd or possible to rely on economical value (McCauley 2006). This begs the question, where does this leave the thousands, if not millions of species that are not easily encompassed by the preceding arguments? Often conservationists are able to contrive uses for species, especially particularly charismatic ones (Ehrenfeld 1976). However, if our aim is to develop a strong and

honest argument for biodiversity conservation, ideally we should develop a stance that incorporates all biodiversity. Consequently, this leaves us with the paradigm of nature for nature's sake, in that wildlife has its own intrinsic worth, beyond that of human values and motives (Norton 2000).

Whilst this ethical stance is tempting as an all-encompassing rationalisation of nature preservation, in reality these arguments tend to further confound the discussions. Firstly, it is strongly debated whether morality should even be part of a scientific discipline. Noss (2007) ardently argues that it is the very passion for the intrinsic worth of wildlife that has driven many to become conservation biologists and, as such, have a responsibility to advocate for what is right for biodiversity. However, others contend that, as intrinsic value cannot be easily defined or compared to instrumental-value, it should not be involved in conservation decision making (Justus *et al*. 2009). In contrast, other advocates of instrumental-value reason that such an approach should include non-monetary values, such as the value of simply knowing that a species exists, and thus will incorporate much of what intrinsic worth describes (Macguire & Justus 2008). Practically, ethical values have many impediments, such as varying moral views and debate over the extent to which they are applicable, for example do ethical concerns only apply to certain species, or should they extend to all living beings? (Hector *et al*. 2001, Oksanen 1997). If the former, how do we define in an unbiased manner which organisms are worthy of ethical consideration? An obvious delineation would be only sentient beings, but this is based on our human value of intelligence and self-awareness, and we should consider other measures for the ethical rights of living things (Fox 1989). If the latter, how are any conservation decisions to be made, where there are endless conflicts between different species? This would likely lead to political, legal, conservation and emotional disarray, such as in the case of protected golden eagles, *Aquila chrysaetos,* preying on, and therefore threatening the survival of, endangered island foxes, *Urocyon littoralis,* (Courchamp *et al*. 2003). Further, ethical dilemmas occur where there are conflicts between wildlife conservation and human welfare (Hill 2002, McShane *et al*. 2011).

These and many other philosophical issues are highly problematic and threaten the use of intrinsic worth in justifying nature conservation. However, basing all conservation decisions on pure science and instrumental values, for want of better

articulation, feels instinctively wrong. Science without a soul may rigidly maintain objectivity, but removes the integral component of compassion from nature conservation. Morality may appear to have an extra-somatic origin, but in fact it is becoming increasingly apparent that it is a product of evolution and a necessary adaptation for the human species (Ruse & Wilson 1986). Further, Jepson & Canney (2003) view conservation as a social movement that aims to develop particular ideals concerning the relationship between humans and nature, with the aim of making a "better" world. They suggest, therefore, that conservation must retain its roots in an ethical basis, otherwise the discipline risks becoming alienated from the general public, threatening effective conservation practices.

It is apparent that justifying wildlife conservation is far from clear cut. Initially this may seem disheartening and harmful to the defence for conservation, whereby such conflicting arguments weaken the cause. However, aiming for an all-encompassing panacea to nature conservation justification is most likely an unattainable goal (Hector *et al*. 2001). On reflection, that justifications can be made from such a variety of stances surely demonstrates how truly important biodiversity is. Nature is many things to many people and it is the very diversity of living beings that leads to a diversity of good reasons to protect it. Rather than just concerning ourselves with the problems of conflicting opinions, we should consider them in unison, in order to build a solid argument for nature conservation and implement conservation practices to best serve these contrasting stances.

How to conserve?

Beyond the conflicts highlighted above, the challenges of actually implementing conservation are even greater. Indeed, just the initial practice of defining and measuring biodiversity alone is hugely complex (Purvis & Hector 2000). However, this is vital in order to set aims and objectives and assess outcomes of conservation practices (Pereira & Cooper 2006). Conservation planning has been likened to triage, where limited resources must be allocated in the most efficient manner. This requires conservationists to make decisions on which biodiversity components to prioritise, a

multifaceted task involving conflicts between science, politics, socio-economic, culture and philosophy (Bottrill *et al*. 2008).

The quantification of species' extinction risk is often one of the primary steps in this process, such as the Red List of Threatened Species (International Union for Conservation of Nature 2011). However, the use of these lists alone to set conservation priorities should be executed with care. For example, concentrating on species with the highest extinction risk may not be the best use of limited resources, as it is expensive and difficult to succeed (Possingham *et al*. 2002). In addition to difficulties involved with prioritising species, the single-species approach is limited due the practical impossibility of conserving all biodiversity on a species-by-species basis, and therefore the ecosystem approach has been advocated (Franklin 1993). Further, one of most embraced strategies is to focus on biodiversity hotspots, which if conserved would preserve the greatest species richness (Myers *et al*. 2000). However, single-species conservation strategies such as keystone species (Mills *et al*. 1993) and surrogates such as umbrella, flagship and indicator species are still widely used and can be successful if implemented carefully (Caro & O'Doherty 1999). It is likely that an effective conservation strategy should be a combination of both species and ecosystem-focussed practices (Lambeck 1997).

In response to global biodiversity loss, an international treaty, the Convention of Biological Diversity was formed in 1992, in order to promote the comprehensive protection, and sustainable and fair use, of biodiversity. Each ratifying member state is obliged to implement a national strategy in order to fulfil the requirements of the convention (United Nations Environment Programme 1992). For example within the UK this was implemented through Biodiversity Action Plans (BAP) for priority habitats and species (UKBAP 1994). However, despite some local successes, the 2010 target to slow the rate of biodiversity loss has generally failed and pessimism is now growing, with frustration at the lack of strength in the convention and fear that it has stagnated (Butchart *et al*. 2010, Harrop & Pritchard 2011, Kursar 2011).

Despite this, worldwide interest in conservation remains, with huge public membership of various conservation organisations and much conservation work being carried out by local, national, regional and international conversation groups (Rands *et*

al. 2010). These organisations are diverse in their methods and success in achieving set goals, leading to many debates on how best to conserve biodiversity. As a demonstration of the wide range of complex problems that face conservationists, dilemmas include, but are by no means limited to: the relative roles of community-based conservation and protectionism (Berkes 2004); whether ecotourism does more harm than good (Krüger 2005); contrasting efficiency of direct and indirect payments for conservation (Ferraro & Kiss 2002); whether trade bans protect endangered species or encourage increased illegal trade (Rivalin *et al*. 2007); and the multitudinous problems regarding how to deal with human-wildlife conflicts (Woodroffe *et al*. 2005).

In the light of the continual reports of biodiversity loss - combined with a plethora of complex, moral and practical challenges, inertia of political will and burgeoning human population size and consumerism - it would be very easy to sink into cynical despondence and resign ourselves to the impending biodiversity crisis. However, there is still plenty that can be done to prevent biodiversity loss, although it will require a multitude of strategies and a shift in society's attitude to nature. The alternative is a sixth mass extinction that could only be rectified by millions of years of evolution (Ehrlich & Pringle 2008).

Terrestrial mammal conservation in the UK

Mammals, as charismatic animals that humans can relate to relatively easily, are often designated flagship species. Even so, about a quarter of the terrestrial mammal species are at risk of extinction and we have little data on mammal distribution that is essential for conservation planning (Ceballos *et al*. 2005). Within Europe - and especially Britain - there is a relatively low diversity of mammals. Although this is partly due to the relatively high latitude of these areas, it has undoubtedly been exacerbated by the extermination of many larger mammal species several hundred years ago (Ceballos *et al*. 2002). The main anthropogenic threats to mammals in the UK are: habitat degradation, loss and fragmentation; predation, competition, disease and hybridisation due to introduced species; unsympathetic agricultural practices; climate change; direct mortality on roads and the effects of pollutants and pesticides (Harris *et al*. 1995).

However, there is still a strong mammal conservation interest in the UK. Much mammal research is promoted by The Mammal Society, which since its inception has increasingly developed its role in mammal protection and conservation, in line with conservation biology being recognised as a scientific discipline (Flowerdew 2004). Its aims are achieved through promoting mammal distribution surveys and monitoring, scientific and general interest publications, training, engaging the general public, conferences and advising on legislation and conservation planning (Flowerdew 2004). The Mammal Society also works in close partnership with other UK conservation organisations, such as the People's Trust for Endangered Species, The Vincent Wildlife Trust, and governmental conservation agencies, as well as local mammal groups, to name just a few. Despite efforts from these many organisations, population data on many mammals in the UK is lacking (Harris *et al*. 1995). Therefore, the Tracking Mammals Partnership was formed, with the aim of bringing together a coordinated monitoring programme for mammals in the UK, using the British Trust of Ornithology as a model (Battersby & Greenwood 2004).

In 2009 a Tracking Mammals Partnership report highlighted that of the 54% of terrestrial mammal species it was currently monitoring, 20% were showing a decline in population numbers, whilst 40% were increasing. However, this latter figure includes non-native and invasive species, namely the grey squirrel, *Sciurus carolinensis*, common rat, *Rattus norvegicus*, Reeves muntjac, *Muntiacus reeves*i, and sika deer, *Cervus nippon* (Tracking Mammals Partnership 2009). Of the 60 non-marine mammal species found in the UK, 18 are designated priority Biodiversity Action Plan species, seven of which are bats. This is an increase from the ten species that were on the original BAP list produced between 1996 and 1998 (Biodiversity Reporting and Information Group 2007).

The hazel dormouse

The hazel dormouse, *Muscardinus avellanarius,* is a member of the Gliridae (synonym Myoxidae), or dormouse family, within the rodentia (Daams & De Bruijn 1995). The present nine genera and 28 species of Gliridae are distributed across Europe, Africa and Asia (Daams & De Bruijn 1995, Nunome *et al*. 2007). Gliridae are

one of oldest extant rodent families and its origins likely date back to the late Paleocene-early Eocene (Daams & De Bruijn 1995, Holden 2005, Storch & Seiffert 2007). The family share a common origin with the Sciuridae, or squirrels, rather than the mouse-like, myomorphous, rodents, as may be assumed from the common name (Holden 2005, Kramerov *et al*. 1999). It is thought that the name, "dormouse", has its roots from the word "dormir", meaning "to sleep". This is in reference to the propensity of many of the species in this family for exhibiting torpid behaviour when energy resources cannot sustain homeothermy (Angilletta *et al*. 2010).

The hazel dormouse is the only extant member of the genus *Muscardinus* (Daams & De Bruijn 1995). Its distribution ranges across Europe and into northern Asia minor (Amori *et al*. 2008, figure 1). It is an elusive, nocturnal, arboreal species, found predominantly in deciduous and mixed woodland, with higher prevalence in ancient woodland, but also in scrub, hedgerows and coniferous forests (Bright *et al*. 1994, Eden &Eden 2001, Juškaitis 2008,). Compared to other small woodland rodents, hazel dormice live at low population densities, ranging from one to ten adults per hectare depending on habitat quality (Bright & Morris 1996, Bright *et al*. 2006). In contrast wood mice, *Apodemus sylvaticus,* and bank voles, *Myodes glareolus,* have been recorded at population densities of over 40 and 130 per hectare, respectively (Corbet & Harris 1991).

The dormouse lacks a caecum, and the resulting inability to digest cellulose means that it must sustain itself on a diet of nutritious food such as fruits, nuts, flowers, insects and buds (Juškaitis 2007, Richards *et al*. 1984). These dietary requirements imply that dormice must live and forage in a habitat with a high diversity of trees and shrubs, so that throughout the season there is a succession of food available (Bright *et al*. 2006, Richards *et al*. 1984). As such, dormice have adapted to live at forest edges and coppices with a dense, well-developed understory and canopy, rather than shaded high forest (Amori *et al*. 2008, Bright & Morris 1993, Bright *et al*. 2006, Richards *et al*. 1984). However, they are increasingly being found in non-typical habitats, which is a reflection either of a more generalised habit than previously appreciated, or the forced use of sub-optimal habitat (Bright *et al*. 2006, Juškaitis 2008).

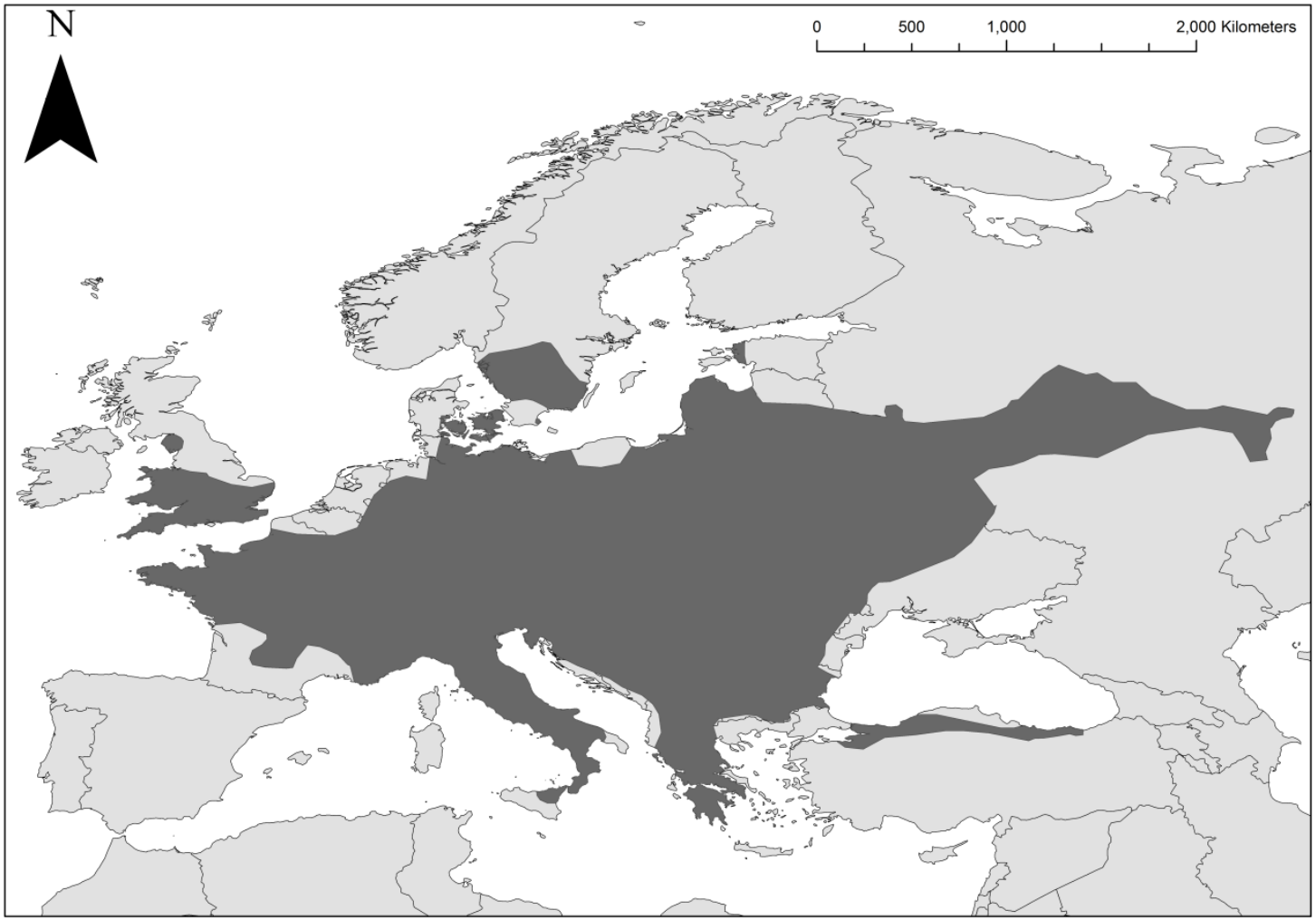

Figure 1. Map showing the geographical distribution of hazel dormice, *Muscardinus avellanarius*, in dark grey (Amori *et al*. 2008).

As an arboreal species, hazel dormice spend the majority of their time amongst the vegetation, rarely venturing down to the ground, where they are generally reluctant to cross open spaces (Bright 1998, Bright & Morris 1991). They build intricate, tightly woven nests, using stripped honeysuckle bark or other suitable material, and then often add an outer layer of freshly picked leaves. The skilled craftsmanship and presence of green leaves very obviously differentiate a dormouse nest from those of other small mammals (Bright *et al*. 2006).

The home ranges of males are larger than that of females, and often overlap with two or more females (Bright & Morris 1991).The breeding season of the hazel dormouse extends across the majority of the active season, and both males and females are territorial during this period. Females will have one, or occasionally two litters a year, with an average of ca. 4 juveniles per litter (range 1-9) (Juškaitis 2008). This relatively low reproductive potential, further distinguishes hazel dormice from other small rodents (Bright *et al*. 1994). In the autumn, with the exception of those in the Mediterranean part of the range, dormice prepare for the hibernation period by accumulating fat reserves, increasing body weight by around 40% from the steady average weight in summer (Juškaitis 2001). It should be noted that even in the active season dormice will often enter a torpid state, or aestivation, when air temperature falls or if food availability is low, in order to conserve energy (Juškaitis 2005). During the hibernation period in the winter months, the timing of which varies with latitude, dormice abandon their lofty habitat and make shallow nests below the leaf litter on the ground (Bright & Morris 1994). Whilst dormice spend up to 60% of their lives in a torpid state, their slower life-styles are compensated by a much higher longevity that other small rodents, and dormice have been recorded living to at least five years in the wild (Bright & Morris 1996).

The hazel dormouse is listed on Appendix III of the Bern Convention and Annex IV of the EU Habitats and Species Directive. In 1996 it was listed as lowest risk/near threatened on the IUCN Red List, however has now been changed to of least concern (Amori *et al*. 2008). This reflects the species' relatively common distribution across its range. However conservation concern remains for this species in the north-westerly parts of its range, such as in the UK, Netherlands, Sweden, Germany and Denmark, where populations are declining. This has led to the hazel dormouse being included in

several national Red Lists in this northern range (Amori *et al*. 2008, Juškaitis 2008). Within Britain, it is reported to have become extinct in approximately 50% of its range in the last 100 years, and a national decline of 19% from 1991 to 2000, and therefore is a Biodiversity Action Plan species (Bright *et al*. 2006).

Dormice are thought to be unlikely to persist in wood patches of less than 20 hectares, or that are isolated by more than 1km (Bright *et al*. 1994). Due to the dormouse's arboreal nature, poor dispersal ability and habitat specialism it is vulnerable to habitat loss, degradation and fragmentation (Harris *et al.* 1995, Bright & Morris 1996). Such threats may occur due to human development and land-use change from urbanisation, roads, agriculture and forestry (i.e. conversion of ancient woodland to conifer plantations) (Amori *et al*. 2008, Bright *et al*. 1994, Juškaitis 2008). The change in management practices of woodlands, leading to less sympathetic regimes, has also likely affected dormouse populations (Bright & Morris 1989). Examples include clear-felling, the decrease of coppicing practices and general neglect that leads to heavy shading and loss of the understory (Bright & Morris 1989, Bright & Morris 1993). The dormouse is sensitive to climate, and due to its influence on productivity and phenology of dormouse food, the extent of torpor behaviour and climatic stochasticity may detrimentally affect their small populations (Bright & Morris 1996). Further research is required to investigate the potential effect of climate change on hazel dormouse populations.

Due to the need for conservation actions, several tools for the presence detection and population trend monitoring of the elusive dormouse have been developed. Primarily, nest boxes and nest tubes (a cheaper nest box equivalent) are used, whereby dormice and/or their characteristic nests are found in artificial nesting sites (Bright *et al*. 2006). Another survey method is to search for feeding remains, specifically gnawed hazelnuts, as different small mammals leave characteristic marks on these nuts, allowing identification of the species' field sign (Bright *et al*. 2006). Hair tube surveys and nest searches are also utilised, although they have a lower detection rate (Capizzi *et al*. 2002).

A variety of conservation actions have been implemented in order to protect the hazel dormouse, which I discuss here in relation to the UK specifically. Many of

these conservation actions are part of the Species Recovery Programme which was set-up in 1992 and run by now Natural England (Mitchell-Jones & White 2009). Due to their legal protected status, dormouse presence must be ascertained and mitigated for, during any development and habitat management practices that may detrimentally affect their populations (Bright *et al*. 2006). To this end, one of the major roles of environmental consultants is the surveying and planning for hazel dormouse presence (Bright *et al*. 2006). Mitigation actions may include ensuring habitat connectivity is retained between habitat patches, habitat creation, the provision of nest boxes (which are thought to augment scarce breeding sites), dormouse bridges over roads and translocation (Bright 2006, Department for Transport 2001). A generally successful captive breeding and reintroduction program was instigated; with the aim of reintroducing dormice to areas of their range where they have been extirpated (Mitchell-Jones & White 2009). In the UK a National Dormouse Monitoring Program (NDMP) was set-up in order to monitor national population trends (Bright *et al*. 2006). This program comprises of dormouse nest box schemes at woodlands across the UK, which are monitored by licensed volunteers at least twice a year during the active season of dormice (Bright *et al*. 2006). Several national hazelnut hunt surveys have also been run nationally, encouraging the general public to search for dormouse-eaten hazelnuts in their local woodlands.

The NDMP and national nut hunts, in combination with other work by the People's Trust for Endangered Species, The Mammal Society and local mammal groups have led to an extensive interest in dormice. Such monitoring and research projects have greatly benefitted from substantial volunteer survey effort. Concurrently, hands-on engagement of members of the public with wildlife is a rare opportunity in British mammal conservation. As such the hazel dormouse, beyond requiring conservation actions due to anthropogenic threats, can be considered as a flagship species, and therefore an important species for promoting wildlife conservation in the UK.

Outline of thesis

Whilst there has been a plethora of scientific studies on hazel dormice, from across its range (reviewed in Juškaitis 2008), there is still much to discover regarding this elusive species. As such, the aims and objectives of this thesis are to make a major contribution to existing information, in order to facilitate continued monitoring, habitat management and conservation practices. Ultimately it is hoped that this will strengthen conservation actions that endeavour to ensure the persistence of this species into the future. Advantageously, ongoing progress and increased accessibility in technology, research techniques and analyses are providing conservation biologists with ever more sophisticated tools for monitoring and studying their focal species, and the research in this thesis is no exception.

In brief, the aims of this thesis are:

- Carry out a pilot study to investigate the effectiveness of alternative techniques to determine the presence of hazel dormice, with the aim of developing methods that are rapid, suitable for a range of habitat types, non-invasive and objective.
- Develop a suite of hazel dormouse microsatellite markers for use in molecular ecology analyses, both for this thesis and for other researchers in the future.
- Describe patterns of population genetics of hazel dormice at a regional scale. It is hypothesised that populations that are at the edge of the range, and more isolated, will demonstrate lower genetic diversity and higher genetic differentiation compared to those found in the core range.
- Quantify the rate of multiple paternity in litters sampled in southwest England and compare results to published data, which reported a rate of multiple paternity in hazel dormice - amongst the highest across mammal taxa. Such a high rate deserves further investigation, as we hypothesise that, due to hazel dormouse testes size and breeding behaviour, the frequency of multiple paternity would not be this extreme, relative to other rodent species.
- Investigate the effect of food availability and natural temperature fluctuations on the hibernation behaviour of hazel dormice. It is hypothesised that dormice that frequently arouse from hibernation, and hence are able to respond to food

availability, will reduce the extent to which they exhibit torpor where food availability is relatively high. This is predicted to the hypothesis that, as hibernation is physiologically and ecologically costly, it should be avoided if energetically feasible.

In Chapter 2 alternative dormouse survey techniques are piloted, namely the use of camera traps and footprint sampling at bait stations. Whilst the use of field signs such as footprints in a centuries-old technique, camera-traps are a relatively new technology, and until recently have mainly been used for larger mammals. Footprint tracking has been used in some studies for small mammals, but rarely for arboreal species. Therefore the aim of this research was two-fold: firstly to encourage the use of these survey methods for a wider range of species, in this case a small, arboreal mammal; and secondly to develop additional, alternative hazel dormouse monitoring tools. Existing dormouse survey methods have specific limitations, such as seasonal and habitat-type dependence that can restrict their usefulness. As such, there is room for methods that avoid these constraints, and provide much more rapid results. This would be particularly useful for surveys related to human development, in order to provide more rapid survey results and therefore reduce human-wildlife conflict.

In Chapter 3 the problem of objectively identifying collected small mammal footprints is addressed. Traditionally, the identification of footprints is performed by experts, with experience in tracking the community of species under study. This need for expertise and the element of subjectivity may preclude ecologists from fully exploiting the potential of footprint tracking. Small mammal footprints specifically are potentially daunting to the uninitiated and therefore a simple algorithm for the discrimination of hazel dormouse and sympatric wood mouse footprints has been developed and tested by volunteers with no tracking expertise. The methodology used in this study comprises Linear Discriminant Analyses, developed in the powerful and open-source statistical programme R.

Chapter 4 considers the use of molecular techniques in dormouse research. Such studies were once restricted to the domain of well-funded research projects that investigated model organisms, and as such were largely inaccessible and inappropriate

for conservation biology. However, as costs have reduced and more work has been carried out using molecular ecology techniques in wild and rare species, the field of conservation genetics has grown. Microsatellites, neutral and highly polymorphic repeat motifs, are one of the main techniques currently used in molecular ecology. The molecular ecology of hazel dormice has only recently begun to be investigated and therefore this chapter describes the isolation and characterisation of dormouse microsatellite markers, which not only aids further work in this thesis, but will also be of great use to other researchers investigating hazel dormouse molecular ecology.

In Chapter 5 these microsatellites are utilised to investigate the population genetics of hazel dormice in south-west England. This research harnesses a range of analyses to describe the population genetic diversity, differentiation and structure of dormice across the study area. These data are used to compare genetic diversity of populations across the sampled range, to determine if those on the edge-of-range are genetically less diverse and therefore potentially at higher risk of extinction. Some analyses, such as F-statistics date back to the early 1900s, when population genetics concepts were first being developed, whilst other techniques use relatively new analyses such as Bayesian clustering software.

Further, in Chapter 6 the microsatellite genotype data is used to infer multiple paternity in sampled dormouse litters using the powerful software programme COLONY. Polyandry in wild populations is a hotly-debated topic. Until relatively recently, the very existence of multiple mating by females was considered a rare event, and it was only with the advent of molecular techniques that finally it was revealed that it is common across a range of animal taxa. Debates continue regarding the proximate and ultimate causes for such phenomena. Little is known about the intra-specific variation in multiple paternity and what consequences this may have for population dynamics.

Chapter 7 investigates the effect of diet and natural fluctuations in temperature on the hibernation behaviour of dormice, using a population of captive dormice at Paignton Zoo. This was carried out using temperature loggers that recorded dormouse nest temperatures, as a proxy for dormouse body temperature. In the light of hazel dormouse sensitivity to climate stochasticity and their particular dietary

requirements, this research forms an essential component of predicting the likely effects of future climate change on dormouse populations.

Lastly, in Chapter 8 the findings of this thesis are summarised in the broader context of hazel dormouse ecology and conservation, the limitations of the studies are discussed and suggestions for further work are made.

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Chapter 2: Take only photographs leave only footprints:

Novel applications of non-invasive survey methods for small arboreal animals

Abstract

The development of appropriate survey techniques is essential to promote more effective and efficient monitoring of species of conservation concern. Here we demonstrate the utility of two rapid-assessment, non-invasive methods to detect presence of small, arboreal animals. Specifically, camera traps and footprint tracking, which are well-established tools for monitoring elusive larger mammals, but are rarely used for small species, such as rodents, or in arboreal habitats. We use the hazel dormouse, Muscardinus avellanarius*, a rodent of conservation concern, as our focal species. Prevailing hazel dormouse survey methods are prolonged, seasonal and habitat dependent or have low detection rates, therefore alternatives would be of use for the great number of ecologists who survey dormice for mitigation purposes, as legally required for building development projects.*

In trials of both these adapted methods, over a total of 405 trapping nights, hazel dormice visited bait stations and were successfully detected by both camera traps and tracking equipment at each of two woodland study sites. Both camera trap images and footprints were of adequate quality to allow discrimination between two sympatric small mammal species (hazel dormouse and wood mouse Apodemus sylvaticus*). Across the two sites the number of nights to first detection was 10.6 nights (range 2-21 nights). Using Cohen's kappa and correlation analyses, we determined that there was substantial agreement between camera trap and footprint tracking in the detection of small mammals. There was evidence, at one site, that as time since-installation increased, the frequency of dormice detected also increased.*

We discuss the relative merits of these methods with respect to research aims, funds, time available and habitat. We conclude that both of the techniques have great potential for use as flexible, rapid-survey methods to detect hazel dormice, as well as other arboreal, small mammal species. As such, they deserve further development to facilitate their utility by a wide range of ecologists and conservationists.

Introduction

Biological surveys and monitoring programs are essential for acquiring knowledge of natural systems. Objectives include identifying trends in population size and range, habitat modelling, habitat use studies, evaluating ecological management approaches and biodiversity assessment (Marsh & Trenham 2008, Tyre *et al.* 2003). Such monitoring is becoming increasingly important, due to intensifying pressures on natural habitats and the continued global biodiversity loss, due largely to anthropogenic effects (Stokstad 2010).

However, establishing abundance estimates in wild populations are costly in time and effort (Joseph *et al*. 2006). The urgent need for information on threatened species, coupled with a general lack of funds encourages the development of techniques that allow the economic and rapid assessment of key species, such as presence-absence surveys, which have lower financial and effort costs than abundance surveys. Additionally, more sample units can be surveyed for the same cost, yielding a more efficient survey design, especially for rare species (Joseph *et al*. 2006, Mackenzie & Royle 2005). There have also been important advances in statistical analyses for presence-absence data, particularly those that incorporate variation in detectability (Joseph *et al*. 2006, Marsh & Trenham 2008, Wintle *et al*. 2004). Several approaches have also been developed to analyse presence-only data (Pearce & Boyce 2006).

The wide variety of field techniques currently employed to establish species presence-absence vary in efficacy, accuracy, effort, cost, invasiveness and ecological constraints. The detection of organisms that are particularly rare, cryptic or inaccessible often requires specific sampling methods. Continuing innovative development and the adaptation of existing monitoring methods will provide a greater choice of survey techniques, for a wider range of species. In turn this will be of benefit for both wildlife conservation and human activities. For example, decisions to grant planning permission are often influenced by the presence of species of conservation concern. The more efficient assessment of the presence of such species will reduce fiscal and time costs to developers and lessen the prevalence of accidental loss of populations of endangered species.

Technologically advanced monitoring tools, such as remote camera traps, are

being increasingly utilised, as they become more accessible and affordable (Rowcliffe & Carbone 2008). The advantages of camera trapping include non-invasiveness, low surveyor time required and the provision of relatively unambiguous, permanent records, for species that are difficult to observe. However, equipment failure, user-error and initial expense can be problematic (Cutler & Swann 1999, Silveira *et al*. 2003, Tobler *et al*. 2008). Despite the increased use of camera traps, they are still not meeting their potential in ecological research (Cutler & Swann 1999, Rowcliffe & Carbone 2008). We conducted a search of the ISI Web of Knowledge database, for the term "camera trap" (in the subject areas: Environmental Science, Zoology and Biodiversity and Conservation) and selected those concerning at least one terrestrial mammal species. Of the 367 citations, 91% of the studies focussed on medium/large species only, 6% on multi-species surveys and just 3% on small mammals (<200g) alone. Whilst smaller vertebrates have a reduced capture probability (Kelly 2008, Tobler *et al*. 2008), camera trapping has been shown to be feasible for small mammal surveying (De Bondi *et al*. 2010) and therefore warrants further research and utilization.

More traditional techniques, such as distance and point sampling, remain important monitoring methods, especially when budgets are limited, equipment security is of concern or a large survey effort is required. One such technique involves point sampling track collection. To circumvent the difficulty of finding footprints in the environment, animals are attracted to track collecting equipment, often utilising lure or bait (e.g. Connor *et al*. 2005, Glennon *et al*. 2002, King & Edgar 1977, Mayer 1957). Tracking stations have been used to survey many terrestrial species including rodents (Brown *et al*. 1996), insectivores (Huijser & Bergers, 2000), mustelids (Ratz 2000), and insects (Watts *et al*. 2008). Additionally, tracking tunnels have been adapted for aquatic mammals (Reynolds *et al*. 2004), but to date have rarely been employed in arboreal habitats (but see Carey & Witt 1991, Palma & Gurgel-Gonçalves 2007). These methods are relatively cheap and easy to set-up, therefore allowing a large survey effort, but require expertise and time for footprint identification. Recent advances in the statistical analysis of footprints for species and even individual identification (e.g. Alibhai *et al*. 2008, Russell *et al*. 2009) are providing new, objective and rapid tools for such analysis, which is likely to greatly increase the potential of tracking monitoring in

the future.

Our goal was to test and promote the use of camera trapping and footprint tracking methods for determining the presence of small, arboreal mammals, using the hazel dormouse, *Muscardinus avellanarius* as our focal species. The hazel dormouse is difficult to study, owing to its elusive nature, small size, low population densities and nocturnal, arboreal behaviour (Bright *et al*. 1994). As a European protected species, the impact of development, land-use change or habitat management upon dormice must be assessed and mitigated (Bright *et al*. 2006), often with some urgency. Current dormouse survey techniques are seasonal, habitat dependent and often prolonged (Bright *et al*. 2006, Chanin & Woods 2003). Nest boxes and nest tubes are the established tools for monitoring dormice in the UK, but the lag between their introduction to a habitat and uptake by dormice can be months or even years. The efficacy of nest boxes and tubes can vary with habitat, since they may be used infrequently if many natural nesting sites are available (Chanin & Woods 2003). A record of the animal in a nest box or tube is reliable, but is invasive and requires a handling licence (Bright *et al*. 2006). Hair tube surveys and nest searches are more economical, but have a low detection rate (Capizzi *et al*. 2002). Searches for evidence of dormouse feeding signs on hazelnut shells are only suitable at sites with sufficient fruiting hazel trees and are best carried out in the late summer to early winter (Bright *et al*. 2006). Therefore, when standard dormouse monitoring methods are used commercial pressures and contractual obligations, along with time and budget constraints, may result in conflicts between development requirements and Ecological Impact Assessments (Treweek 1996). There is, therefore, a pressing need for simple, inexpensive and accurate methods for the rapid detection of hazel dormice.

In light of these constraints, the hazel dormouse is an excellent species to test novel survey methods, in order to provide professional ecologists with alternative survey tools, which additionally may be applicable for other small and/or arboreal animals. Therefore, we test adapted camera trap and footprint tracking methods to detect hazel dormice at bait stations within the tree canopy.

Materials and Methods

Study Sites

The investigation was conducted at two Cornwall Wildlife Trust reserves located in mid-Cornwall, UK, where dormice were known to be present from monthly checks of dedicated nest boxes. Cabilla (50°27’32.50”N, 4°37’49.76”W) is a site of ancient mixed woodland with areas of oak and hazel coppice. Red Moor (50°25’45.12”N, 4°43’08.47”W) is a reserve of heath and grassland with areas of woodland, including hazel coppice. The frequency of nest box use by dormice was significantly higher at Cabilla than Red Moor prior to, and during, the survey year, which suggests a greater density of dormice at the former site (People's Trust for Endangered Species, *pers. comm.*). Therefore, initial trials of the ability of camera traps and footprint monitoring to detect dormouse presence were conducted at Cabilla It should be noted however, that unmeasured differences in habitat, such as natural nesting site availability, may also account for the variation in the use of nest boxes by dormice between sites.

Camera traps

Five Scoutguard SG550 (HCO Outdoor Products, Georgia, USA) trail camera traps were used in this study. These are passive infrared heat and motion triggered cameras with an infrared flash, which unlike a light flash are not detected by animals. Prior to field trials, the camera traps were piloted in a garden setting to ascertain whether the image quality would be sufficient to detect and discriminate between small mammal species. It was determined that camera traps should be placed approximately 1-1.5 meters from the bait, to produce a clear image large enough to identify small species. At this proximity the infra-red flash over exposes the image, and so was covered with opaque parcel tape to reduce flash intensity. Camera traps were set to take video footage of 20 seconds duration once triggered, with a delay of 1 minute between triggers to conserve memory. Video was chosen over stills as the former allows the capture of many frames of images, increasing the chances of species detection and identification, which may be more problematic for small species. The trade-off associated with video capture is that camera trap memory cards are filled more rapidly, forcing more regular checks.

Small mammal species, specifically the hazel dormouse and wood mouse, *Apodemus sylvaticus*, were identified based on morphological features such as ear size, tail length, head shape and the presence of fur on the tail. We did not identify any other small mammal species during this study, although it is plausible that other species such as bank voles, *Myodes glareolus*, may have been captured.

Tracking cages

We designed and built five baited tracking cages to collect small animal prints in the tree canopy (figure 1a-b and figure 2), using adapted 8-inch squirrel blocking cages (Chapelwood, Worcestershire, UK). The cage was required to prevent non-target grey squirrels, *Sciurus carolinensis*, from depleting bait and inundating tracking cards with footprints. A platform inside the lower half of the cage was constructed by supporting a piece of round rigid corrugated plastic sheeting by a framework of wire. A plastic box fitted tightly into a hole in the platform and its lid was covered in tracking medium, which comprised graphite powder mixed with sunflower oil to a viscous consistency. A hole in the box lid allowed small animals access to the bait inside. A replaceable square of white card (180gsm) with a hole that fitted around the plastic box rim was placed on the platform and secured in place by the box lid. Plastic sheeting was attached to the ceiling of the cage to protect the platform from rain. Animals small enough to fit into the cage are attracted to the food in the box, walk over the tracking medium and then leave tracks on the card when they depart. Cards are later retrieved, tracks fixed and replaced with new card.

All tracking cards with footprints were scanned by eye to identify clear prints with a minimum of 4 toe marks visible. These were photographed next to a precision scale and identified, using reference footprints as a comparison (figure 3). Dormouse reference footprints were collected from captive animals at Paignton Zoo. Wood mouse and bank vole reference footprints were collected from animals live-trapped during other studies. Animals were placed in a small container at one end of a tunnel which had a small pad of tracking medium at its entrance and was lined with tracking card. Animals walked over the tracking pad and through the tunnel, leaving behind tracks.

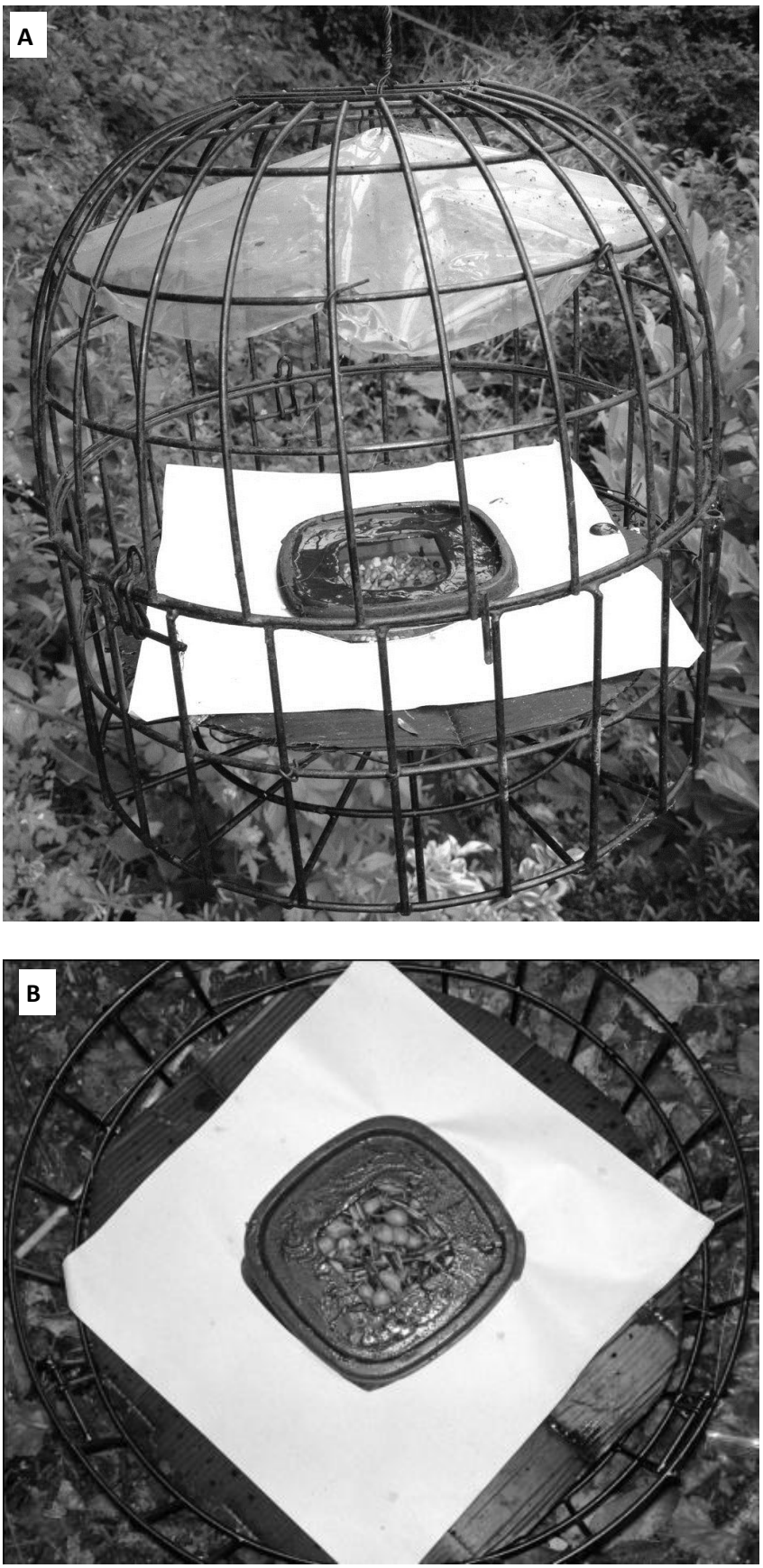

Figure 1. Photographs of assembled footprint tracking cage: a) Side-view of tracking cage; and b) view looking down on tracking cage, with cage lid removed

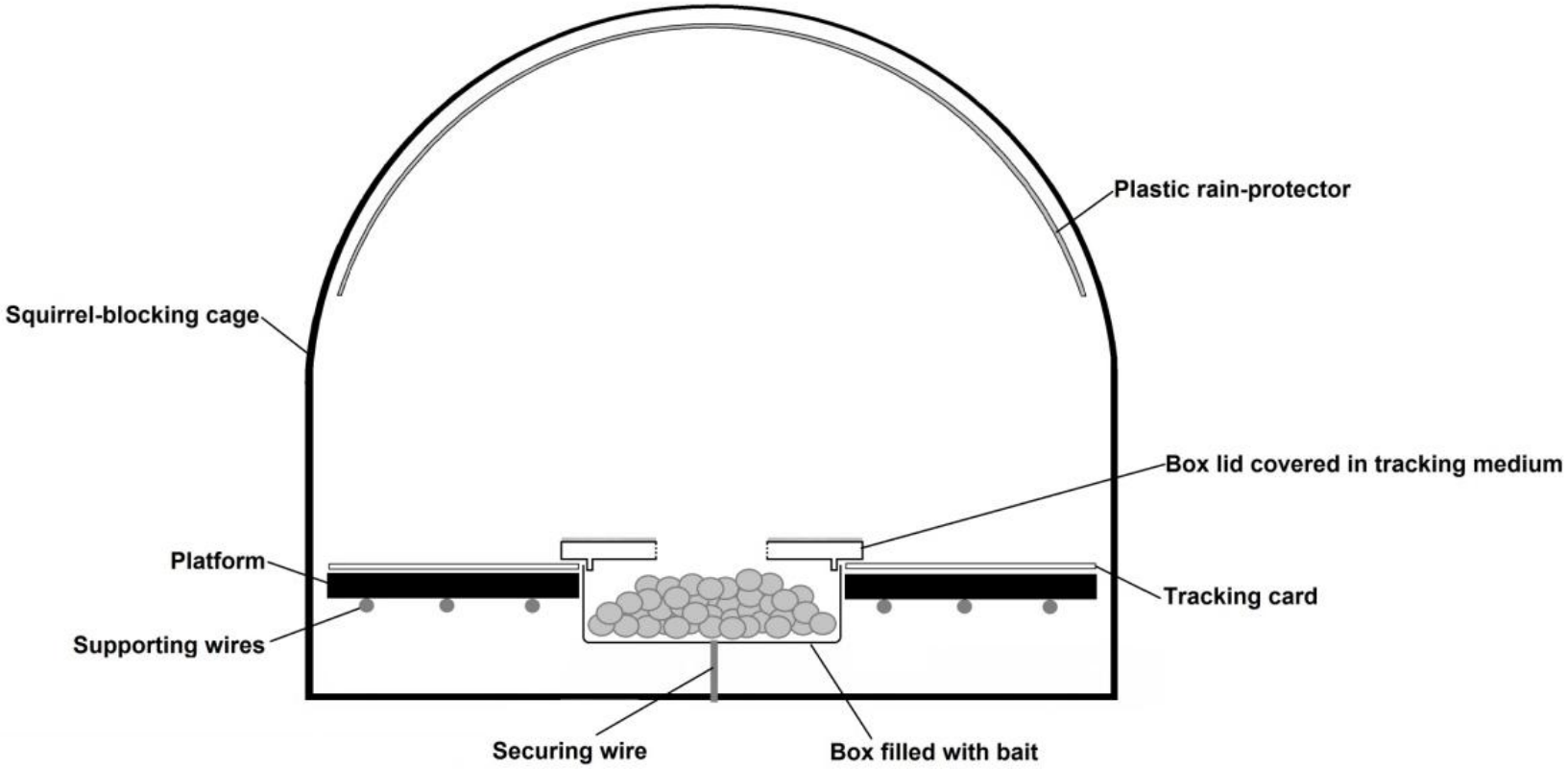

Figure 2. A schematic diagram of a longitudinal section through the footprint tracking cage. The securing wire holds the bait box to the bottom of the cage. A network of supporting wires provides a frame for the platform. A hole in the lid of the bait box provides allow small mammals access to the bait. The tracking card is a square piece of card with a hole in the middle which fits around the bait box. The inner edge of hole in the tracking card fits underneath the outer rim of the box lid, which helps to hold it in place. The plastic cover protects the tracking equipment from rain damage.

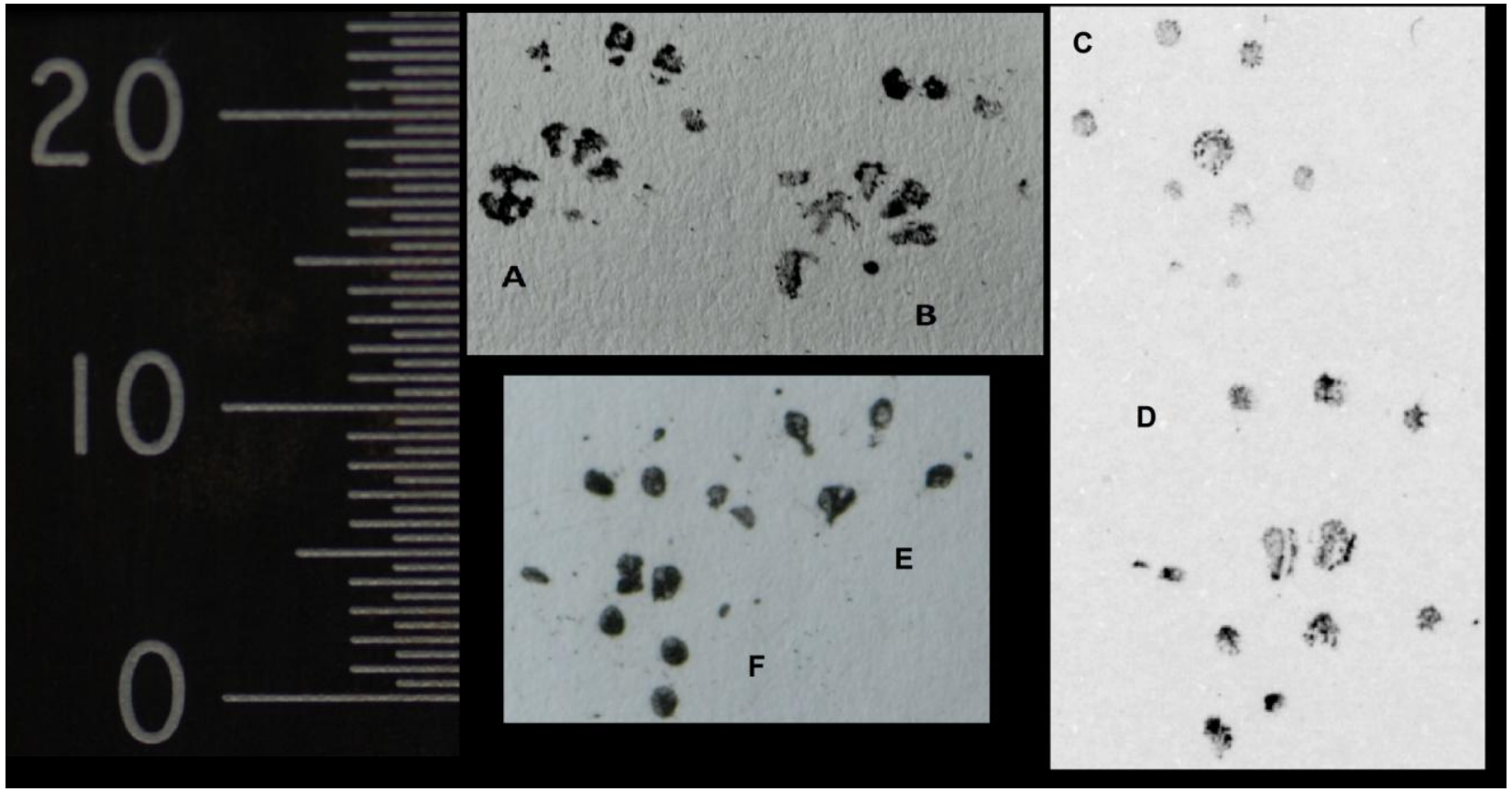

Figure 3. Photographs of reference footprints of the hazel dormouse, fore foot (A) and hind foot (B), for comparison to wood mouse fore foot (C) and hind foot (D), and bank vole fore foot (E) and hind foot (F). All prints are positioned with toes at the top, the scale bars represent 0.5mm graduations. Note the distinctive three triangular metacarpal pads found in dormouse prints, presumably due to arboreal behaviour leading to highly adapted fleshy feet for gripping. In all species there are four toes on forefeet and five on hind feet.

Study Programme

The field testing of baiting, camera traps and tracking equipment occurred in three phases, in order to investigate several questions regarding the survey of small arboreal mammals: 1) Were bait stations able to attract such species? 2) Were camera trap images sufficiently clear to identify species? 3) How soon after installation of bait stations, and how frequently, were small arboreal mammals detected visiting monitoring stations? 4) Were tracking cages collect tracks of adequate quality to allow discrimination between species? 5) How did the detection rates compare between camera traps and tracking cages?

At both survey sites monitoring stations were distributed within the nest box survey site at a minimum of 60 metres apart. A trapping night comprised a 24-hour monitoring session, and was defined as a "trapping night" as only camera trap footage recorded during dark hours, where infra-red was required, were analysed Trapping sessions comprised a variable number of trapping nights, whereby the survey equipment was left monitoring unattended. During all phases of the study, trapping sessions comprised an average of 2.7 trapping nights (range one to six trapping nights. Bait (sunflower seeds, peanuts, apple and honeysuckle fragrance), tracking consumables and camera trap batteries were replenished, and camera trap footage and/or tracks were collected for later analysis where appropriate.

During phase one our objectives were to establish whether small, arboreal mammals would visit bait stations and investigate the ability of camera traps to provide sufficiently clear images to allow species discrimination. Between 8th July and 3rd August 2010 five monitoring stations with camera traps were installed at Cabilla. Each station comprised of a bait tray (a wooden frame with a mesh floor to hold bait), hung from tree branches approximately 2.5 meters above ground level and one camera trap aimed at the tray. Camera traps were secured with Python^{TM} adjustable locking cables (Masterlock, Neuilly-sur-Seine, France).

In phase two, once the effectiveness of the bait trays and camera traps was confirmed, we introduced tracking cages at the existing stations. This allowed us to determine if tracking cages could collect clear, identifiable footprints from small, arboreal mammals. Distinct phases one and two were used to ensure the novel

tracking equipment did not bias objectives of phase one. Between 5th August and 3rd September 2010, five tracking cages replaced the bait trays at the monitoring stations at Cabilla. The camera traps remained monitoring at the stations, to allow comparison of detection rates between camera traps and footprint cages.

In phase three, all equipment was moved to a second site for testing, which allowed further comparisons of camera trapping and tracking cages, at a site where the animals would not have been habituated to any of the equipment. From 11th September to 13th October 2010, the monitoring stations of camera traps with footprint bait cages were moved to Red Moor.

Analysis

The number of trapping nights, starting from installation, required to first detect dormice and wood mice using camera traps was calculated, in order to determine how rapidly small arboreal mammals start utilising bait stations and therefore how soon presence may be inferred. This was combined with descriptive statistics to indicate the frequency of visits of small mammals to bait stations.

The effect of time from initial installation of camera trapping equipment, site (including an interaction between these two effects), and the number of nights per trapping session on dormouse presence detection by camera traps, were investigated using a linear mixed effects model. Station was included as a random effect. Model simplification was performed using Chi-squared test comparisons of maximum likelihood versions of the mixed effects model.

We compared camera trap and tracking cage detection rates for the period when both techniques were running simultaneously at each site. Further analysis was conducted using Cohen's Kappa statistic, a measure of inter-observer variability. This descriptive statistic indicates if any observed agreement in the detection, (or not) of each species, between the two monitoring techniques was due to chance alone (Cohen 1960). This allowed an assessment of the degree of agreement between the two techniques and suggested the rate of detection failure for the two methods. Additionally, to test for a correlation of detection rates between the two techniques, a

Pearson's correlation test on the number of sessions where small mammals were detected by paired camera traps and tracking cages was performed in R version 2.14.1 (R Foundation for Statistical Computing 2011).

Results

Survey effort

Overall, we carried out 30 trapping sessions at 10 different bait locations, across two sites in south west England. Over a total of 81 nights, this resulted in a grand total of 405 trapping nights. Table 1 provides a summary of survey effort over the three testing phases.

Success of baiting and camera traps

We successfully demonstrated arboreal small mammals were attracted to bait and that camera traps captured images sufficiently clear to identify small mammal species (figures 4a-b). Over the three phases 3732 night-time video shots were recorded. Of these, 8.3% captured dormice, and 38.0% wood mice. Conversely, the percentage of shots where no species was identified, due to a false trigger, the animal not being present in the shot or the image being of too pure quality to allow species identification was across sites 53.7%.

Dormice and wood mice were readily distinguished from each other, due to dormice having a much more furry tail, smaller ears and blunter snout. No bank voles were recorded, but due to their smaller ears and shorter tails it is unlikely that they would be mistaken for either wood mice or hazel dormice. Species were only recorded as detected if confidence in species identification was high.

Table 1. Summary of the survey effort and study programme piloting camera traps and footprint tracking techniques, in order to detect hazel dormice.

Phase	Dates	Site	Survey method	Trapping sessions	Trapping nights	Average number of nights per trapping session (range)	Number of trapping nights
1	8/07-27/07	Cabilla	Bait trays and camera traps	10	20	2 (1-3)	100
2	5/08-2/09	Cabilla	Tracking cages & camera traps	9	29	3.22(1-6)	145
3	11/09-12/10	Red Moor	Tracking cages & camera traps	11	32	2.91 (2-4)	160

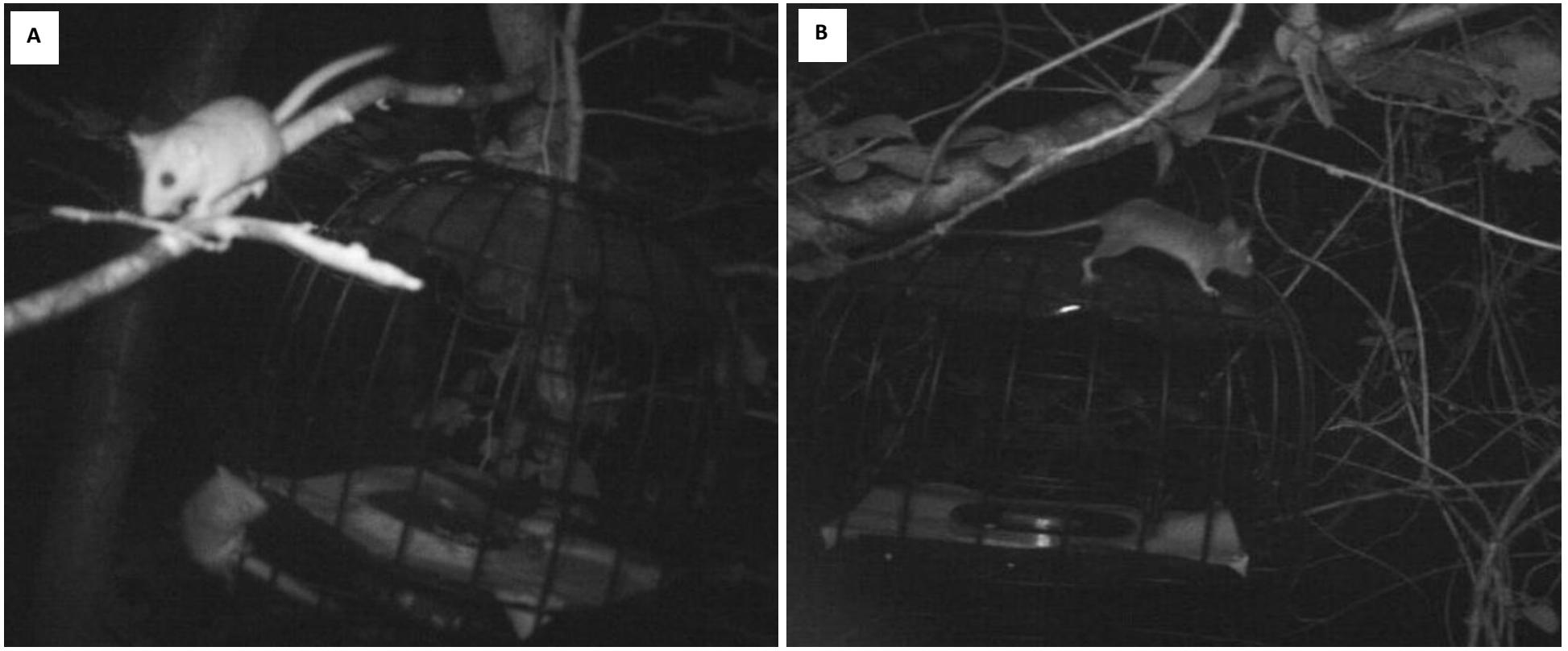

Figure 4. Camera trap shots. Frame from camera trap video footage during phase two of study, (therefore includes tracking cages in shots) of: a) two dormice and; b) wood mouse, to demonstrate video quality sufficient to allow species identification.

Time to first detection and frequency of visits

To investigate how soon after the installation of bait stations small arboreal mammals were likely to utilise bait stations we analysed time to detection rates at Cabilla (phase one and two combined) and Red Moor (phase three), using camera trap data. Across both sites the average number of nights to first detection of dormice was 10.6 nights (range 2-21 nights) and for wood mice 10.0 nights (range 2-40 nights), see table 2 for further breakdown of data.

An indication of the relative frequency of visits to bait stations by dormice and wood mice at the two sites can be shown by the average number of video shots caught per session of the two species, when that species was detected (table 2). The overall averages were 6.2 (SD 5.7) and 2.3 (SD 1.6) for dormice, and for wood mice 13.3 (SD 20.5) and 25.3 (SD 29.0) at Cabilla and Red Moor respectively.

There was a significant interaction between time from initial installation and site on the presence of dormice detected by camera traps ($X^2 = 8.39$, df = 1, p = 0.004). At Cabilla, dormice were more frequently detected in trapping sessions as time increased from initial installation, however this effect was not seen at Red Moor. There was no effect of the number of nights per trapping session on the presence of dormice detected by camera traps ($X^2 = 0.12$, df = 1, p = 0.73).

Success of tracking cages

It was also successfully demonstrated that tracking cages were able to collect tracks of small mammals and that they were of adequate quality to attempt species identification. Figure 5 shows some foot prints obtained from the tracking cages whilst in the field. The blocking cage also effectively prevented bait disruption by grey squirrels during our study, with only two out of a total of 100 trapping sessions being disrupted due to squirrels being able to open the cage.

Over phases two and three of the study, out of the 100 trapping sessions (20 sessions with five monitoring stations), 65% resulted in tracking cards with at least one print that was sufficiently clear to allow an attempt at species identification at each bait station. Of the remaining 35% no prints were present; this would be due to either

Table 2. Detection rates at each monitoring bait station, using camera trap data, at Cabilla (phase one and two combined) and Red Moor (phase three). Data summarised by the time to first detection at and, where a species was detected, the average number of video shots caught of the respective species per session.

	Time to first detection (nights)		Average number of shots per session	
Station trap #	**Dormouse**	**Wood mouse**	**Dormouse**	**Wood mouse**
Cabilla Phase 1&2				
1	4	8	3.0 (2.5)	1.0 (0.0)
2	11	14	2.4 (2.6)	2.5 (1.7)
3	4	40	9.6 (6.5)	7.7 (10.2)
4	18	4	1.0 (0.0)	23.2 (27.5)
5	14	6	5.9 (5.0)	6.6 (7.3)
Average (SD)	**10.2 (6.2)**	**14.4 (14.8)**	**6.2 (5.7)**	**13.3 (20.5)**
Redmoor Phase 3				
6	-	-	-	-
7	-	2	-	14.4 (23.4)
8	-	8	-	31.0 (16.0)
9	2	4	1.3 (1.6)	21.8 (16.1)
10	21	4	3.0 (1.8)	32.3 (47.5)
Average (SD)	**11.5 (13.4)**	**4.5 (2.5)**	**2.3 (1.6)**	**25.3 (29.0)**

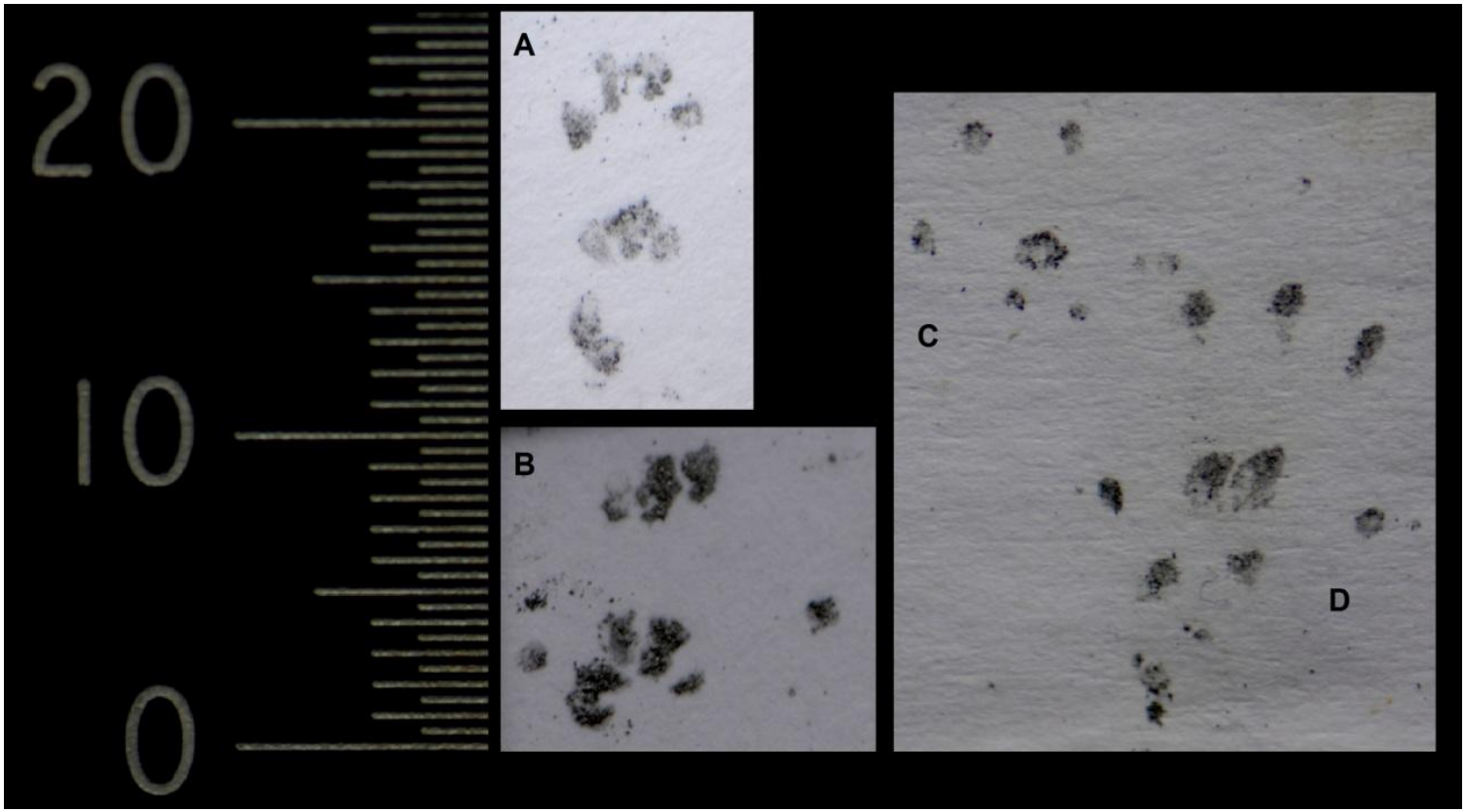

Figure 5. Footprints from wild animals, captured using the footprint tracking cage. Subsequently identified as: hazel dormouse fore foot (A) and hind foot (B); and wood mouse forefoot (C) and hind foot (D). All prints are positioned with toes at the top, scale bars represent 0.5mm graduations. Note the distinctive three triangular metacarpal pads found in dormouse prints, which in some prints merge into each other, such as in print

no animals visiting the tracking cage, or the tracks failing to collect any visiting animal prints. Whilst there were many overlapping prints, an average of 4 prints per tracking card (SD 3.73, range 1-23 prints) were sufficiently clear to allow an attempt at species identification from a visual scan of each tracking card. These 306 prints were identified by eye. This was achieved by comparing unknown prints to the known reference prints (See figure 3 and supplementary figures S1 and S2).

Camera trap and footprint technique comparison

We compared the detection rate between camera traps and tracking cages, by calculating the percentage of trapping sessions that detected the two species, comparing monitoring stations (table 3). This could not be calculated for tracking cages in phase one as they were not employed during this phase. For phase two the detection rates were 37.8% (SD 40.5) and 40.0% (SD 33.0) for camera traps, and 40.0% (SD 30.0) and 35.6% (SD 38.0) for tracking cages for dormice and wood mice respectively. During phase three detection rates for wood mice was higher, and lower for dormice; at 12.7% (SD 17.7) and 60.0% (SD 34.4) for camera traps, and 3.6% (SD 8.1) and 65.5% (SD 36.6) for tracking cages for dormice and wood mice respectively.

There was substantial agreement between the two survey methods in detecting both species (Cohen's kappa = 0.61), with 82% of the trapping nights having an overall agreement. When looking at species separately there was substantial agreement between techniques for identifying wood mice (Cohen's kappa = 0.65, table 4) and moderate agreement for dormice (Cohen's kappa = 0.48, table 4). The reduction in agreement for dormice is probably due to less dormice being detected at Red Moor and therefore there was an increased chance that by random both techniques would fail to detect dormice during a trapping session. If we assume that discrepancies were not caused by false-positives and that there were no occasions where both techniques missed small mammal activity, we can conclude that tracking cages failed to detect visiting small mammals in 7% of trapping sessions, and camera traps in 11% of trapping sessions (table 4). There was a highly significant positive correlation for the number of sessions that detected wood mice and dormice, comparing tracking and cages (Pearson’s correlation = 0.89, df = 18, *p*-value <0.001, figure 6).

Table 3 Comparison of detection rates between camera traps and tracking cages.

	Percentage of sessions species detected by camera traps (%)		Percentage of sessions species detected by tracking cages (%)	
Station trap #	**Dormouse**	**Wood mouse**	**Dormouse**	**Wood mouse**
Cabilla Phase 1 (n sessions = 10)				
1	60.0	10.0	-	-
2	30.0	30.0	-	-
3	70.0	0.0	-	-
4	10.0	80.0	-	-
5	30.0	50.0	-	-
Average (SD)	**40 .0 (24.5)**	**34 .0 (32.1)**	-	-
Cabilla Phase 2 (n sessions = 9)				
1	22.2	11.1	22.2	0.0
2	0.0	11.1	22.2	0.0
3	100.0	33.3	77.8	33.3
4	11.1	88.9	11.1	88.9
5	55.6	55.6	66.7	55.6
Average (SD)	**37.8 (40.5)**	**40.0 (33.0)**	**40.0 (30.0)**	**35.6 (38.0)**
Red Moor Phase 3 (n sessions = 11)				
6	0.0	0.00	0.0	0.0
7	0.0	63.64	0.0	81.8
8	0.0	72.73	0.0	81.8
9	27.3	81.82	0.0	81.8
10	36.4	81.82	18.2	81.8
Average (SD)	**12.7 (17.7)**	**60 (34.38)**	**3.6 (8.1)**	**65.5 (36.6)**

Table 4. Summary of agreement between the two monitoring techniques to detect dormice, wood mice and both species combined. Failure to detect is based on the assumption that a technique failed to detect a species if it had no record but the other method had a positive record for a session.

		Cohen's	Failure to detect	
	Agreement %	**Kappa**	**Camera trap**	**Tracking cage**
Dormice	83	0.48	0.08	0.09
Wood mice	81	0.65	0.14	0.05
Species combined	82	0.61	0.11	0.07

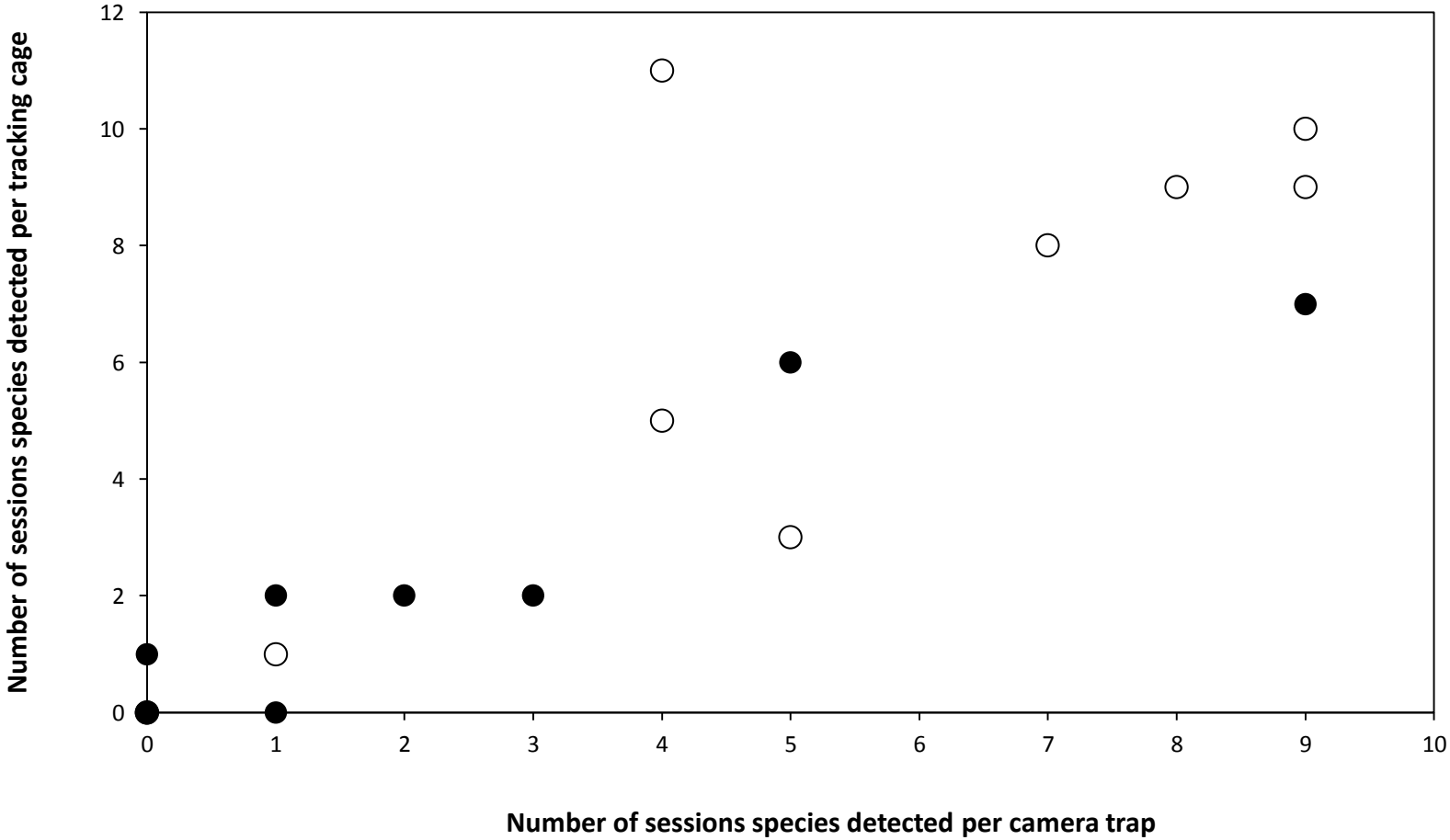

Figure 6. Correlation between of the number of sessions where small mammals were detected, by camera trap and footprint tracking cages, at each monitoring station, for dormice (solid circles) and wood mice (open circles).

Discussion

We have established that both camera traps and tracking cages are able to detect the presence of small, arboreal mammals at bait stations. Camera traps have rarely been used for small animals and we anticipate that our findings will encourage other researchers to utilise camera traps for a wider range of species, including smaller animals. As camera traps continue to become cheaper and increasingly accessible, this will become more feasible (Rowcliffe & Carbone 2008). We have also shown that tracking stations can be adapted for use in arboreal habitats, demonstrating the importance of continued adaptation and development of existing techniques to provide solutions for surveying elusive species.

More specifically, both methods have the potential to determine hazel dormouse presence much more rapidly than current dormouse survey techniques and can be employed at any time throughout the active dormouse season. Our results have shown that dormice may visit bait stations and hence be detected, as soon as two days after installation. Of the stations that detected dormice, all were visited within three weeks. Whilst our methods require more regular visits than the recommended monthly nest box/tube checks (Bright *et al.* 2006), the total number of visits required are comparable, and would greatly reduce time-associated conflict with developers. Additionally these methods can be used in a greater variety of habitats than existing survey methods, as they do not require the presence of any specific vegetation species and should not be affected by the availability of nest sites.

At Cabilla the probability of detecting dormice increased with increasing time-since-installation However, this relationship was not observed at Red Moor, perhaps due to the low statistical power afforded by low rates of detection. The increasing probability of detection implies a certain level of 'trap-happiness' among resident dormice (perhaps not surprising since the bait stations are deliberate attempts to attract subjects), or an aggregation of dormice to bait stations following attraction of the first subject(s). For presence-absence surveys this suggests that a pre-baiting period may be a simple way to maximise the probability of detection. However for studies employing this technique to investigate dormouse ecology, such temporal changes in detectability must be considered. The effect of number of trapping nights

may have no significant effect on the ability of camera traps to detect dormice, as whilst longer trapping sessions may increase the chance of a dormouse visiting the monitoring station, the efficiency of the equipment may be compromised due to equipment failure, for example. Further studies are required to investigate this.

The camera trap and footprint tracking techniques provided similar results for the majority of the trapping sessions. Where there was disagreement, the estimated proportion of assumed detection failure from the two techniques was very similar, which suggests one technique did not appear to detect small mammals more effectively than the other. The cause of failure to detect small mammal activity may be attributed to several factors, dependent on the technique in question. A qualitative comparison of the two techniques is given in table 5.

Table 5. A comparison of the pros and cons for camera trap and tracking stations as survey techniques, with the objective of detecting the presence of hazel dormice.

	Camera trap	Footprint tracking
Invasiveness	No need to handle animals	No need to handle animals
	Infra-red flash is not detectable	Tracking medium adheres to animal's feet, but is non-toxic
Survey effort	Positive records of dormice are acquired within days to weeks	Positive records of dormice are acquired within days to weeks
	Bait, batteries and memory requires replacing	Bait, tracking card and medium requires replacing
	Can be left out for long periods of time before collection as stores data safely	Must be collected regularly as overlapping tracks make footprint identification difficult
	Higher cost reduces potential survey effort	Lower cost allows larger survey effort
Accuracy	Animal can be detected even if doesn't make direct contact with bait	Animal must make contact with bait and tracking medium to leave prints
	False-negative results may result from the camera trap failing to trigger, or the delay from trigger to recording	False-negative results may result from environmental conditions damaging tracking cards
	False-positives if species incorrectly identified	False-positives if species footprint incorrectly identified
Flexibility	Can be used in any habitat where camera traps can be secured	Can be used in any habitat where tracking cages can be secured
	Can be used any time throughout the active dormouse season	Can be used any time throughout the active dormouse season
Reliability	Weather conditions may increase the number of false triggers, depleting memory and battery	Weather conditions damage tracking medium and cards, preventing tracks being left by animals
	Equipment waterproof so rain and wind should not damage records	Weather conditions may destroy tracks
	Potential equipment failure and user-error	Simple system reduces likelihood of equipment failure and user-error
Expertise	Data analysis requires minimal expertise	Data analysis requires footprint identification expertise
	The placing of the camera trap relative to bait requires skill to optimise results	Placing of tracking cage can be more *ad hoc*
Record	Permanent	Permanent
Costs	High initial and maintenance costs and therefore increased security concerns	Low cost and therefore less security concerns

Future directions

Whilst the principle of both techniques has been proven, further work is required to establish a standardised protocol with guidelines on experimental design (Kelly 2008). A survey effort that minimises the risk of false absences and takes detection probability into consideration should be determined (Mackenzie 2005).

Various ecological factors such as: food availability; abundance of bait competitors; habitat type; and weather conditions may influence the effectiveness of these baited methods and therefore requires further investigation. The population density of the study species is also likely to affect the detection rates of these methods. Our surveys at Red Moor detected less dormouse activity at bait stations, but it is unclear if this is due to lower dormice density, seasonality, the competitive exclusion of dormice by wood mice, other variables or just due to our small sample size. Calibration of detection rates to accurate abundance estimates may allow the establishment of methods to determine indices of relative abundance (Kelly 2008, Rovero *et al*. 2009).

The use of pre-baiting and an increased number of bait stations may reduce the temporal survey effort required. However, a subsequent increase in bait competitors attracted to pre-bait may dissuade focal species from visiting the bait. As rate of bait taken may vary for locations, we recommend a pilot study to determine the required rate of replenishment at each site. This would also allow the rate of checking required to be determined, depending on chosen camera settings. Whilst we used video clips of 20 seconds duration, if memory card become full species may be missed, reducing video duration to 10 seconds would lessen this.

In our study we only detected, and distinguished between, hazel dormice and wood mice. The camera trap data suggest that few or no other small mammal species visited the bait stations during our study. However, it is important to note that other species such as voles and shrews may visit arboreal tracking cages. This is of particular note for footprint identification, as wood mice prints may be confused with other rodent species, as these rodents have a similar general morphology (Van Apeldoorn *et al*. 1993). However, the characteristic metacarpal pads of the hazel dormouse result in it being more distinctive. The adoption of statistical algorithms for footprint

identification, such as those employed by Alibhai *et al*. (2008) and Russell *et al*. (2009) would provide a more automatic and objective method, could include a wider range of small animal species and provide additional information, such as age and sex. We envisage that the continuing develop of such techniques will lead to an expansion in the use of point sampling of footprints for many species groups.

Conclusion

Our study successfully demonstrated proof-of-concept for the use of camera traps and tracking cages to detect the presence small, arboreal animals. As wildlife monitoring technology becomes more sophisticated and the urgent need for cheap and quick monitoring techniques heightens, it is likely that the employment of presence-absence surveys will continue to increase. Therefore, future studies should consider these techniques when surveying for such species. We believe that these novel survey methods may also be used for other research interests, such as temporal and spatial activity patterns and food preferences.

We have demonstrated that there is value in adapting and creating new survey techniques, even if established survey methods exist. Alternative techniques increase the range of potential survey methods, providing ecologists with greater flexibility to choose a technique most suitable for their particular time and financial constraints. Presence/absence survey techniques need not be expensive, as exemplified by the simplicity of footprint tracking, but can dramatically reduce the delay in detection of species of conservation concern in threatened habitats.

Acknowledgements

Our thanks go to Paignton Zoo and Julian Chapman for assistance with the collection of dormouse reference footprints, the Cornwall Wildlife Trust for allowing us access to the study sites and David Groves for comments on an earlier draft. C. Mills received funding from the People's Trust for Endangered Species for her research on dormice behaviour and population genetics.

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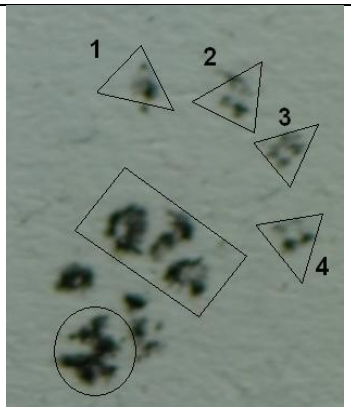
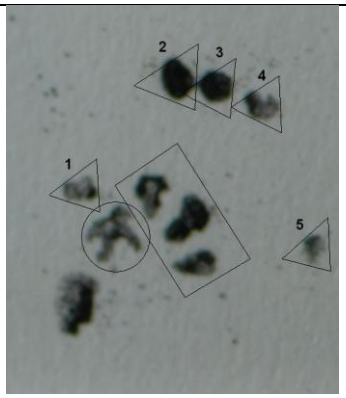
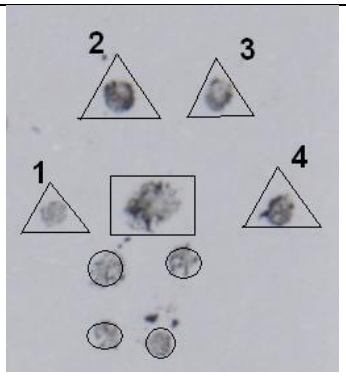
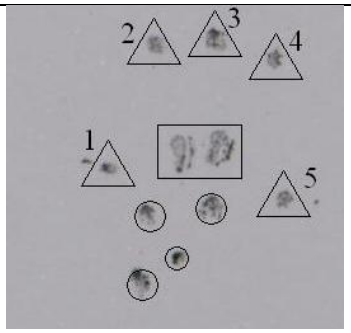
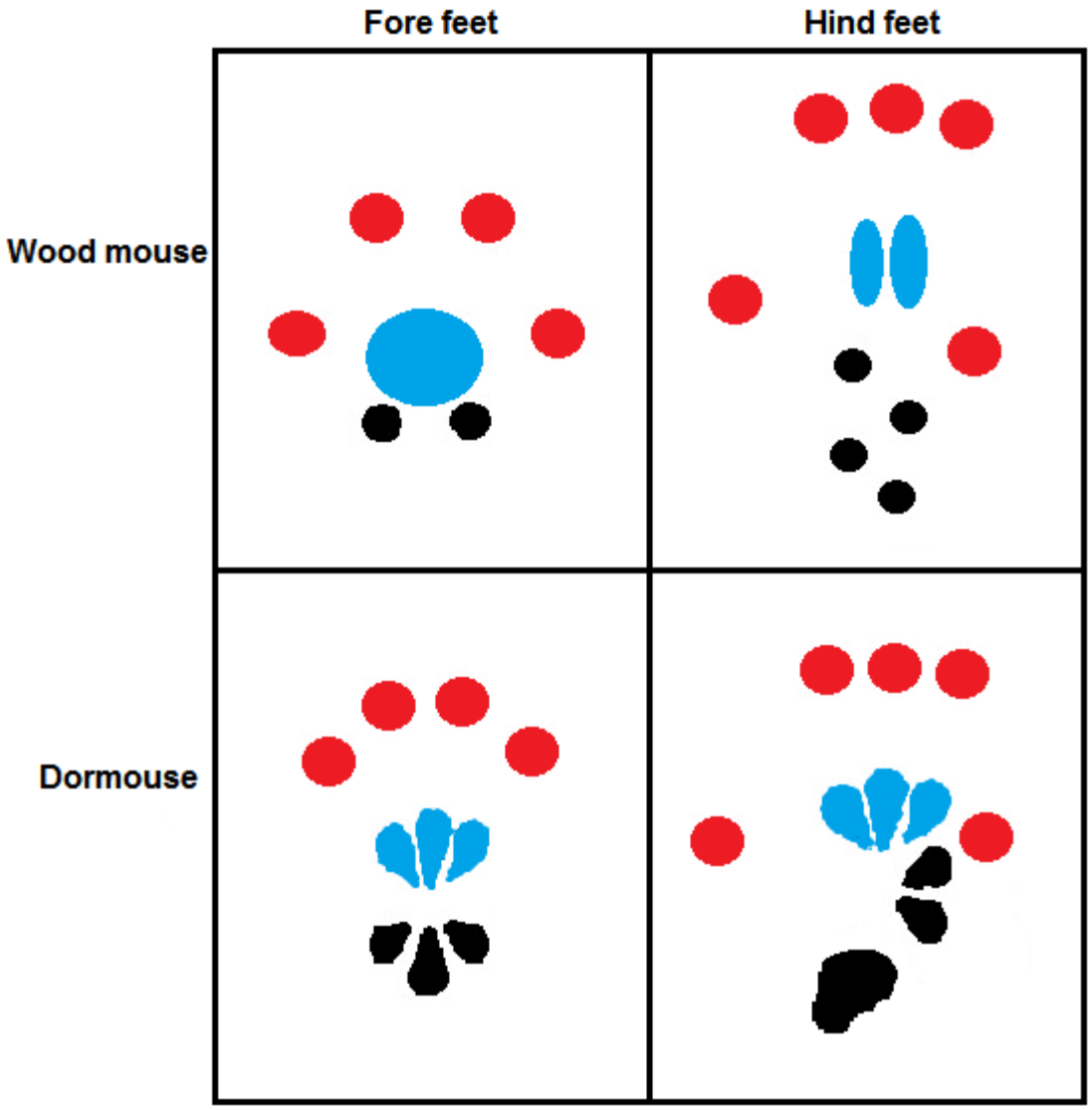
Wintle, B.A., McCarthy, M.A., Parris, K.M. & Burgman, M.A. 2004. Precision and bias of methods for estimating point survey detection probabilities. *Ecological Applications*, **14**, 703-712.

Supplementary Figure S1. A description of the morphological features of small mammal feet: a comparison of hazel dormice and wood mice. Main features of the feet are marked by: triangles (toes), rectangles (metacarpal pads) and circles (heel pads).

	**Dormouse fore foot** *Toes* Four toes. Toes form a symmetrical, shallow arch. A line drawn between toes 1 and 4 would cross well above the metacarpal pad. *Metacarpal pads* Three triangular/oval pads close together. If excess ink may merge into an oblong. *Heel pads* Irregular concave polygon shapes.
	**Dormouse hind foot** *Toes* Five toes, sometimes four as outer toe often doesn't leave a print. Toes 1 and 5 at an obtuse angle from middle three toes. Angle between toes 1,2 and 5 and 1,3 and 5 generally more than 75 degrees. *Metacarpal pads* Three triangular/oval pads close together. If excess ink may merge into an oblong. *Heel pads* Irregular concave polygon shapes.
	**Wood mouse fore foot** *Toes* Four toes. Toes form a symmetrical pattern. A line drawn between toes 1 and 4 would transect the metacarpal pad. *Metacarpal pads* One circular pad. *Heel pads* One to four small round pads below metacarpal pads.
	**Wood mouse hind foot** *Toes* Five toes, sometimes four as outer toe often doesn't leave a print. Toes 1 and 5 at an obtuse angle from middle toe. Angle between toes 1,2 and 5 and 1,3 and 5 generally less than 75 degrees. *Metacarpal pads* Two oval pads. Can merge into each other if excess ink. *Heel pads* One to four small round pads below metacarpal pads

Supplementary Figure S2. Diagram of the dorsal surface of wood mouse and dormouse front and hind feet, showing the toes (red), metacarpal pads (blue) and heel pads (black).



Chapter 3: Keeping track of hazel dormice:

an objective method for discriminating small mammal footprints

Abstract

As a European protected species, there are legal procedures in place which require the mitigation of development effects on hazel dormice, Muscardinus avellanarius. Hence, a prime role of environmental consultants is to detect the presence of these mammals in order to implement conservation initiatives. Established methodologies vary in effectiveness, and habitat and temporal constraints, that impact on their execution. A recent study (Chapter 2) successfully detected wild dormice and wood mice, Apodemus sylvaticus, *through the collection of footprints in their arboreal habitat. However, the need for expertise in footprint identification is often a barrier to ecologists utilising such tracking techniques. Naïve surveyors may find footprint identification challenging, therefore an objective system for discrimination between wood mice and dormice is required.*

Suitable morphometric measurements from reference images of hazel dormouse and wood mouse footprints were selected. An algorithm based on Linear Discriminant Analysis (LDA) using four measurements was then developed and tested by non-experts, through a random selection of 39 reference footprints, and 212 identification attempts.

Of the four measurements, three were found to be highly repeatable and contribute to LDA functions that resulted in high proportions of correct identifications. However, the inclusion of one measure (B) resulted in an increase in incorrect identifications and therefore was dropped from the final LDA algorithm. This final algorithm was compared to identifications made manually by experienced footprint surveyors. For footprints where both methods made an attempt at species identification agreement was 99%. However, using the 95% confidence interval with the LDA function led to 24% of prints being reported as unidentified, compared to just 7% of prints examined manually. Our methodology could easily be adapted to include additional small mammal species as well as applied to other animal communities.

Introduction

Throughout human history, the identification and interpretation of animal signs has been a fundamental skill, enabling ancestral and present-day hunters to detect and follow focal species (Stander *et al*. 1997). More recent scientific, ecological and conservation motives, across the range of mammals, has prompted the increased uptake of field sign monitoring (Twigg 1975). Field signs surveying is non-invasive, relatively inexpensive, begets a permanent record in collected signs and photographs, requires less survey effort compared to live trapping for example, and enables spatially extensive studies (Van Apeldoorn *et al*. 1993).

Footprints and tracks are widely used field signs for surveying across a range of taxa, including large carnivores (Gusset & Burgener 2005, Hussain 2003), ungulates (D'Eon 2001), erinaceids (Huijser & Bergers 2000), mustelids (Ratz 2000) and rodentia (Connors *et al*. 2005). This is possible because evolutionary adaptations frequently produce inter-specific variation in foot morphology and because animals often leave behind tracks in various stratum within their environment (De Camargo *et al*. 2012, Stander *et al*. 1997).

Footprints can be acquired by searching for tracks within a species' natural habitat where the ground is likely to retain prints, such as along the edge of water bodies (Sidorovich 1992), sandy roads (Gusset & Burgener 2005) or in the snow (D'Eon 2001). Whilst these methods will have little or no surveyor effect, intuitively they may be habitat and/or seasonally restrictive, time consuming and have low detection rates. Therefore, often artificial tracking stations are employed, whereby animals leave footprints on a specially prepared tracking medium, frequently attracted by bait or lure. A variety of techniques have proved successful at collecting mammal footprints, including scent-stations surrounded by sand (Conner *et al*. 1983), tracking tunnels (Drennan *et al*. 1998, Glennon *et al*. 2002, King & Edgar 1977), floating rafts for aquatic mammals (Reynolds *et al*. 2004), tracking plates (Connors *et al*. 2005) and tracking cages for arboreal mammals (Chapter 2).

However, once tracks are acquired, the task of reliably identifing prints to species is challenging and requires expertise. This is particularly difficult for morphologically similar, sympatric species. This constraint has potentially impeded the

further expansion of tracking techniques for surveying. However, there have been advances in objective identification techniques, such as discriminant and canonical analyses (e.g. Alibhai *et al*. 2008, De Angelo *et al*. 2010, Palma & Gurgel-Gonclaves 2007 and Sharma *et al*. 2005), automatic image recognition (Russell *et al*. 2009) and unsupervised neural-network and Bayesian methods (Riordan 1998).

The focus of this study is the hazel dormouse, *Muscardinus avellanarius*. As a flagship species protected under European Directives, much effort is put into surveying, monitoring and mitigation for this species (Bright *et al*. 2006). Many existing hazel dormouse survey methods are seasonal and/or habitat dependant, or require prolonged survey periods (Bright *et al*. 2006). Therefore, the development of more efficient and rapid survey techniques for hazel dormice would be beneficial. It has been shown that it is possible to acquire footprints from dormice using a baited tracking system placed in their arboreal habitat (Chapter 2). However, non-target, wood mouse, *Apodemus sylvaticus,* tracks were also recorded. Hazel dormouse feet are morphologically different from wood mice, and it is possible, with training, to distinguish them by eye. However, this is subjective, requiring expertise and therefore prone to error if attempted by less experienced surveyors.

Measurable morphometric features of known hazel dormouse and wood mouse prints were analysed using linear discriminant analysis (Fowler *et al*. 1998) to develop an accurate algorithm with a 95% confidence threshold. The ability of naïve surveyors to accurately record these measures from reference footprints and apply the algorithm was assessed. These results were used to select the most accurate and simple numerical algorithm for the objective identification of species from footprints. Species determinations calculated using this algorithm were then compared to those made subjectively by surveyors who are familiar with hazel dormouse and wood mouse footprints.

Materials and Methods

Reference footprint collection

Reference footprints were collected from 15 captive hazel dormice from Paignton Zoo and 16 wild wood mice live trapped during other studies. Tracks were obtained by allowing animals to freely walk through a tracking tunnel sited in a large container, into which the animal was released. A cardboard pad covered in tracking medium (a viscous mixture of graphite powder and sunflower oil) was positioned at the tunnel entrance. A strip of white card lined the floor of the tunnel. As an animal walked over the pad and through the tunnel, footprints were left on the tracking card. These were retrieved and sprayed with a fixative (i.e. hairspray) to prevent smudging. Each reference footprint was photographed using a digital camera, with a 0.5mm increment precision scale in the frame. Data on date, species and sex were recorded. Other small mammal species such as voles and shrews were not included in this study, as these species were not detected in the testing of arboreal tracking cages (Chapter 2).

Ethical Note

Hazel dormice were kept by Paignton Zoo as part of a conservation reintroduction scheme, licenced by Natural England and at all times acting within the laws of the UK and abiding by all ethical policies of the British and Irish Association of Zoos and Aquariums, the European Association of Zoos and Aquaria and the World Association of Zoos and Aquaria. Collection of footprints took place during normal husbandry practices, when animals would normally be removed from their enclosures, to ensure no additional disturbance to the animals occurred. All wild wood mice were live trapped following recommended guidelines (Gurnell & Flowerdew 2006), and footprints were collected in the field and the animal immediately released at its capture site.

Morphometric measurement selection

The image processing software ImageJ v1.44 (Abramoff *et al*. 2004), was used to collect morphometric measurements from randomly selected reference footprints. To avoid pseudoreplication only one fore and one hind print from each individual was used. Measurements incorporating angles and distances between all combinations of toes and the central pad was carried out on a small subset of the prints (n=4 per species per fore/hind foot position). Distances were converted into ratios in order to control for variation in footprint size. Additional characteristics, such as distinctive pad shapes were also recorded. Those measurements that showed no variation, based on exploratory data were eliminated from further analyses. The remaining measurements were taken randomly from the additional reference images, resulting in a suite of measurements for one fore and one hind foot from each individual (dormice n=15, wood mice n=16).

We further selected measurement parameters that were independent of foot position (i.e. hind/fore and left/right). This ensured the same methodology could be implemented on any print irrespective of foot position, a feature that will usually be unknown for prints on tracking cards. Both species have four toes on forefeet and five toes on hind feet and so we selected measures that were based on the corresponding four toe positions only. Where five toes were present, the outer toe that was closest to the central pads was arbitrarily designated as the toe that should be ignored. We used this definition, as this is most likely to be the thumb-equivalent and so more likely to alter biometric features between feet. This left four toes for further measurements and allowing hind and forefeet to be treated by the same analysis.

A total of four measurements (A-D) were selected and used throughout the following methodology. In brief, A = width to height ratio of the area encompassing the four toes, B = ratio between the distance between the outer two toes, and the centre of that line to the middle toe which has the greatest distance between itself and the adjacent outer toe, C = angle between the two outer toes and the middle toe which has the greatest distance between itself and the adjacent outer toe, D = qualitative description of metacarpal pad morphology. Figure 1 provides a diagram of each measurement for both dormice and wood mice, and supplementary figure S1 includes

more detailed instructions on recording these measurements.

Original Linear Discriminant Analysis

To determine which measurements were most accurate in determining species identity, separate linear discriminant analysis (LDA) models were performed on the data for each of the possible combinations of the measurements (A-D), using R v2.10.0 (R Foundation for Statistical Computing 2010). LDA finds a linear combination of the response variables that maximises the probability that a new observation joins one of the predefined clusters. It assumes normally distributed data with equal co-variances between measurements (among clusters). The proportion of footprints correctly assigned by each LDA model was ascertained, using the "leave-one-out" method (Everitt 2005).

LDA algorithm testing by non-experts

Twenty groups of volunteers (range one to four people per group) from biological and non-biological backgrounds, but none with experience of tracking were used to trial the LDA algorithm. Volunteers were given a brief introduction to footprint morphology and provided with instructions on how to take the measurements and use the LDA algorithm (supplementary figure S1). The ability of volunteers to record the all four measurements and calculate the corresponding LDA function was then assessed through blind testing with a selection of reference footprint images. Images were randomly rotated so that volunteers were required to ascertain the correct direction of travel for a footprint and determine the layout of toes and pads, as this would not necessarily be clear from the tracking card. Volunteers either took measurements manually using a rule and protractor, or with ImageJ and then recorded their data in a spreadsheet, along with their calculated LDA value and the species identification.

LDA measurement combinations

For each LDA model of the different combinations of measurements A-D, the lower and upper threshold values were determined that gave 95% confidence that the footprint was either dormouse or wood mouse, respectively. This was carried out using the original morphometric measurements data. Then using the raw data from the volunteers' measurements, for each combination of LDA model we determined the

proportion of samples that were identified correctly before applying the 95% confidence threshold, the proportion of samples that provided an identification estimate with at least 95% confidence, the proportion of these "confident" identifications being correct and the proportion of false identifications of hazel dormice.

LDA versus experienced surveyors

Three hundred and six unknown footprints collected during trials of tracking cages (Chapter 2), were subjected to the final LDA algorithm. The identification results using the algorithm were compared to the identifications made subjectively by experienced small mammal footprint surveyors, as determined in the original study analysis (Chapter 2).

Repeatability analysis

Intra and inter-observer consistency in recording the footprint measurements were investigated through repeatability analyses. Five observers each measured a set of eight footprints twice, with a minimum of fifteen minutes between repeat measures. For each of the four measurements, observer's data were analysed using a linear model, with mixed effects, whereby observer and print were included as random effects. Correlations between the continuous intra-observer repeat measurements were assessed using model simplification via Chi-squared tests to compare nested Maximum Likelihood models. The distribution of variance amongst the fixed (intra-observer measures) and random effects (print and inter-observer measures) was investigated to determine the relative contributions to the total variance from each of these effects.

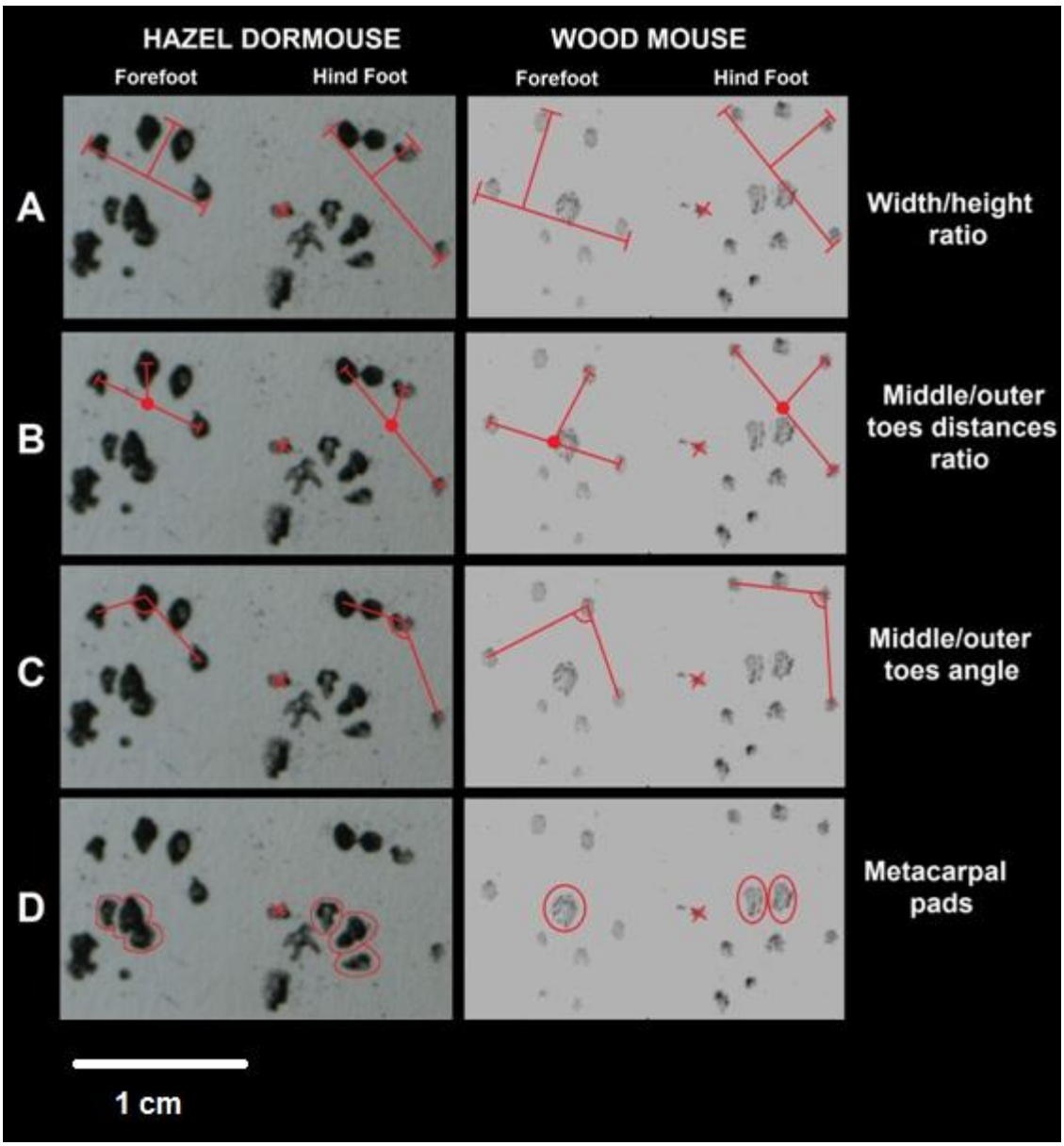


Figure 1. Diagrammatic descriptions of the morphometric measurements A-D, shown on prints from both, hazel dormice and wood mice, as well as fore and hind feet. Crosses denote the hind toe that is ignored, as per our methodology.

Results

Original Linear Discriminant Analysis

Linear discriminant analysis based on the original measurement data demonstrated that all combinations of the four reference footprint measurements provided high proportions of correct species identifications, ranging from 0.92 to 0.95 (table 1). The full model, ABCD, did not provide the highest proportion of correct identifications.

LDA algorithm testing by non-experts

From a random selection of 39 known footprints, volunteers made a total of 212 identification attempts. When volunteers used all four measurements and calculated the LDA value based on the ABCD LDA model, 90% of identification attempts were correct, which compares to 94% for the same model when calculated using the original measurements. However, there was substantial error in some models, and significant variation in the success of the different models, ranging from 44% to 91% correct. Note, that this is prior to the application of confidence thresholds.

LDA measurement combinations

Further analysis of the volunteers' raw measurement data allowed us to determine in which LDA models the greatest error lay, using the 95% confidence threshold values. This analysis demonstrated that there was a large variation between models that provided confident identifications in contrast to these confident identifications that were also correct. Additionally, there was some variation in the proportion of false-positive identifications of hazel dormice (table 2), although in models where this was low, this is due to very few samples being identified confidently and correctly as dormice.

By contrasting these values, it is possible to determine that the LDA model using measurements ACD provided the best compromise between the proportion of confident and correct identifications (figure 2).

Table 1. LDA results for measurements taken by ourselves, showing the proportion of correct identifications, based on the "leave-one-out" method (Everitt 2005).

Measure combination	Proportion of correct identifications
AB	0.92
ABC	0.92
ABCD	0.94
ABD	0.95
AC	0.92
ACD	0.94
AD	0.94
BC	0.92
BCD	0.94
BD	0.95
CD	0.94

Table 2. Comparison of success rates for the different combinations of LDA measurements, A-D. Volunteer measurement data were used for all combinations of LDA models and tested for: the proportion of identifications that were correct; the proportion that provided confident identifications; using the 95% confidence threshold; the proportion of these confident values that were correct; and the proportion that provided false dormouse identifications.

Measure	Proportion correct across all data	Proportion providing "confident" LDA values	Proportion of correct "confident" identifications	Proportion of false dormouse identifications
AB	0.44	0.99	0.45	0.00
ABC	0.66	0.66	0.72	0.00
ABCD	0.90	0.83	0.93	0.01
ABD	0.46	0.92	0.48	0.00
AC	0.82	0.64	0.89	0.14
ACD	0.91	0.86	0.94	0.02
AD	0.87	0.78	0.95	0.02
BC	0.56	0.77	0.64	0.00
BCD	0.88	0.82	0.91	0.01
BD	0.45	0.99	0.45	0.00
CD	0.91	0.85	0.95	0.02

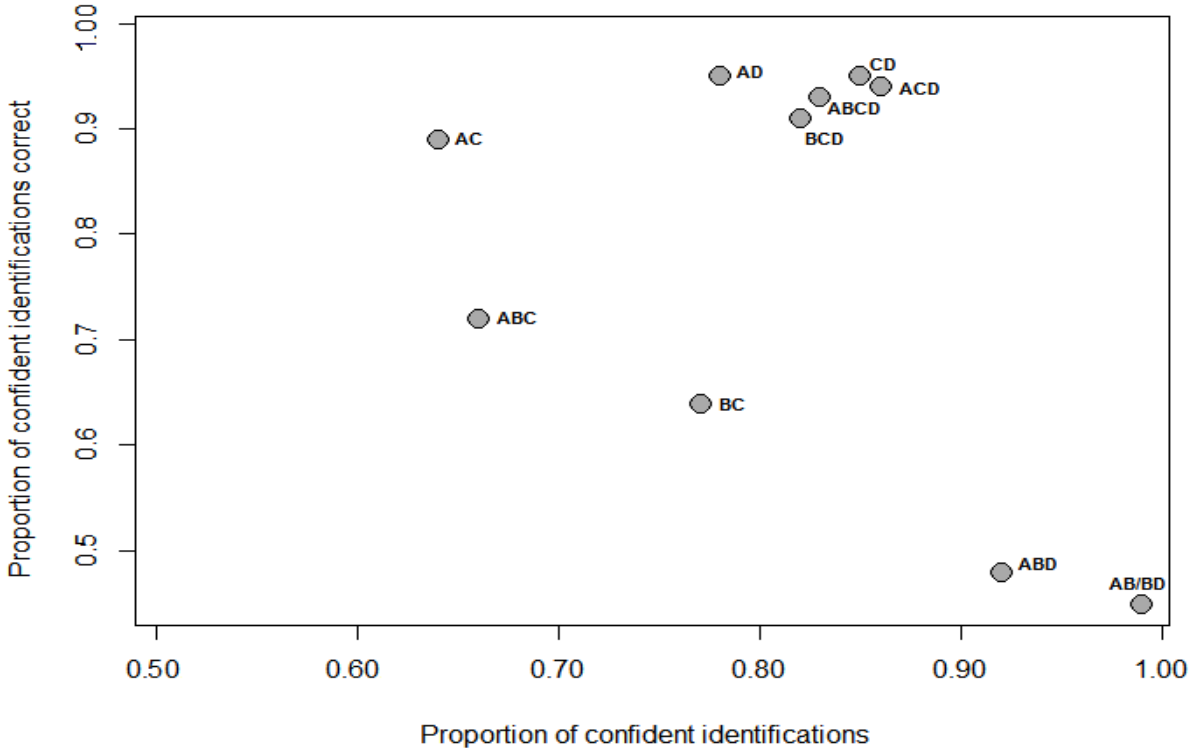


Figure 2. For each LDA model and using the volunteer's data, a plot of the proportion of confident identifications, against those that were confident and correct. LDA models that lie in the furthest top right will provide the highest number of most accurate identifications.

When the volunteers' measurement data are compared to the original measurements, there is a larger mean and variance for measurement B for the latter trial group. This indicates that this measure was responsible for much of the error in the volunteers' data, as the other measurements were similar in mean and variation between trial groups (figure 3 a-d). The final LDA function, based on measurements ACD, is:

$$\mathbf{- 8.417 + (A*-2.132) + (C*0.090) + (D*1.145)}$$

For a footprint to be identified as dormouse, with 95% confidence, this function must exceed 0.89. To be identified as wood mouse with 95% confidence, it should be lower than -0.87. The frequency of ACD model LDA scores from the original data for the two species is shown in figure 4. Using this LDA function and 95% confidence threshold, for the 212 identification attempts by volunteers, 81% were identified correctly, 14% were classified as unknown and 5% were incorrectly identified.

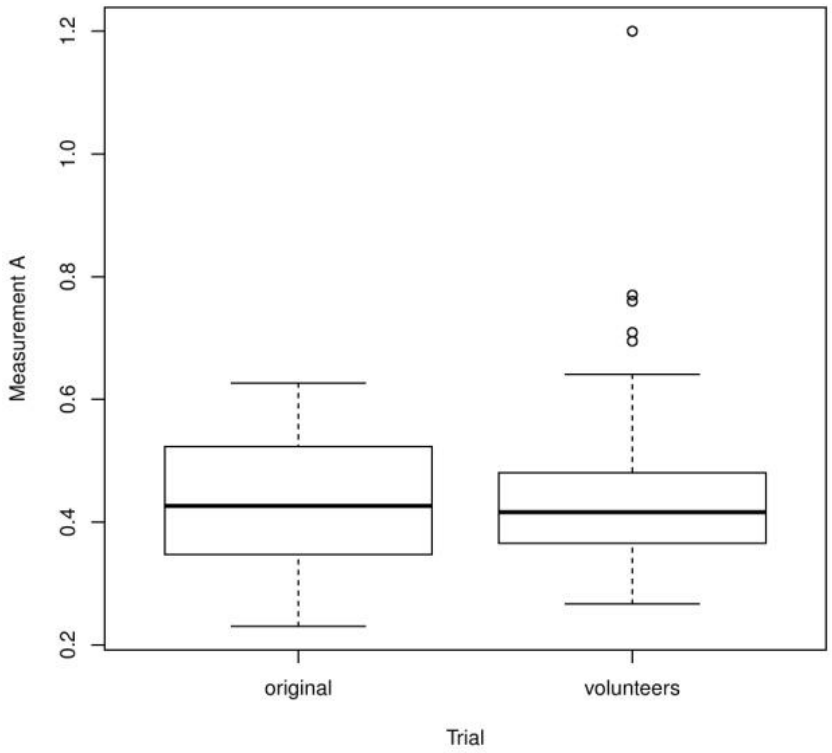
Footprint bait cages

The 306 prints collected from unknown animals using tracking cages, which were identified manually by eye by an experienced small mammal ecologist (Chapter 2) were then subjected to the final LDA algorithm methodology for the measurements ACD. For prints where both techniques made an identification prediction, 99% of the predictions were in agreement. However, the LDA algorithm was more conservative, with 24% of prints being reported as un-identified, whilst manually by an experienced ecologist only 7% were considered unidentifiable.

Repeatability analysis

All three continuous measurements demonstrated highly significant intra-observer correlations (measure A: $X^2 = 31.33$, df = 1, $p<0.01$; measure B: $X^2 = 33.22$, df = 1, $p<0.01$; measure C: $X^2 = 16.19$, df = 1, $p<0.01$; table 3). Variance distribution amongst the effects for all four measurements was predominantly explained by the fixed effect of intra-observer measurements (table y). Measurement A alone had a substantial percentage (23.02%) of variance explained by the inter-observer effect (table 4).

A



B

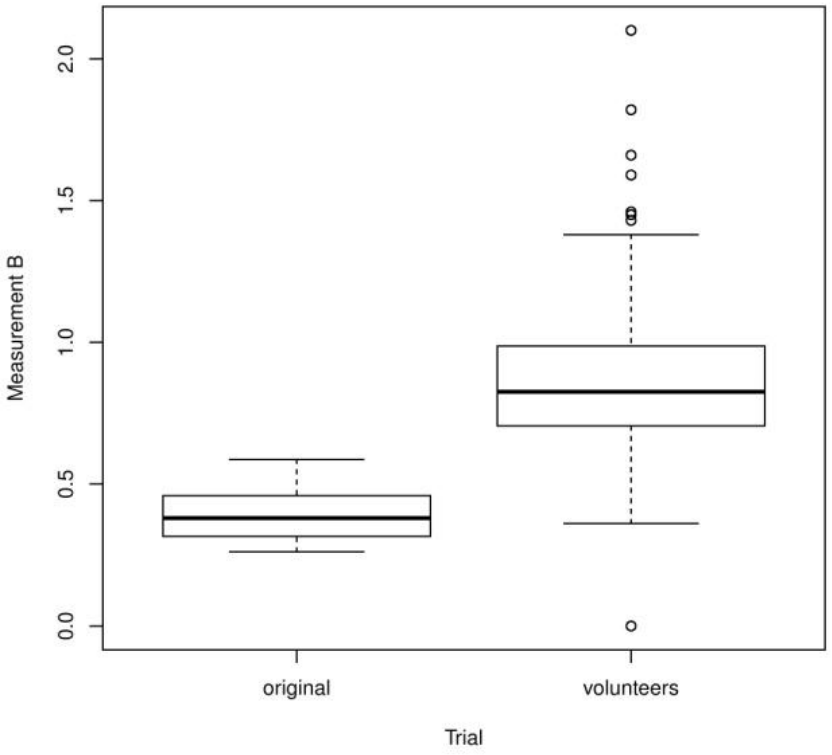
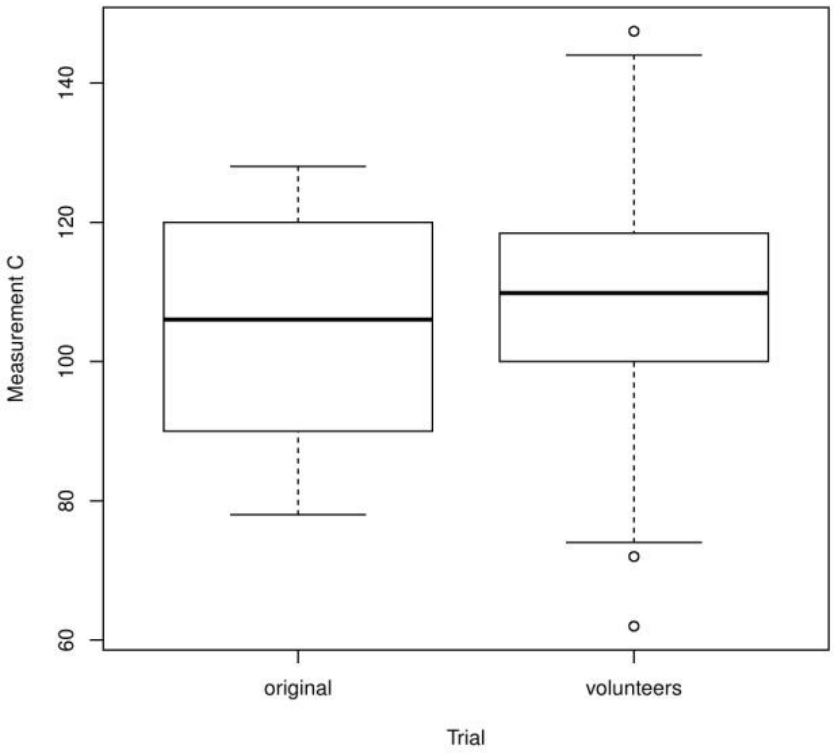


Figure 3a-b. Box and whisker plots of measurement-values taken from footprints, comparing the original measures with those taken by volunteer trial groups, for measurements A to D.

C



D

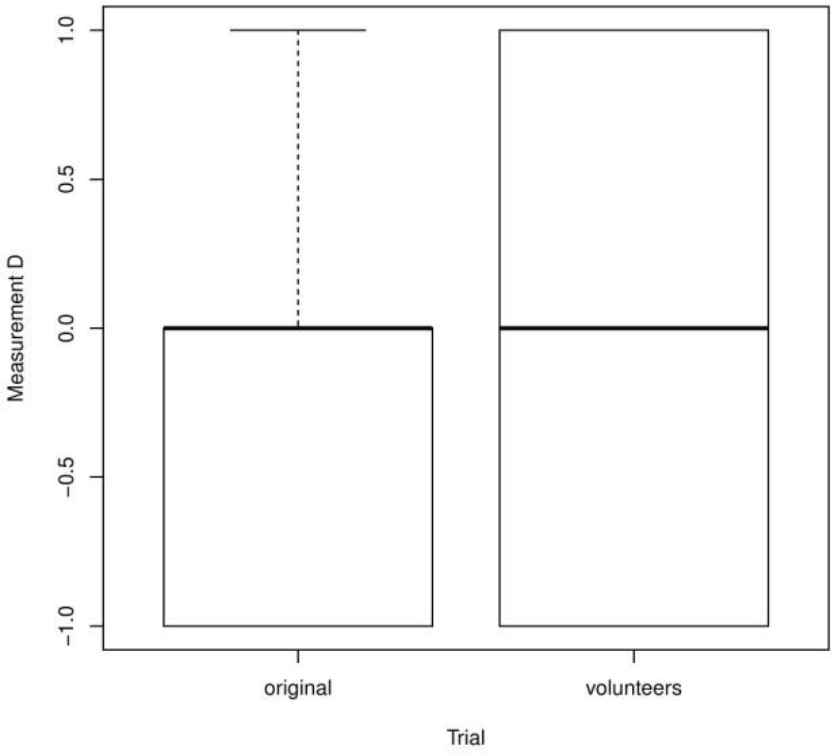


Figure 3c-d. Box and whisker plots of measurement-values taken from footprints, comparing the original measures with those taken by volunteer trial groups, for measurements C to D.

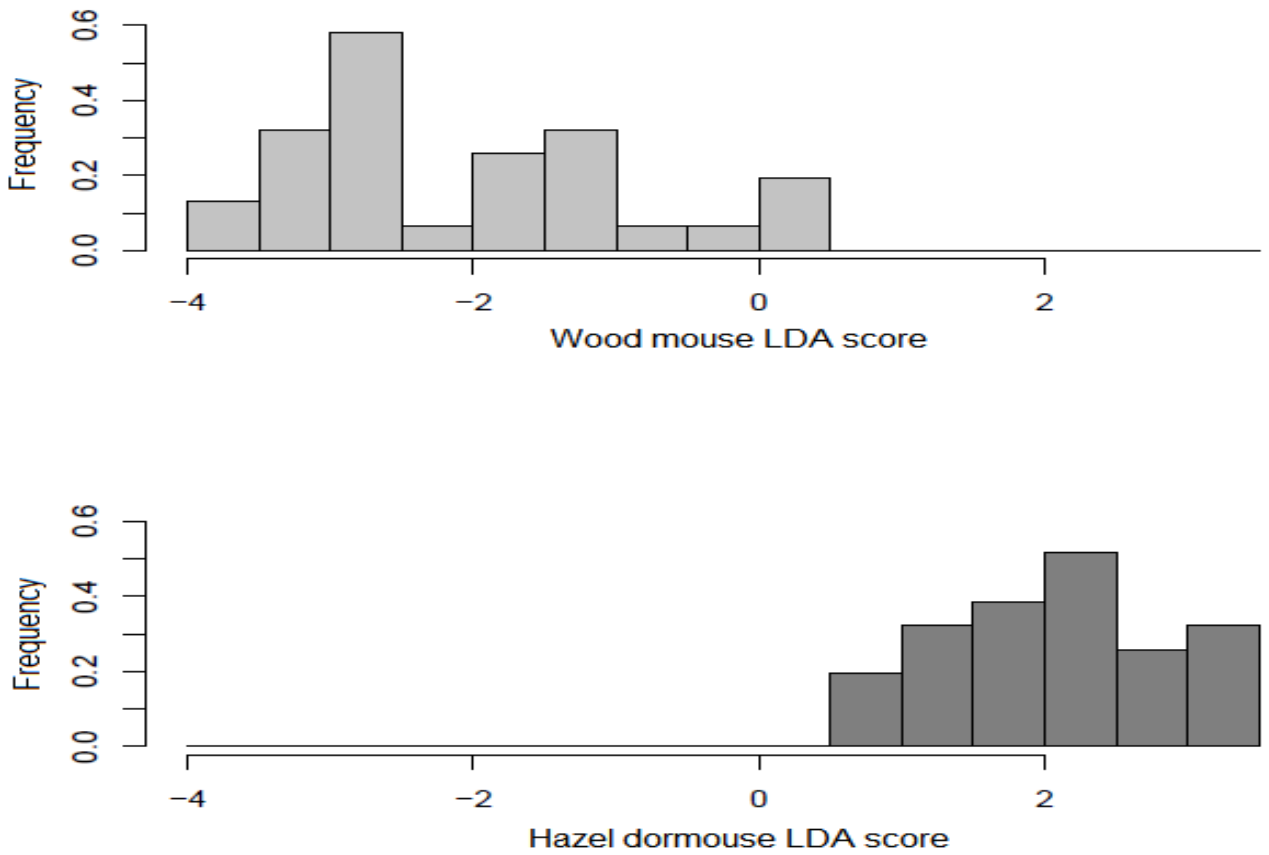


Figure 4. Frequency plots showing the range of LDA scores for wood mice and dormice, using the original measurements for the ACD model.

Table 3. Results of the analyses of correlations between intra-observer repeated measures of eight small mammal footprints, for the measurements A-C.

Measurement	Slope between repeated measures	Measure 1~ Measure 2 Chi2 (df)	Measure 1~ Measure 2 Pr
A	1.01	31.33 (1)	<0.01
B	0.84	33.22 (1)	<0.01
C	0.98	16.19 (1)	<0.01

Table 4. The distribution of variance amongst prints, amongst observers and within observers, for the measurements A-D.

	A		B		C		D	
	Variance	**%**	**Variance**	**%**	**Variance**	**%**	**Variance**	**%**
Print	1.1864e-20	<0.01	0	0	1.6163e-01	1.08	4.0312e-07	<0.01
Inter-Observer	3.8559e-04	23.02	0	0	2.1827e-13	<0.01	1.4269e-07	<0.01
Intra-Observer (Residual)	1.2893e-03	76.98	0.0087	100	1.4829e+01	98.92	4.9999e-02	99.99

Discussion

We have designed and tested a simple and objective technique for non-experts to use for the confident and accurate discrimination between hazel dormouse and wood mouse footprints collected in tracking cages. This methodology proved to be as accurate as manual identification by experts. Due to the allocation of a confidence value it is more conservative and so provides fewer identification attempts. We envisage that the two methods would complement each other, as well as assist new surveyors in gaining experience in how to identify small mammal prints.

The LDA algorithm, ACD, was our chosen model as it had the best compromise between the proportion of confident identifications and correct species allocations of these, as well as having a low false dormouse identification rate. We demonstrated extremely high repeatability for measurements A, C and D within individuals. However, the slope value for measurement B indicates a slightly weaker correlation between individuals repeat measurements. This concurs with the findings that measurement B was responsible for the greatest amount of error, and this is likely due to it being particularly difficult to record consistently. Many of the LDA models were less accurate due to measurement B. From our data the exact cause of this remains unexplained. It may be due to the methodology for this measure being poorly explained to volunteers (see figure 1 and supplementary figure S1). Irrespective of the cause, these inaccuracies support the removal of this measurement. It could be argued that the simpler model CD, which showed similar performance to ACD, may be preferable; however we consider the provision of three measurements more beneficial for cases where a poor quality print negates the possibility of taking one of the measures accurately.

Our footprint identification methodology only allows discrimination between hazel dormice and wood mice. For hazel dormouse-focused surveys this is not of concern, as this species' footprints tend to be relatively characteristic compared to other British small mammals (Chard 1936). However, the inclusion of more sympatric small mammal species would further extend the usefulness of this methodology. Additionally, other variables such as sex and age may be incorporated.

Here we demonstrate that it is relatively straight forward to establish a simple algorithm that can be used for footprint identification and therefore similar methodology could be applied to other research projects for any group of species for which tracking surveys are used. We envisage that the development of these techniques, will lead to an expansion in the use of point sampling of footprints for many species groups.

Acknowledgements

Our thanks go to Paignton Zoo and Julian Chapman for assistance with the collection of dormouse reference footprints, the Cornwall Wildlife Trust for allowing us access to the study sites and all the many volunteers who helped with reference footprint collection and took part in footprint identification trials. C. Mills received funding from the People's Trust for Endangered Species for her research on dormice behaviour and population genetics.

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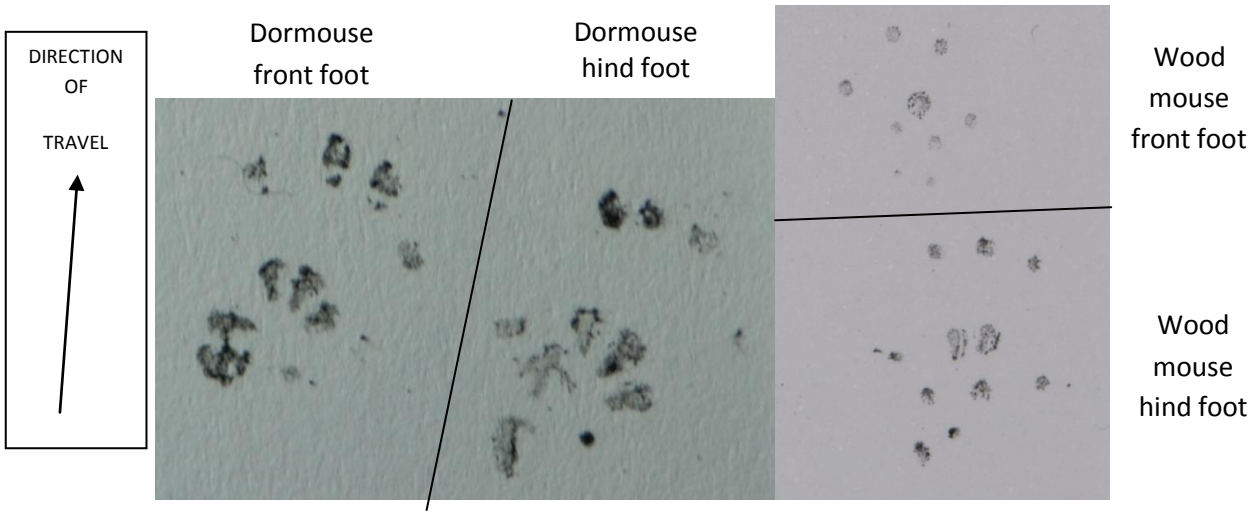
Supplementary Figure S1: A copy of the instructions given to the volunteers who conducted the trial of collecting data for and implementing the LDA algorithm. Note that the equation at the end of this figure is therefore not the final LDA algorithm defined in the results section.

Discriminating between dormouse and wood mouse footprints.

- Hazel dormice are a European protected species and so many ecologists survey for their presence.
- Current methods can take up to 1 - 2 years to complete.
- As part of my PhD, I am investigating alternative, more efficient survey methods.
- This trial involves the collection and identification of small mammal footprints.
- Collection has successfully been achieved by attracting small mammals to food, the access to which requires walking over safe "ink" and then card where they leave footprints.
- I have designed a method to identify the prints, but need you to trial it - to check that it is accurate and easy to use.
- Your results will demonstrate which combination of the four measurements are best
- Please use the following instructions, and don't hesitate to contact me if you have any questions

1. Look carefully at the example prints of dormice and wood mice below and read through the main points. This will give you an introduction to what small mammal prints look like.



Main things to notice:

i. Prints are small - approximately 1 cm in height.

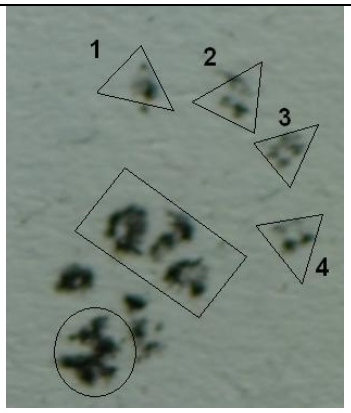
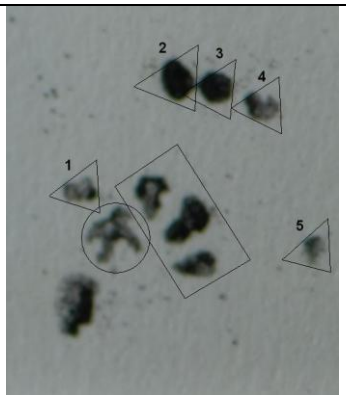
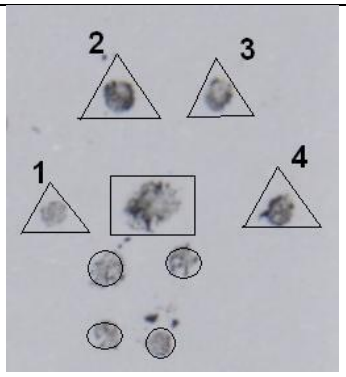
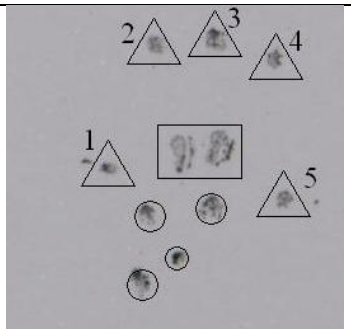
ii. Both species have four toes on their front feet and five toes on hind feet.

iii. Metacarpal pads are the pads just below the digits

iv. Below the metacarpal pads are heel pads.

v. These training prints are "ideal" prints, but other prints may be missing toes or pads and excess ink, slippage and overlapping prints may create unclear foot prints.

2. **The table below highlights the main parts of the footprints and explains the differences between the species and front/hind feet. It is vital that you can visualise the general pattern of small mammal footprints, such as position of toes, metacarpal and heel pads.**

	**Dormouse fore foot** *Toes* Four toes. Toes form a symmetrical, shallow arch. A line drawn between toes 1 and 4 would cross well above the metacarpal pad. *Metacarpal pads* Three triangular/oval pads close together. If excess ink may merge into an oblong. *Heel pads* Irregular concave polygon shapes.
	**Dormouse hind foot** *Toes* Five toes, sometimes four as outer toe often doesn't leave a print. Toes 1 and 5 at an obtuse angle from middle three toes. Angle between toes 1,2 and 5 and 1,3 and 5 generally more than 75 degrees. *Metacarpal pads* Three triangular/oval pads close together. If excess ink may merge into an oblong. *Heel pads* Irregular concave polygon shapes.
	**Wood mouse fore foot** *Toes* Four toes. Toes form a symmetrical pattern. A line drawn between toes 1 and 4 would transect the metacarpal pad. *Metacarpal pads* One circular pad. *Heel pads* One to four small round pads below metacarpal pads.
	**Wood mouse hind foot** *Toes* Five toes, sometimes four as outer toe often doesn't leave a print. Toes 1 and 5 at an obtuse angle from middle toe. Angle between toes 1,2 and 5 and 1,3 and 5 generally less than 75 degrees. *Metacarpal pads* Two oval pads. Can merge into each other if excess ink. *Heel pads* One to four small round pads below metacarpal pads

3. Now you are ready to work through the Footprint identification methodology:

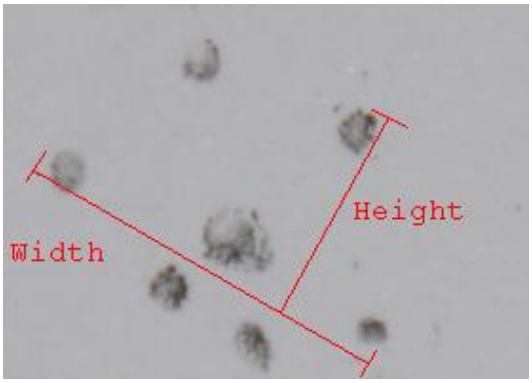
i. Open a footprint jpeg image, which can be found in the folder "Test prints". Open in either Paint or ImageJ. (Can be downloaded for free from: http://rsbweb.nih.gov/ij/). Alternatively, print the image off in black and white.
ii. Be careful to not change the image in any way that stretches or skews the image, the ratio of height:width must stay the same.
iii. If using Paint or print-outs you will need a ruler and protractor to take measurements.
iv. As you go along taking measurements, put your data into the Excel file "YOUR NAME HERE_footprint data". Save regularly, replacing file name with your name.
v. Include the image file name under "Photo ID", so I know which image the measurements relate to.

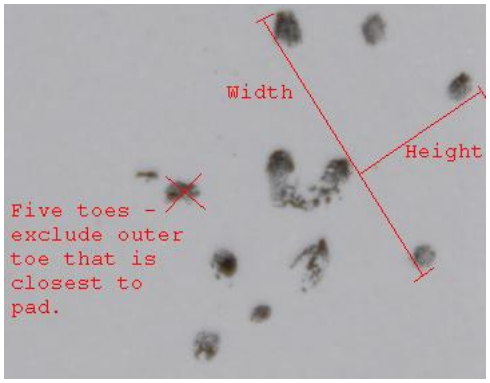
STEP 1: Exclusion of fifth toe.

i. Count the number of toes. If four toes, go to Step 2.
ii. If five toes, look at the toe outer toes, choose the one that is closest to the central pads and cross this one out so that you are left with the other four toes.
iii. The one you crossed out can now be ignored for the rest of the methodology. The reason is the method has been designed to work with four toes only, so it can be used on front and hind feet.

STEP 2: (A) - Height:width ratio.

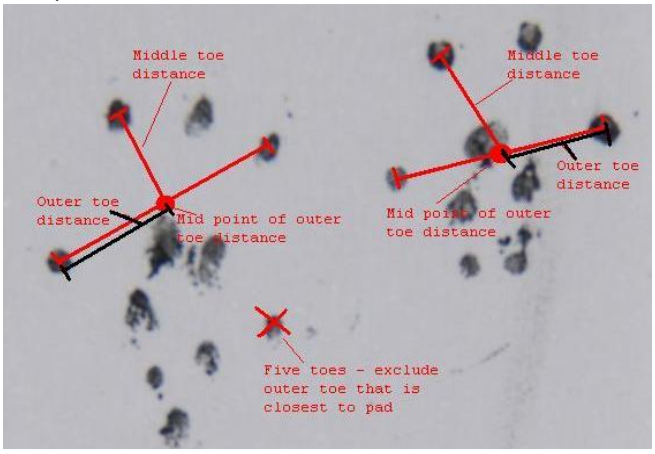
i. Imagine you are drawing the smallest possible box that contains the four toes.
ii. Width of the line just below and between the outer two toes, and that extends to their outer edge.
iii. Identify the middle toe that is furthest from the width line.
iv. Height of the line from the top edge of the middle toe identified in iii, down to and perpendicular to the width line.
v. Ratio= height/width.





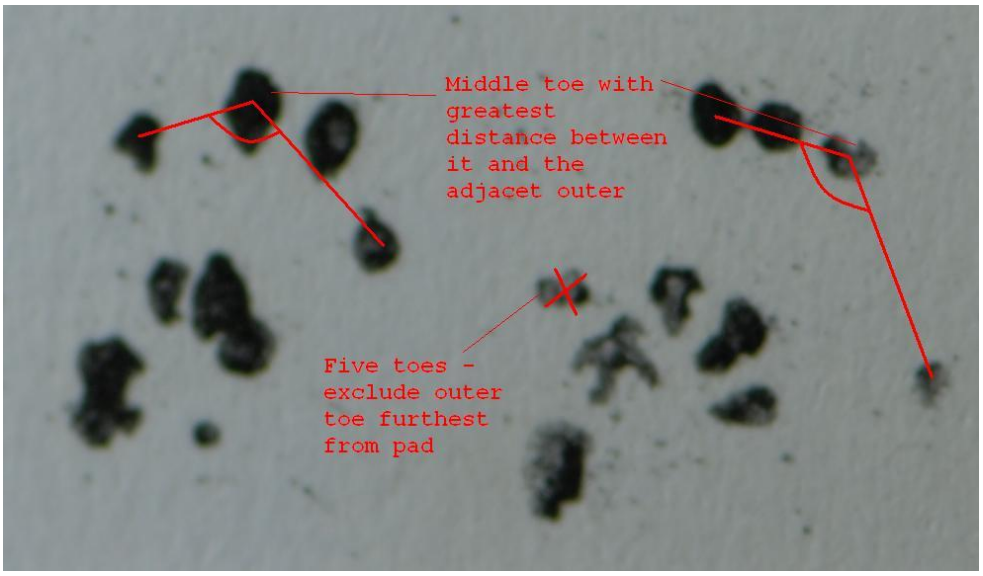
STEP 3: (B) - Middle/outer toes distances ratio.

i. Mark the mid-point distance half way between the outer two toes.
ii. Measure the distance between this mid-point and the centre of one of the outer toes.
iii. Look at the two middle toes, select the one which has the largest distance between it and the adjacent outer toe.
iv. Measure the distance between the mid-point and the centre of the middle toe you selected in iii.
v. Ratio = Middle toe distance/outer toe distance.



STEP 4: (C) – Angle

i. Mark the centre of the two outer toes
ii. Look at the two middle toes, select the one which has the greatest distance between it and the adjacent outer toe.
iii. Mark the centre of the middle toe that you selected in ii.
iv. Measure the angle in degrees between these three toes.



STEP 5: (D) - Metacarpal pad score.

i. Identify the metacarpal pad on the print.
ii. Look at the shape, and then give a score based on:
+1 = Three triangular or oval pads in a straight line and close to each other. If there was excess ink on the animal's feet the pads may merge into one oblong, but three pad impressions are still visible.
0= No metacarpal print present, or too poor quality to be able to score confidently. If excess ink has created a blob.
-1= Either one round central pad (wood mouse fore foot) or two small ovals (wood mouse hind foot).

STEP 6: Calculate the footprint identification score.

i. I established this calculation using prints from known species.
ii. Use Excel to work out the score for each footprint you measured, using the calculation below, where the letters correspond to the four (A-D) measurements above:

Score value = -7.09 + (A*-1.92)+ (B*-1.64) + (C*0.08) + (D*1.13)

iii. Above 0 indicates a dormouse print, below 0 indicates a wood mouse.

STEP 7: Repeat steps 1-7 with a new print. Please try to do as many as you can, doing a mixture of images from set 1 and 2 first, and then if you are really keen images from set 3!

STEP 8: Send me your completed Excel file.

THANK YOU!

Chapter 4: Isolation and characterisation of

hazel dormouse microsatellite loci

Abstract

The hazel dormouse, Muscardinus avellanarius L. *(Rodentia; Gliridae) is considered vulnerable due to habitat loss and fragmentation. As such, research on the species' molecular ecology may provide vital insights, which aid its protection. Integral to these studies is the development of DNA markers, which are essential tools in genetic analyses.*

We isolated hazel dormouse microsatellite sequences from enriched genomic libraries. Fifty-three primer sets were designed from 51 newly isolated loci. Additionally, nine primer sets from published hazel dormouse microsatellite sequences (Md Naim et al. 2009) were redesigned and tested. In total, 61 marker sets were initially tested in eight unrelated individuals.

Thirty-nine polymorphic loci amplified in >85% of samples and therefore were genotyped and characterised in further individuals from one of two single populations. Of these, 30 autosomal loci (22 new and eight published) adhered to Hardy-Weinberg equilibrium and displayed an estimated null allele frequency <0.20. Only one pair of loci displayed linkage disequilibrium after correction for multiple tests.

This marker set will facilitate genetic studies of this protected species, such as population genetics and parentage analysis, and provide information for conservation management decisions.

Hazel dormice, *Muscardinus avellanarius,* are vulnerable to habitat fragmentation as they have low population densities, limited dispersal and low reproductive potential (Bright 1993). Population declines have led to protection by European Directives (Amori *et al*. 2008).

Two new microsatellite-enriched libraries were constructed, from one hazel dormouse (ID79) found dead in Cornwall, England, in 2009. Genomic DNA was extracted from liver tissue using an ammonium acetate precipitation method (Nicholls *et al.* 2000). Libraries were constructed using the approach of Armour *et al*. (1994) and enriched for dinucleotide (library 1) or tetranucleotide microsatellite motifs (library 2) and their complements: $(AC)_n$, $(AG)_n$ or $(GTAA)_n$, $(CTAA)_n$, $(TTTC)_n$ and $(GATA)_n$. Motifs were denatured and bound to magnetic beads following Glenn & Schable (2005). Following enrichment, the dinucelotide- and tetranucleotide-enriched fragments were PCR amplified separately in parallel, three times each to obtain sufficient DNA (c5µg) for next generation sequencing. Each 25µl PCR contained 2.0µl dinucleotide or tetranucleotide-enriched DNA, 1x reaction buffer (Bioline), 25µg/ml BSA, 150µM dNTPs, 0.5µM Sau-L-A linker/primer (Royle *et al.* 1992), 2.0 mM $MgCl_2$ and 1 unit of DNA Taq polymerase (Bioline). The PCR program used was as in Glenn & Schable (2005), but omitting the final 15°C hold. The three dinucleotide and three tetranucleotide PCRs were pooled into a single tube and purified using a QIAquick PCR purification column (Qiagen) and eluted in 40µl to create a concentration of c125 ng/µl. DNA concentration was measured on the Nanodrop 8000 (Thermo Scientific). Pooled PCR-amplified enriched fragments were 454-pyrosequenced (Roche, FLX) at the NERC Biomolecular Analysis Facility at the University of Liverpool. Six different species' enriched libraries were tagged and sequenced together on a quarter of a plate.

Additionally, microsatellite loci previously characterised for *M. avellanarius* (Md Naim *et al*. 2009) were redesigned as amplification failure led to the identification of errors in the published primer sequences. The reverse primer sequences of *MavB5*, *MavG3* and *MavH3* were not present in the corresponding full sequences cited by the authors and two bases were missing from the middle of forward primer of *MavE3*. We redesigned nine of the ten loci to correct these errors and to create a set of markers all within the same melting temperature range. Locus *MavC42* was not redesigned as it proved to be monomorphic in our populations.

We checked for duplication among all microsatellites using BlastN 2.2.4 (Altschul *et al*. 1997). Over 500 new unique hazel dormouse microsatellite sequences were isolated, however, approximately 100 had >20bp of sequence on both sides of the repeat region. Fifty-three primer sets were designed from singleton (single-read) sequences of 51 microsatellite loci using PRIMER3 (Rozen & Skaletsky 2000).

These 53 primer sets, plus the nine redesigned primers, were tested in two wild populations, near Bodmin, Cornwall and Okehampton, Devon, England. Plucked hair samples were taken from dormice residing in nest boxes and stored dry in microcentrifuge tubes at -20°C. Genomic DNA was extracted from hair using a Chelex method (Walsh *et al.* 1991).

Primer sets were assessed for amplification and polymorphism by typing eight unrelated hazel dormice from the Cornwall population. Additional individuals were genotyped for polymorphic loci, totalling 26 unrelated individuals (males *n*=9, females *n*=10, unknown sex *n*=7). Any monomorphic loci in this population were characterised in 22 unrelated individuals from the Devon population (males n=9, females n=12, unknown sex n=1).

Genotyping was performed in 2-µl PCR reactions, containing <10ng of lyophilised genomic DNA, 0.2 µM of each primer and 1 µl QIAGEN multiplex PCR mix (QIAGEN Inc.; Kenta *et al*. 2008). PCR amplification was performed using a DNA Engine Tetrad PTC-225 thermal cycler (MJ Research, Bio-Rad, Hemel Hempstead, Herts., UK) with the following touch-down program: 95°C for 15 minutes; followed by 13 cycles of 95°C for 30 seconds, primer annealing for 30 seconds (decreasing by 1°C every cycle from 67°C to 55°) and 72°C for 45 seconds; then 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds and, 72°C for 45 seconds, and a final elongation at 60°C for 10 minutes. Amplified products were loaded on an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, California, USA) and GENEMAPPER v3.7 (Applied Biosystems, California, USA) was used to assign allele sizes. Observed and expected heterozygosities, and estimated null allele frequencies were calculated using CERVUS v3.0.3 (Kalinowski *et al*. 2007). Tests for departures from Hardy-Weinberg equilibrium and assessment of linkage disequilibrium were conducted in GENEPOP v4.0.10 (Raymond & Rousset 1995, Rousset 2008). The False Discovery Rate correction for

multiple tests (Verhoeven *et al*. 2005) was applied to *p*-values obtained from Hardy-Weinberg equilibrium and linkage disequilibrium tests.

Of the 59 loci tested in eight individuals, 18 amplified in less than 85% of the samples and three were monomorphic in both populations. Of the remaining 39 loci (31 new plus eight redesigned), all except *Mav034* were polymorphic in the Cornish individuals and therefore characterised in 26 individuals from this population. Locus *Mav034* was polymorphic in the Devon population and characterised in this population instead.

All 39 polymorphic loci were confirmed to be autosomal, based on their successful amplification and presence of heterozygotes in both sexes. In the Cornwall population, the number of alleles per locus ranged between two and nine (mean=3.34, SD=1.63), and the mean observed and expected heterozygosities were 0.42 (SD=0.23) and 0.44 (SD= 0.21), respectively (table 1). Nine loci deviated from Hardy-Weinberg equilibrium, four of which also showed a high estimated null allele frequency (>0.2, Table 1). Twenty-six loci pairs displayed linkage disequilibrium. This may be due to the inclusion of relatives: whilst known parents/offspring/siblings were not included, related individuals may have been included as samples were taken from nest boxes, which are distributed over a relatively small area. However, only the locus pair *Mav017/Mav042* showed evidence of linkage disequilibrium in the Cornwall population after FDR correction.

These loci will enable parentage and population analyses for the hazel dormouse, Gliridae and related species and ultimately aid conservation.

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Table 1 Characterization of 39 hazel dormouse (*Muscardinus avellanarius*) microsatellite loci.

Locus	EMBL accession no.	Repeat motif	Primer sequence (5'-3') and fluro label	Tm (C)	Exp. allele size (bp)₸	Observed allele size range (bp)	Pop	n	A	H_O	H_E	P_{HWE}	Estimated null allele frequency
Mav002	HE819184	$GA_{(15)}$	F:[HEX]CTACCATGTGCTTGGCTGAG	59.5	108	103–111	COR	26	6	0.5	0.56	0.01	0.05
			R:CAGCCTGAACCACTCCAAG	59.4									
Mav003	HE819185	$GTT_{(16)}$	F:[HEX]TTCTCAATTGCCTTCAGCTC	58.2	226	228–231	COR	26	2	0.42	0.38	1.00	-0.06
			R:TTAGTGAGGCCTTCTGCAAC	58.1									
Mav005	HE819187	$CTTT_{(12)}$ $CCTT_{(1)}$ $CTTT_{(1)}$ $CTT_{(1)}$ $CTTT_{(3)}$	F:[6-FAM]AGGCATATGGCAGCAGAGC	61.5	314	318–342	COR	25	7	0.72	0.76	0.35	0.02
			R:TTCGTGGACAGCCTCAGC	61.2									
Mav009	HE819190	$CA_{(14)}$	F:[6-FAM]GGCTGGTATGTAGCTCAGTGG	59.8	167	162–164	COR	26	2	0	0.145	**<0.01**	**0.85**
			R:GACCAGCCATTGACACCTG	60.1									
Mav011	HE819192	$CTTT_{(2)}$ $CT_{(2)}$ $CTTT_{(2)}$ $CT_{(2)}$ $CTTT_{(13)}$	F:[HEX]CCCGAGTACTGGGATTACAGG	60.7	184	186–214	COR	26	5	0.50	0.55	0.19	0.04
			R:AAGGCTGAGGGTTTAGTTCAGAG	60.3									
Mav015	HE819196	$GT_{(5)}$ $GC_{(3)}$ $AC_{(2)}$ $GT_{(12)}$	F:[HEX]ACACCAGCCTCTGCAACTTAG	59.6	248	244–250	COR	25	3	0.52	0.54	0.61	0.01
			R:GAGAATGGCCTCTGACGAAG	60.0									
Mav017	HE819198	$CTTT_{(2)}$ $CT_{(1)}$ $CCTT_{(2)}$ $CTTT_{(1)}$ $T_{(1)}$ $CTTT_{(10)}$ $CTT_{(2)}$ $C_{(1)}$ $CTT_{(2)}$ $GT_{(1)}$ $CTTT_{(3)}$	F:[6-FAM]ACCATAAGCGAAGGGTGAG	57.8	246	244–260	COR	26	4	0.54	0.72	0.05	**0.13**
			R:TCTCCGTCTTGCTTTACAGG	58.1									
Mav020	HE819201	$CTTT_{(12)}$	F:[6-FAM]TCAAGCCCTGGGATTACAAG	60.1	281	285–293	COR	25	4	0.52	0.54	0.03	0.02
			R:ATAGCCCGGAGGTAGAAAGC	59.7									
Mav021	HE819202	$CTTT_{(1)}$ $TT_{(1)}$ $CTTT_{(13)}$ $CTT_{(1)}$ $CTTT_{(1)}$ $CCTT_{(1)}$ $CTTT_{(3)}$	F:[HEX]AACTTGCTAGGCCAGACCAC	59.4	168	162–174	COR	26	4	0.58	0.65	0.64	0.06
			R:CCTGAGCAACTTAGCAAGTCC	59.1									

Locus	EMBL accession no.	Repeat motif	Primer sequence (5'-3') and fluro label	Tm (C)	Exp. allele size (bp)₮	Observed allele size range (bp)	Pop	n	A	H_O	H_E	P_{HWE}	Estimated null allele frequency
Mav023	HE819204	$\underline{GT}_{(18)}$ $GC_{(1)}$ $GT_{(3)}$	F:[HEX]GGGAGTATAGCCCGGAGGT	60.3	149	145–162	COR	26	9	0.65	0.77	0.09	0.06
			R:GGCCCTACTTCAAACACATGA	60.0									
Mav024	HE819205	$\underline{CA}_{(12)}$	F:[HEX]GGGTGCAGCTGTGAGGTAG	59.4	128	117–123	COR	25	4	0.6	0.57	0.03	-0.06
			R:ACCGGGTGATGAAAATTGAG	59.8									
Mav026	HE819207	$GT_{(6)}$ $GC_{(1)}$ $GT_{(1)}$ $GC_{(1)}$ $\underline{GT}_{(11)}$	F:[6-FAM]AGCTTCAGCACCTTAATCCAC	58.5	242	244–246	COR	25	2	0	0.08	0.02	**0.67**
			R:GCAAGTGAGCTGAGGAAGG	58.7									
Mav027	HE819208	$\underline{CA}_{(15)}$	F:[6-FAM]CACGACCATCCCAACCTC	59.9	146	137–141	COR	26	3	0.04	0.11	0.02	**0.44**
			R:GGGCGATATATGTCTCAGGTG	59.4									
Mav028	HE819209	$CT_{(4)}$ $\underline{CTTT}_{(13)}$	F:[HEX]CCTGCTCTGGCTGTAGGC	59.7	215	213–245	COR	26	6	0.77	0.76	0.03	-0.02
			R:GGAGGTAGAAAGCCCTGGAC	60.1									
Mav030	HE819211	$\underline{CTTT}_{(16)}$	F:[6-FAM]GGGAACCATCCAACCTATTG	59.1	157	138–157	COR	26	2	0.54	0.48	0.69	-0.06
			R:AAATGTCTCTGGGTTCCATACC	59.2									
Mav032	HE819213	$GATA_{(2)}$ $AATA_{(1)}$ $GATA_{(1)}$ $CATA_{(1)}$ $GAT_{(1)}$ $GCAA_{(1)}$ $\underline{GATA}_{(9)}$	F:[6-FAM]AGGGTTTCCCACAGAAACAG	59.0	147	145–149	COR	26	2	0.42	0.47	0.68	0.05
			R:GAATCTATAACCAGTTAGCAAATCTCC	59.1									
Mav033	HE819214	$CA_{(4)}$ $CG_{(5)}$ $G_{(1)}$ $\underline{CA}_{(14)}$	F:[HEX]GTGAAGCCCAGAGACTTTGC	60.0	230	221–223	COR	26	2	0.15	0.15	1.00	-0.03
			R:AGGGAGAATTGTCTCCTTAGTGG	60.0									
Mav034	HE819215	$\underline{GT}_{(15)}$	F:[6-FAM]ATGTACACAACGCGGAAGTG	59.6	236	239–241	DEV	22	2	0.08	0.08	1.00	-0.01
			R:GGCAGCTCAGAAGTAGAATGC	59.2									
Mav036	HE819217	$CG_{(5)}$ $\underline{CA}_{(15)}$	F:[HEX]GGTTCTGTGAAAGCCTGAGC	60.0	209	203–207	COR	24	3	0.67	0.51	0.13	-0.18
			R:TGGTCAGAAGCTGTCAATGC	60.0									
Mav038	HE819219	$\underline{GT}_{(18)}$ $GC_{(2)}$ $GT_{(7)}$	F:[HEX]TCTTGTCTCAGCTTCCCAAGT	59.1	267	263–267	COR	26	2	0.27	0.34	0.29	**0.11**
			R:AGCACCTCAGAGGGAGTTGT	58.9									

Locus	EMBL accession no.	Repeat motif	Primer sequence (5'-3') and fluro label	Tm (C)	Exp. allele size (bp)₮	Observed allele size range (bp)	Pop	n	A	H_O	H_E	P_{HWE}	Estimated null allele frequency
Mav039	HE819220	$GATA_{(2)}$ $GAT_{(1)}$ $GATA_{(5)}$ $GAT_{(1)}$ $GATA_{(2)}$ $GAT_{(1)}$	F:[HEX]AATTTACCGAGGCCCACAG	59.9	174	174–178	COR	26	2	0.69	0.48	0.04	-0.19
			R:TTGAACCAAGACATTCTACCTCTG	59.7									
Mav040	HE819221	$GTAAT_{(4)}$ $\underline{GT}_{(13)}$ $GC_{(4)}$	F:[6-FAM]GTGCTGAGTGGTGGAGTGAG	59.4	183	176–178	COR	26	2	0.31	0.32	1.00	0.00
			R:TTTATAGCTAGAATCATGCTGTCTTTG	59.5									
Mav042	HE819223	$\underline{CTTT}_{(11)}$	F:[HEX]TCGCAGCAACATAGCAAGAC	60.2	234	231–239	COR	26	3	0.42	0.6	0.11	**0.18**
Mav043	HE819224	$CTTT_{(1)}$ $CTT_{(1)}$ $\underline{CTTT}_{(11)}$ $TTT_{(1)}$ $CTTT_{(1)}$	F:[6-FAM]GGCTCTGGTCTCTGTCACTATG	59.0	165	164–180	COR	26	5	0.50	0.58	0.16	0.08
			R:AAGTAGGACCAGGAGGTAGAAGG	59.2									
			R:GAGAAACGCGATTCATGGAC	60.6									
Mav044	HE819225	$C_{(6)}$ $T_{(6)}$ $\underline{CTTT}_{(15)}$	F:[HEX]CAGCACCCACAGCCTCAT	60.9	142	134–142	COR	26	3	0.62	0.56	0.31	-0.06
			R:GGCAGGCTACTGCTGCAC	60.7									
Mav047	HE819228	$CA_{(5)}$ $A_{(3)}$ $\underline{CA}_{(10)}$	F:[HEX]ACTGGGGTGTAGCTCAGAGG	59.3	133	133–135	COR	25	2	0.08	0.08	1.00	-0.01
			R:AATTTTACACAGTATGGGGACTGTT	59.2									
Mav048	HE819229	$\underline{CTTT}_{(14)}$ $TCTTC_{(1)}$ $CTTT_{(1)}$ $CTTC_{(1)}$ $CTTT_{(1)}$ $T_{(1)}$	F:[HEX[CACAATCCTCCTGTGTCAGC	59.3	221	222–230	COR	26	3	0.54	0.62	0.41	0.07
			R:AGGACTCTCCATGCCAAGAG	59.4									
Mav049	HE819230	$\underline{CTAT}_{(11)}$ $CT_{(1)}$ $GT_{(1)}$ $CAT_{(1)}$ $CTAT_{(3)}$	F:[6-FAM]GGGTTTGCAAAGAACCCAAT	61.1	212	208–212	COR	26	2	0.12	0.18	0.19	**0.20**
			R:CCTGGGTTCCGTCCCTAGTA	61.2									
Mav050	HE819231	$\underline{GATA}_{(9)}$	F:[6-FAM]CCAAGCACTCAGCTCTCTTG	58.9	281	279–288	COR	26	3	0.23	0.43	0.01	**0.31**
			R:TCCATAGAAACTAGGTAATTTGAGTGA	58.8									
Mav051	HE819232	$\underline{CA}_{(18)}$	F:[6-FAM]AGTGGTGGCATGTACCTGTG	59.5	235	233–245	COR	26	4	0.69	0.59	0.59	-0.09
			R:ACGATTATTCTGCCACTGAGC	59.4									

Locus	EMBL accession no.	Repeat motif	Primer sequence (5'-3') and fluro label	Tm (C)	Exp. allele size (bp)₮	Observed allele size range (bp)	Pop	n	A	H_O	H_E	P_{HWE}	Estimated null allele frequency
Mav053	HE819234	$CA_{(3)}$ $GA_{(1)}$ $CA_{(3)}$ $GA_{(1)}$ $CA_{(2)}$ $GA_{(1)}$ $CA_{(1)}$ $CG_{(1)}$ $\underline{CA_{(17)}}$	F:[6-FAM]GGCCTTCTATAACTTAGCAAGAACC	60.1	224	219–221	COR	26	2	0.65	0.49	0.12	-0.15
			R:ACGGACGAGTGAGCTGTACC	60.3									
MavSB5*	GF089516	$\underline{CA_{(40)}}$ $C_{(1)}$ $CA_{(2)}$	F:[HEX]GTAGAATGCTTCGAGTTCAATTTC	58.5	145	97–107	COR	24	5	0.625	0.586	0.1573	-0.0475
			R: TGGAGAAGGGTACTAGGATGC	58.3									
MavSE3*	GF089515	$\underline{CA_{(49)}}$	F:[HEX]TGGGAGTATAGCCCAGAGGTAG	59.6	211	151–155	COR	26	3	0.077	0.076	1	-0.0105
			R:CAGGAGTTAGCATCCCGTTC	59.7									
MavSF10*	GF089509	$\underline{CA_{(43)}}$	F: [6-FAM]GCTGAGGGTATAACTTGGAGGTAG	59.6	266	224–230	COR	26	3	0.538	0.594	0.725	0.045
			R:GTCTGAAATGGCTGGTATTGC	59.6									
MavSF12*	GF089514	$GT_{(16)}$ $GG_{(1)}$ $GA_{(20)}$ $GT_{(17)}$ $\underline{GA_{(23)}}$	F: [HEX]CAGGCAAGGAGTTGGATG	57.7	300	224–231	COR	24	4	0.417	0.422	0.0954	-0.0349
			R:TTGAAAGGCAGGAGAATCC	57.8									
MavSG3*	GF089517	$\underline{GT_{(54)}}$	F: [6-FAM]TGTTGACTGATTGAGTGGTGAC	58.6	195	131–159	COR	25	4	0.24	0.255	0.2249	0.0644
			R:GGCTGAAGATGTAGCTCATAGG	58.1									
MavSG6*	GF089511	$\underline{GT_{(38)}}$	F: [HEX]AGCCCTTCACTTTCCCTGTATC	60.8	194	175–177	COR	26	2	0.538	0.401	0.134	-0.1552
			R:TCCTCAGCAACTTAGCAAGACC	60.9									
MavSG9*	GF089510	$\underline{GT_{(34)}}$	F: [6-FAM]AGCCACATCCCAACTACTGG	60	208	192–194	COR	26	2	0.5	0.509	1	-0.0007
			R:AGTGCTGCTGGGACAACC	59.8									
MavSH3*	GF089518	$\underline{GT_{(29)}}$ $CNT_{(1)}$ $GT_{(11)}$	F: [HEX]CTGGGAGTGTAGCTTGAAGG	57.6	246	205–209	COR	25	2	0.16	0.15	1	-0.0338
			R:CAAGGATAGGGATACACCTCAG	57.7									

* denotes newly designed primer sets for the microsatellite loci published by Md Naim *et al*. (2009). Tm, primer melting temperature (given by PRIMER3). ₮, expected allele size based on the sequenced allele isolated from the Cornish hazel dormouse sample used to create the genomic library. Pop, UK populations tested - Cornwall, (COR) and Devon (DEV). n, number of amplifying hazel dormouse samples from a total of 26 (Cornwall) and 22 (Devon) samples tested. A, number of alleles observed. H_O, observed heterozygosity. H_E, expected heterozygosity. P_{HWE}, *p*-value for testing Hardy-Weinberg equilibrium (HWE) and significant values after FDR correction are shown as underlined and bold. Estimated null allele frequency >0.10 is shown as underlined and bold.

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Chapter 5: Dormice on the edge: population genetics of hazel dormice on the southwest peninsula of England

Abstract

Variation in measures of genetic diversity and differentiation across a species' range will be influenced by many ecological and evolutionary factors. The central-periphery hypothesis predicts that edge-of-range populations will have reduced abundances and increased isolation, compared to populations within the range core, due to sub-optimal resources and/or conditions. Dispersal barriers may also isolate populations through a reduction in gene flow. Small, isolated populations are vulnerable to increased genetic drift and inbreeding, leading to lower levels of genetic diversity, population fitness and adaptive potential, as well increased vulnerability to stochastic events. Such effects threaten population persistence, and therefore understanding their mechanistic basis is of utmost importance for conservation management. Here, we describe the genetic diversity, differentiation and structuring amongst populations of the protected hazel dormouse, Muscardinus avellanarius*, from the core to periphery of their range in southwest England.*

Our results reveal moderate to very high genetic differentiation, with three defined regional populations at the highest hierarchical level and further sub-structuring within these populations, which corresponds to geographical location. We then test the effect of region and a continuous core to periphery longitudinal distribution, on two parameters of genetic diversity (frequency of private alleles and allelic richness), genetic differentiation (F_{ST}), isolation by distance and inbreeding (F_{IS} coefficient).We find strong evidence for reduced diversity, increased differentiation and stronger isolation by distance at the edge of the species range, but the pattern tends to be an abrupt change at the regional boundary rather than a longitudinal cline. This implies that these populations at the edge of their range are smaller in effective population size with low gene flow between them. However, there is no significant evidence for higher levels of inbreeding in the peripheral populations or for population genetic bottlenecks.

We discuss the implications of our findings, which are two-fold: first, if limited national funds for dormouse conservation must be allocated to populations of highest genetic diversity, priority should be given to maintaining core populations, such as those in Devon; second, if the preservation of genetic diversity across the species is considered of importance, the more isolated and differentiated populations, such as those in Cornwall, should be conserved.

Introduction

Increasingly, the role of population genetics research is being embraced in order to infer gene flow, genetic diversity and population structure for species of conservation concern (Broquet & Petit 2009, Haig 1998). Conservation genetics facilitates the management of species, through identification of populations with high levels of extinction risk, informing reintroduction and captive breeding programs, describing gene flow patterns that enlighten habitat management and defining genetically distinct conservation units (Frankham 2005, Frankham *et al*. 2002, Paetkau 1999, Petit *et al*. 1998).

Small, isolated populations, such as those of species vulnerable to habitat degradation, loss and fragmentation, will exhibit lower gene flow between them than larger, well connected populations (Slatkin 1987). Through genetic drift and the allee effect, this will lead to reduced genetic diversity and a lower effective population size (Frankham 1996, Stephens *et al*. 1999). These populations will be at risk of inbreeding depression and mutation accumulation, and have reduced evolutionary potential to adapt to environmental change, culminating in an elevated risk of extinction (Charlesworth & Charlesworth 1999, Frankham 1996, Frankham 2005, Lande 1995, Saccheri *et al.* 1998,). Descriptions of patterns in population genetic diversity, differentiation and structure for species of conservation concern are therefore critical (Lawton 1993).

Geographical and temporal variation in ecological and evolutionary parameters will influence population size and predispose certain populations across a species' range to a higher risk of extirpation than others (Diniz-Filho *et al*. 2009, Gotelli &

Simberloff 1987, Gulve 1994). For example, the central-periphery hypothesis states that peripheral populations should have reduced abundances and be more patchily distributed than those at the core, due to sub-optimal habitat at range edges (Brown 1984, Eckert *et al.* 2008). This may lead to reduced gene flow between peripheral populations, and increased genetic differentiation, reduced genetic diversity, smaller effective population sizes, increased risk of inbreeding and subsequently a higher risk of extinction compared to populations within the core (Diniz-Filho *et al*. 2009, Lawton 1993, Vucetich & Waite 2003, but see Channell & Lomolino 2000). Evidence for this hypothesis has been provided for a variety of plant and animal taxa. However, findings have been somewhat inconsistent: in a review of studies on genetic variation across geographical range, only 64.2% identified the predicted pattern (Eckert *et al.* 2008). These mechanisms are also fundamental to an important problem in evolutionary biology: why do species not adapt to local conditions and therefore expand their range margins? (Eckert *et al.* 2008).

In combination with position on the core-to-periphery continuum, habitat configuration and landscape features will affect the ability of species to disperse between populations (Fahrig & Merriam 1985, Quemere *et al*. 2010). Such dispersal is necessary in order to maintain gene flow and re-colonise extirpated habitat patches. Further, anthropogenically-driven habitat loss, degradation and fragmentation, considered the greatest modern threats to biodiversity, may lead to the reduction of population abundance, occupancy and connectivity (Dirzo & Raven 2003, Prugh *et al*. 2008). In order to make informed conservation decisions it is essential to discover which mechanisms are driving population genetic parameters, and identify populations at highest risk of extinction. It is also important to consider geographical variation, as management actions may have different effects, and hence outcomes, at different locations across a species' range (Whittingham *et al*. 2007).

The hazel dormouse, *Muscardinus avellanarius,* is a species of conservation concern due to population declines that have occurred across much of its northern range (Amori *et al*. 2008). Dormice are thought to be particularly vulnerable to habitat fragmentation as they are arboreal habitat specialists (primarily found in woodland, scrub and hedgerows), with low population densities, reproductive potential, and dispersal ability (Bright 1993, Bright & Morris 1996). As such, they are an ideal model

species for the investigation of genetic diversity and structuring (Mortelliti *et al*. 2009). There is an urgent need to establish a baseline measure of population genetic structuring of hazel dormice and determine whether measures of genetic diversity and differentiation are consistent amongst core and peripheral populations. This will assist conservation status assessment and allow future studies to gauge the loss of genetic diversity in response to further habitat fragmentation and climate change (Allendorf & Luikart 2007).

A previous study of population genetics of hazel dormice has shown substantial genetic structuring between populations separated at the landscape scale, i.e. located 15km apart (Md. Naim *et al*. 2012). However, we are not aware of any studies investigating the population genetics of dormice over larger regional scales. We hypothesise that dormice are likely to be particularly vulnerable to edge-of-range effects, due to their specialist habitat requirements and propensity to form small, isolated populations, resulting in sensitivity to clines in environmental and habitat suitability. Dormouse reproductive success is influenced by stochastic climatic effects (Bright & Morris 1996), which may lead to reduced effective population sizes and hence reduced genetic diversity, especially in peripheral populations. We aim to test this hypothesis by comparing genetic diversity, differentiation, and inbreeding along a sequence of populations that extend down a peninsula, from a dormouse stronghold in the east to the westerly edge-of-range for this species. We predict that hazel dormouse populations on the periphery will have relatively higher differentiation and lower genetic diversity and therefore a higher susceptibility to inbreeding, compared to core populations (Lesica & Allendorf 1995).

In addition to the theory of a cline in genetic parameters correlating with reduced habitat suitability towards the edge-of-range, we predict that landscape features will also isolate populations, influence evolutionary mechanisms and consequently affect patterns of population genetics. Due to the poor dispersal ability of dormice, we expect the species' genetic structure to be impacted upon by landscape features that confer significant dispersal barriers. Therefore, we aim to compare genetic diversity, differentiation and inbreeding between regions (assessed via Bayesian clustering analyses), and assess correlations with major landscape features, with the aim of explaining identified patterns in population genetics.

To this end, our study has three aims. First, we will test for and describe population genetic structuring and isolation by distance patterns (IBD) across the study area. Second, we quantify population genetic diversity, differentiation and inbreeding amongst populations of hazel dormice across their southwest range in England. Third, we relate these results to the central-periphery hypothesis and major landscape features, in order to identify the relative importance of these mechanisms on patterns of population genetics within this species.

Methods

Study area

The distribution of hazel dormice within Britain is predominantly in southern England and Wales (figure 1, NBN Gateway 2012). Our study was conducted throughout the southwest range of dormice in England. This area forms a peninsular of approximately 11,400sq km, comprising of the counties (from west to east) Cornwall, Devon and Somerset (figure 2). Despite many presence-absence surveys, there are few reliable dormouse records at the western tip of this peninsula (Bright *et al*. 2006). Further, a survey conducted by us in 2008 using the established nest-tube method of Bright *et al*. (2006), failed to detect any dormice in 20 woodland/hedgerow sites across mid and west Cornwall (see figure 2 for location of survey sites). Therefore, our study area extends from a core area in Somerset and Devon down to the edge-of-range area for this species, namely the peninsula tip in west Cornwall.

The study area is a rural region encompassing a heterogeneous landscape of fragmented woodland and scrub habitat within a matrix of agricultural and urban land use. Many of the woodland patches are connected to varying degrees by hedgerows. Three moorland areas, with little woodland vegetation suitable for dormice are found in the study area. Many rivers dissect the landscape forming potential physical barriers to dormouse dispersal: most significantly, the large river Tamar runs north-south along the Cornwall and Devon border and is at its widest in the south, where it forms an estuary. See figure 3 for location of moorland and deciduous woodland in relation to sampled populations.

Figure 1. Distribution map of the records of hazel dormice within Britain, at a 2km-square resolution, from 1960 to 2012. (Downloaded from NBN Gateway 1st June 2012). The box illustrates our study area location.

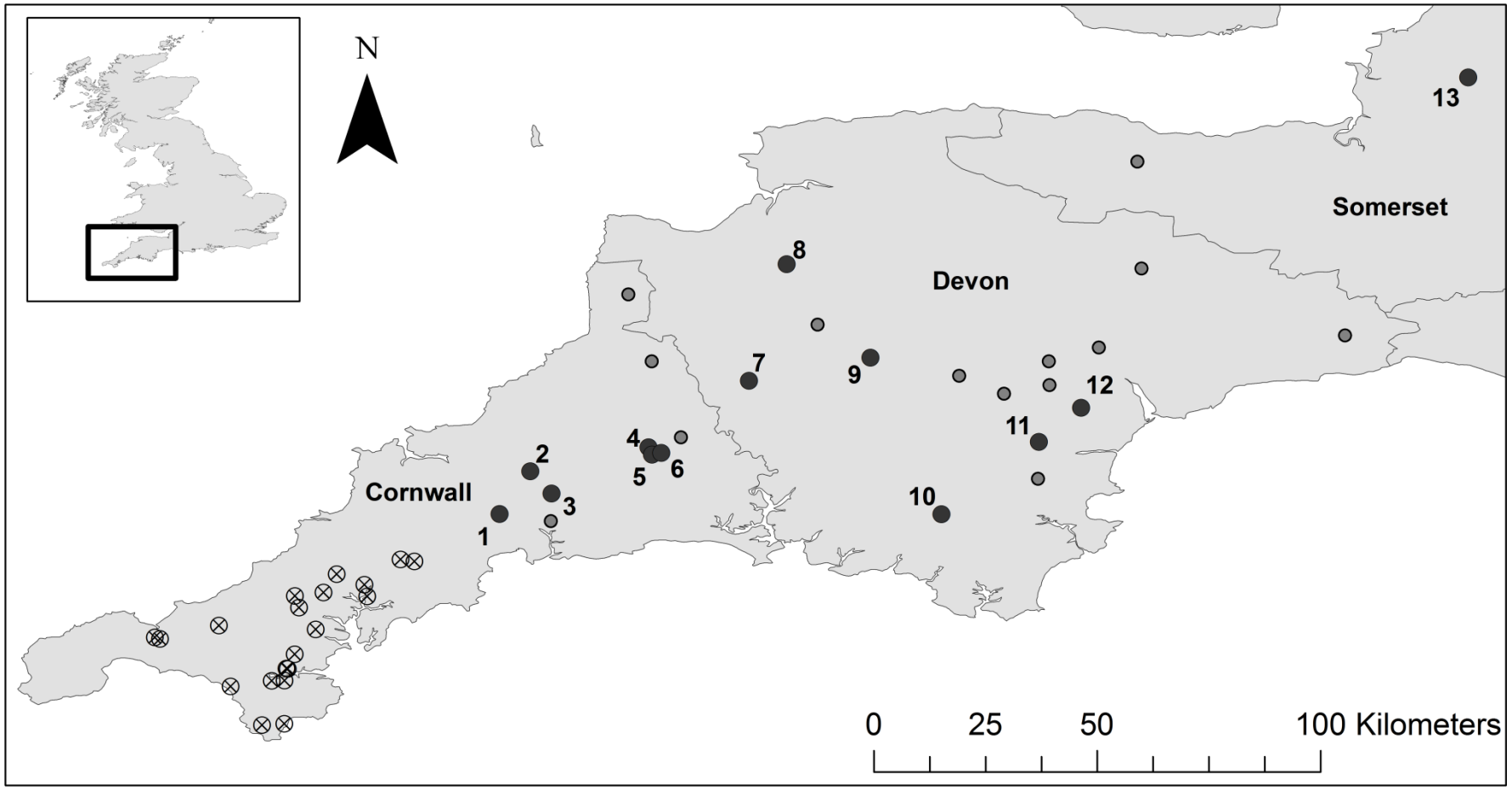

Figure 2. Study area of south west England peninsula: 13 populations (large, numbered black dots) in which we were able to sample n>5 dormouse individuals: 1-HelmanTor, 2-Penlan, 3-West Bodmin, 4-Middlewood, 5-Darley, 6-Stara, 7-Roadford, 8-North Devon, 9-Okehampton, 10-East Ivybridge, 11-Newton Abbott, 12- Haldon, 13- Cheddar; and 14 populations with n≤5 (small grey dots). Additionally, 20 sites surveyed for dormouse presence in 2008 throughout mid and west Cornwall, (but no dormice were detected) are indicated (crossed circles). The three counties, Cornwall, Devon and Somerset are also labelled. Inset map shows extent of entire survey area.

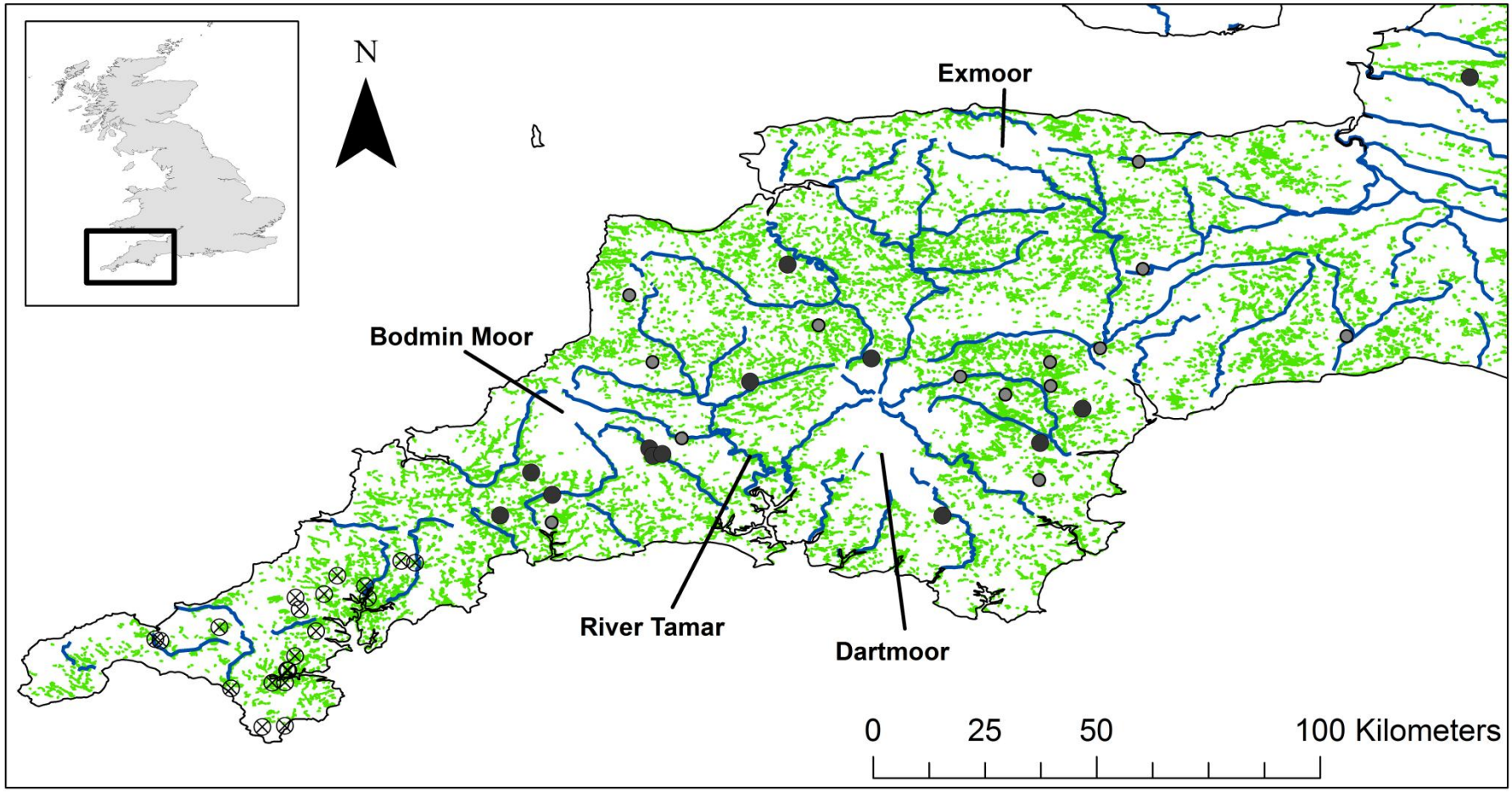

Figure 3. Study area of south west England peninsula, with 13 populations n>5 (solid black dots), 14 populations with n≤5 (grey dots), and surveyed sites with no dormouse detected (crossed circles). Green shaded areas represent deciduous woodland, blue lines indicate waterways. Three major moorlands, Bodmin Moor, Dartmoor and Exmoor are labelled. The approximate area of these moorlands can be recognised due to absence of woodland. Inset map shows extent of entire survey area. Landscape data: www.gis.naturalengland.org.uk, downloaded 18/03/2012.

Sampling

Samples were collected from 27 populations across the study area from April 2009 to October 2011 (figure 2). Plucked hairs were acquired from wild dormice during the routine monitoring of dedicated dormouse nest box schemes that are located in woodlands throughout the study area. The majority of these schemes are part of a long-term national dormouse monitoring programme (Bright *et al*. 2006). Data on sampling date, woodland site (including an OS grid reference), sex, weight, age (juvenile or adult), and reproductive condition were recorded.

Tweezers were used to pluck 1-3 small clumps of fur from individual dormice. Samples were taken under Natural England licence (numbers 20090841, 20100962 and 20111228). Further samples were obtained from *ad hoc* discoveries of dead dormice, usually by members of the public. Data available for the location of these samples were occasionally unavailable; therefore only samples with a definite OS grid reference were used. Genetic material was obtained either from tail ends or liver samples. All fresh samples were stored dry at -20°C, whilst dissected tissue samples were stored in 100% ethanol at -20°C.

Microsatellite amplification

All DNA extractions were performed by incubating samples overnight at 56°C in a 5% Chelex solution with 10mg/ml proteinase-K (Walsh *et al*. 1991). DNA was then denatured at 100°C for eight minutes and the supernatant removed from the Chelex solution/tissue debris and stored at -20°C. DNA samples were genotyped using 28 microsatellite markers selected from Md. Naim *et al*. 2009 and those described in Chapter 4. PCR conditions and profile were performed as in Chapter 4. Amplified products were genotyped in an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems) and allele sizes determined using GENEMAPPER v3.7 software (Applied Biosystems).

The samples in our study were freshly plucked, stored immediately, comprised multiple hairs and showed a low genotyping error rate. It was therefore concluded that the multi-tube technique, of re-analysing samples several times, was unnecessary

(Taberlet *et al.* 1996). This is of more concern with non-invasive genetic samples such as shed hair and faeces, which may produce large genotyping error rates due to allelic dropout and false alleles (Gagneux *et al.* 1997, Taberlet & Luikart 1999). Additionally, Gagneaux *et al*. (1997) demonstrated that plucked compared to shed hairs gave significantly more reliable genotypes.

Note that a glossary of the molecular analysis software packages cited in this chapter is provided on pages 156 to 160, giving a brief introduction to the functions of the programme and, where appropriate, details on analysis methodology.

Loci error checking

Preliminary error-checking of genotyped data was carried out using MICROSATELLITE TOOLKIT (Park 2001). In order to calculate the remaining loci error rate, 16% (n=93) of samples were randomly selected to re-PCR and were then compared to the original genotypes. Error-rate was calculated manually as the proportion of mismatches between a) alleles, and b) individual genotypes per loci. PEDANT v1.0 (Johnson & Haydon 2007) was also used to determine maximum likelihood allelic dropout and false allele rates for each locus. Six loci that had an amplification failure rate of above 15% (based only on samples that themselves successfully amplified in a minimum of 50% of the loci) and/or an error rate, allelic dropout and/or false allele frequency above 5% were dropped (supplementary table S1). Additionally, the assumption that no microsatellite loci were under selection was confirmed in LOSITAN (Antao *et al*. 2008, Beaumont *et al*. 1996). A total of 22 loci were found to be suitable for population genetics analyses.

Sample selection

Samples which failed to amplify in more than 3 loci (failure≥15%) were removed to minimise missing data and improve robustness, as poorly amplifying samples are more likely to be problematic and have errors (Morin *et al.* 2001). Due to samples being acquired from animals using nest box schemes, repeat samples from the same individual and close relatives were likely. The presence of family groups within

sampled sites can lead to biased inferences regarding population genetic structure and so their removal is recommended (Anderson & Dunham 2008, Rodríguez-Ramilo & Wang 2012). This was achieved by using ML-RELATE (Kalinowski *et al.* 2006), whereby one sample from pairwise comparisons with a relatedness coefficient above 0.4 was removed, to account for parent-offspring, full-siblings and repeat samples. The inferred relatedness by this method corroborated well with familial data, where sampled nest boxes comprised a mother and offspring, and therefore this was deemed an appropriate strategy. A total of 554 hair samples and 38 tail ends/corpses were collected across the study area. After removing samples with a poor amplification rate, repeat individuals and close kin, 237 samples remained for analysis. 215 samples came from 13 geographical populations each with sample size n>5 (figure 2). The remaining 22 samples were from 14 populations with sample size of n≤5 and so were used only in clustering analyses and not for inter-population analyses, such as genetic diversity and genetic differentiation between populations.

Genetic diversity

GENEPOP v4.0.10 was used to test for deviations from Hardy-Weinberg and genotypic equilibrium per locus, per population (Raymond & Rousset 1995, Rousset 2008). Resulting *p*-values were manually corrected for multiple tests using the False Discovery Rate (Verhoeven *et al.* 2005). The estimated frequency of null alleles was determined per locus per population in CERVUS v3.0 (Kalinowski *et al*. 2007).

For each of the 13 populations across all loci, we calculated deviations from Hardy-Weinberg due to heterozygosity deficiency, using the multi-sample score test (Rousset & Raymond 1995). We also calculated Weir & Cockerham's (1984) inbreeding coefficient (F_{IS}). These two parameters were calculated in GENEPOP v4.0.10. We calculated the mean number of alleles, and observed and expected heterozygosities per population, using ARLEQUIN v3.5 (Excoffier & Lischer 2010). The rarefaction method in ADZE v1.0 (Szpiech *et al.* 2008) was employed to determine allelic richness and frequency of private alleles. Allele frequencies were summarised in MICROSATELLITE ANALYSER v4.05 (Dieringer & Schlötterer 2003).

Population genetic structure

Bayesian clustering analyses were used to investigate genetic population structure across all 27 populations, and compare this to the geographical distribution of samples. Genetic structuring was analysed using STRUCTURE v2.3.3 (Falush *et al*. 2003, Pritchard *et al*. 2000) and BAPS v5 (Corander *et al*. 2008). The comparison of results from different clustering algorithms has been recommended to reduce the likelihood of acceptance of biased results (Frantz *et al*. 2009). For example, patterns of IBD may result in an overestimation of structure in clustering analyses, although, this is more of a concern when samples are evenly distributed (Frantz *et al*. 2009).

STRUCTURE analyses were carried out based solely on genotype data with no *a priori* information on sample location. An admixture model with correlated allele frequencies was used for varying numbers of possible clusters (K), using *K*=1-25, with 10 runs for each value of *K*, and 1,000,000 MCMC iterations following a burn-in of 500,000. STRUCTURE HARVESTER v0.6.92 (Earl & vonHoldt 2011), was used to generate plots of *K* against a) estimated log-probability of data value and b) delta *K* (Evanno *et al*. 2005). This latter interpretation method highlights the uppermost hierarchical level of clustering, and therefore we ran each of the highest level clusters (that we call "regions") separately to investigate further structuring within them. The same parameters as described above were used, except the range of K was adjusted to the appropriate number based on the number of populations in each region. CLUMMP v1.1.2 (Jakobsson & Rosenberg 2007) was then used to summarise the data over runs for models with selected K values. Parameters used were the LargeKGreedy algorithm with 1000 repeats. DISTRUCT v1.1 then enabled the construction of labelled bar plots (Rosenberg 2004). The 27 populations were also analysed in BAPS, using the population mixture model, and latitude and longitude spatial data were incorporated with individual clustering analysis (Corander *et al*. 2008). A range of maximum K of 1 to 25 with 10 repeats each was run, to determine the probability of the K which the analysis returns as the most optimal.

A neighbour-joining tree was generated to assess the degree of genetic divergence between the 13 geographical populations, based on Nei's standard genetic distance (Nei 1972). Four hundred bootstraps over loci were used in the program

POPULATIONS v1.2.32 (Langella 1999). The tree figure was plotted in TREEVIEW v1.6.6 (Page 1996).

Genetic differentiation

A pair-wise F_{ST} matrix for the 13 geographically defined populations was calculated using the Weir & Cockerham (1984) method and tested against the null hypothesis that there is no genetic differentiation between populations using permutations. Calculations were performed in MICROSATELLITE ANALYSER v4.05 (Dieringer & Schlötterer 2003), which subjects the corresponding *p*-values to Bonferroni corrections (Rice 1989). A one-way ANOVA was performed in R v2.14.1 (R Foundation for Statistical Computing 2011) in order to compare F_{ST} values between the highest hierarchical clusters, as determined by STRUCTURE analysis.

Several locus-by-locus analyses of molecular variance (AMOVA), using 20,000 permutations, were performed in ARLEQUIN v3.5 (Excoffier & Lischer 2010, Excoffier *et al.* 1992). AMOVAs were compared for four different population structure models: a) 13 geographically defined populations, b) 13 geographically defined populations divided into the three genetically defined higher hierarchical regions determined by STRUCTURE analysis, c) 13 genetically defined clusters grouped into the 3 higher hierarchical regions assigned by STRUCTURE, and d) 12 genetically defined clusters assigned by BAPS.

Isolation by distance (IBD)

Within the 13 populations (with samples n>5), there were 21 spatial categories, due to different sampling locations within a population, with sample size ≥5 for a total of 209 individuals, which were used in the IBD analysis. Geographical coordinates for each of the 21 spatial categories and the corresponding genotypes were input into SPAGeDi v1.3 (Hardy & Vekemans 2002). Pair-wise genetic differentiation was calculated using F_{ST} (Weir & Cockerham 1984) with 10,000 permutations (which is equivalent to a Mantel test) and jack-knifing to report a standard error. A regression of the spatial distance against $F_{ST}/(1-F_{ST})$ was performed (Rousset 1997).

Patterns of IBD were also compared between Cornwall and Devon, as a proxy of peripheral versus core populations respectively. Regions defined by clustering analyses were not used as the two Cornwall populations alone were of insufficient sample size, and therefore were combined. In order to control for spatial range, populations from central Devon were selected to match the spatial distance covered by the populations in Cornwall (Eckert *et al*. 2008). The difference in strength of IBD patterns and intercepts between the two geographical areas was explored using the "MantelPiece" function which uses permutation tests to estimate null-hypothesis-distributions of Mantel test correlations and ordinarily least square regression coefficients (Hamilton & Eckert 2007). Calculations were performed in R v2.14.1 (R Foundation for Statistical Computing 2011).

Regional and longitudinal effects on genetic diversity

The effect of longitude (a proxy for continuous distribution along the periphery-core gradient) and region (as defined as the highest hierarchical clusters defined by STRUCTURE) on genetic parameters were compared using ANCOVAs and F-tests in R v2.14.1 (R Foundation for Statistical Computing 2011). The parameters comprised allelic richness, a measure of genetic diversity that is more sensitive to edge-of-range affects than expected heterozygosity (Eckert *et al.* 2008); frequency of private alleles, a measure of distinctiveness; and the inbreeding coefficient.

Bottleneck

The inference of recent bottleneck events for the 13 populations was carried out using two methods. A two-phase 95% step-wise and 5% non-step-wise mutation model, with a Wilcoxon sign-rank test was used in BOTTLENECK v1.2.02 (Cornuet & Luikart 1997). The second method employed was the Garza-Williamson ratio (Garza & Williamson 2001), which was calculated in ARLEQUIN v3.5 (Excoffier & Lischer 2010).

Results

Clustering analysis

The STRUCTURE clustering data are presented at the highest hierarchical level of population genetic structure, and then within each of these main clusters. At the highest hierarchical level of population genetic structure, the Evanno *et al*. (2005) method suggests the data consist of three genetically distinct clusters, or "regions". The log-likelihood and delta *K* for the range of *K* runs are shown in figure 4a. This corresponds to Mid Cornwall, East Cornwall and Devon/Somerset geographical regions and concurs with neighbour-joining tree, results presented below (figure 7). The estimated membership coefficients for each individual for each of the three clusters suggests there is very strong structuring and little admixture between the three clusters (top bar plot in figure 5). Geographical location of these regions, defined by STRUCTURE with no *a priori* spatial information, are shown in figure 6 Further STRUCTURE analyses within each of these clusters, based on the Evanno *et al*. 2005 method, show that there are three clusters each in Mid Cornwall and East Cornwall, and seven clusters in Devon/Somerset. The log-likelihood and delta *K* for the range of *K* runs are shown in figure 4b-d. Again, estimated membership coefficients for each individual, for each of the clusters, suggests there is generally strong structuring. There is some admixture between populations within East Cornwall, probably due to these being within 2-3km distance of each other, within dormouse dispersal distance. Bar plots are shown in figure 5, with a range of *K*, to demonstrate how individuals were clustered between levels of nested hierarchy.

The optimal partitioning of clustering in BAPS suggested K = 12 populations across the entire study area (probability of 0.91). This concurs well with the STRUCTURE analyses, which when considering total the number of subpopulations, which in the latter analysis equals 13 when using the Evanno methodology. It is of note that Okehampton appears to be sub-divided into two populations by STRUCTURE (figure 5). However, BAPS did not concur, therefore the degree of true genetic structuring is somewhat equivocal. Inspection of the local geography demonstrates that a railway track is the largest landscape feature that correlates with this subdivision.

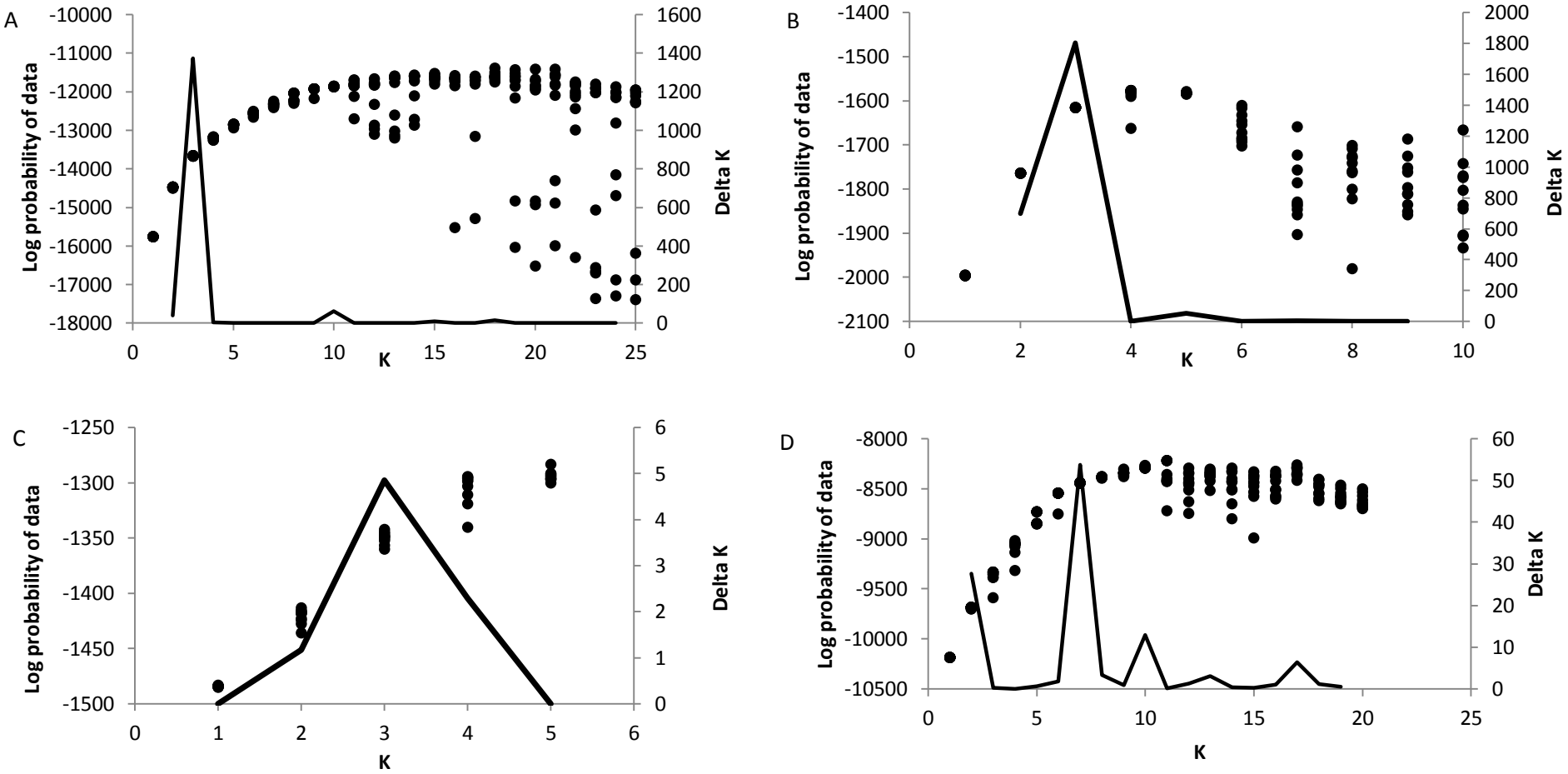

Figure 4. STRUCTURE results for dormouse samples from south west England, showing the estimated log probability of *K* on each primary (left) axis and delta *K* on the secondary (right)axes. A-Highest hierarchical structure level – across entire study area of Cornwall, Devon and Somerset, B-Mid Cornwall cluster, C- East Cornwall cluster, D- Devon and Somerset cluster.

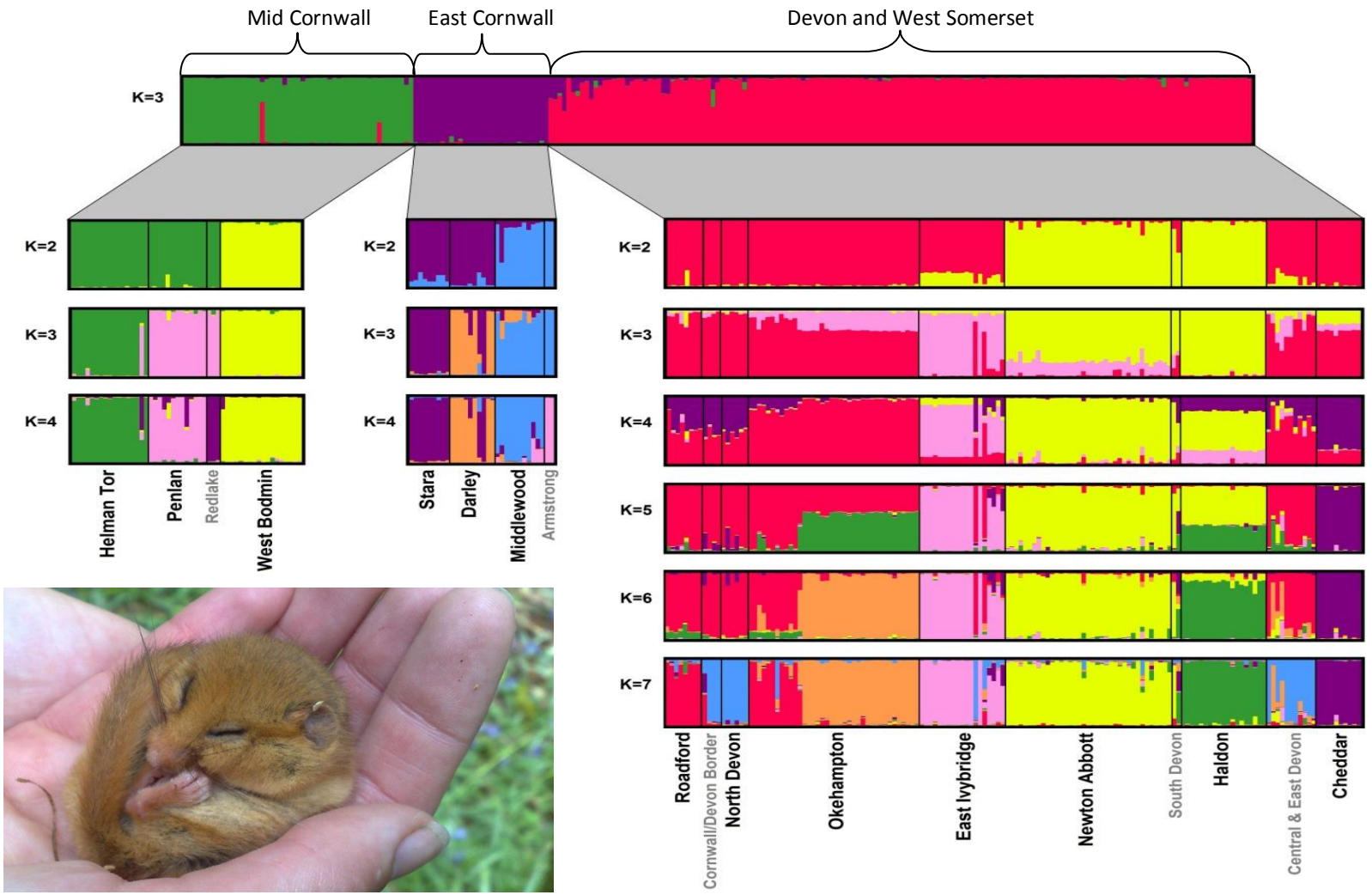

Figure 5. Bar plots showing the estimated membership coefficient (Q) for each individual for all clusters is displayed. Each vertical bar corresponds to one individual and within each run clusters are represented by different colours. Black site name labels correspond to the 13 populations (n>5). The grey labels incorporate 14 populations (n≤5) and for clarity in the Devon/Somerset cluster these are grouped by broad geographical areas. Inset, torpid hazel dormouse during sampling.

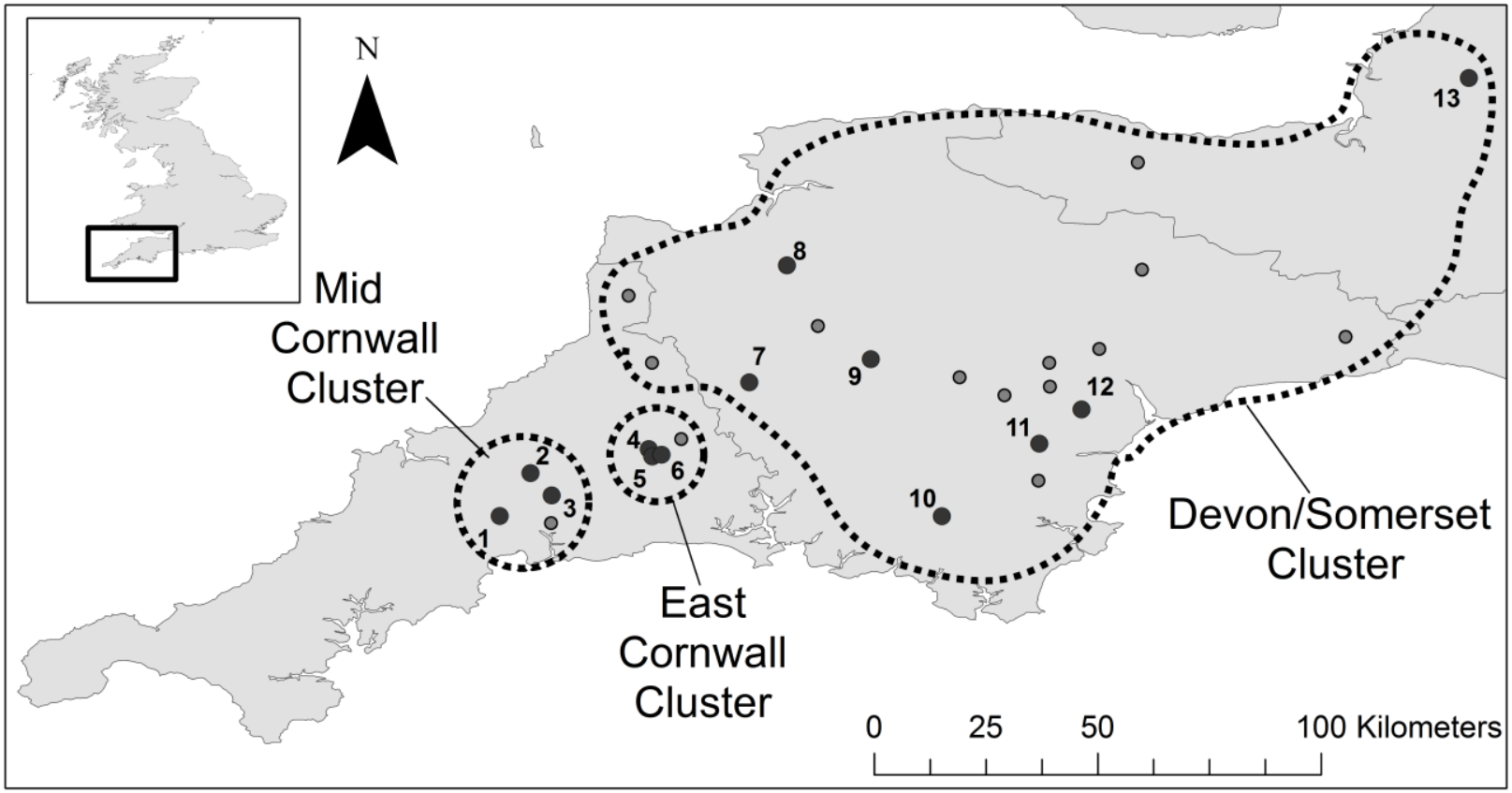

Figure 6. Geographical location of the three highest hierarchical clusters, as defined with no *a priori* spatial data by STRUCTURE analysis. The three clusters are encircled with dashed lines and labelled. The 13 populations (large black dots) with n>5 dormouse individuals samples are numbered; Mid Cornwall cluster: 1-HelmanTor, 2-Penlan, 3-West Bodmin; East Cornwall cluster: 4-Middlewood, 5-Darley, 6-Stara; and Devon/Somerset cluster: 7-Roadford, 8-North Devon, 9-Okehampton, 10-East Ivybridge, 11-Newton Abbott, 12- Haldon, 13- Cheddar and 14 populations with n≤5 (small grey dots).

The genetic distances between populations, as represented in the neighbour-joining tree (figure 7), shows the presence of two clusters of populations that are distinct from the remaining populations. These two clusters correspond to the two regions, Mid Cornwall and East Cornwall

Genetic differentiation

Across the study area, the global F_{ST} was 0.23 (*p*-value=0.0001). All F_{ST} pair-wise comparisons between populations indicated highly significant genetic differentiation after Bonferroni correction, where all pairwise test *p*-values were below 0.025: estimates are shown in table 1. Across pairwise estimates, F_{ST} values ranged from 0.09 to 0.48. The greatest F_{ST} values were for comparisons between regions: average F_{ST} value within regions was 0.18 (SD=0.06) and between regions 0.30 (SD=0.08). Within each of the three regions the average F_{ST} value was; 0.23 (SD=0.03) for mid Cornwall, 0.11 (SD=0.01) for east Cornwall and 0.18 (SD=0.06) for Devon and Somerset. There was a significant difference in mean F_{ST} amongst regions (ANOVA, $F_{2,24}$=4.3562 *p*-value=0.024). This indicates that there was significantly more genetic differentiation within the mid Cornwall region compared to the other regions, despite the Devon/Somerset region encompassing a much larger geographical area.

Genetic structure existed among clusters or geographic populations at all hierarchical levels tested, and for all the population structure models compared (Analysis of Molecular Variance, all *p*-values<0.0001, table 2a-d). This, along with the clustering analyses and F_{ST} tests, confirms that there is substantial population genetic structure in the microsatellites of the sampled dormouse populations. The model describing 13 geographically defined populations explains 23.1% of variation due to differentiation between populations, but this increases to 25.5% when populations are grouped by three regions. The highest percentage of subpopulation differentiation variation, 26.2% (c), is absorbed by the model defined by STRUCTURE analysis, whilst the BAPS analysis absorbs the least variation, at 21.1% (d), of all four models. However, all four models agree with regard to number and distribution of clusters, which suggests the results are robust.

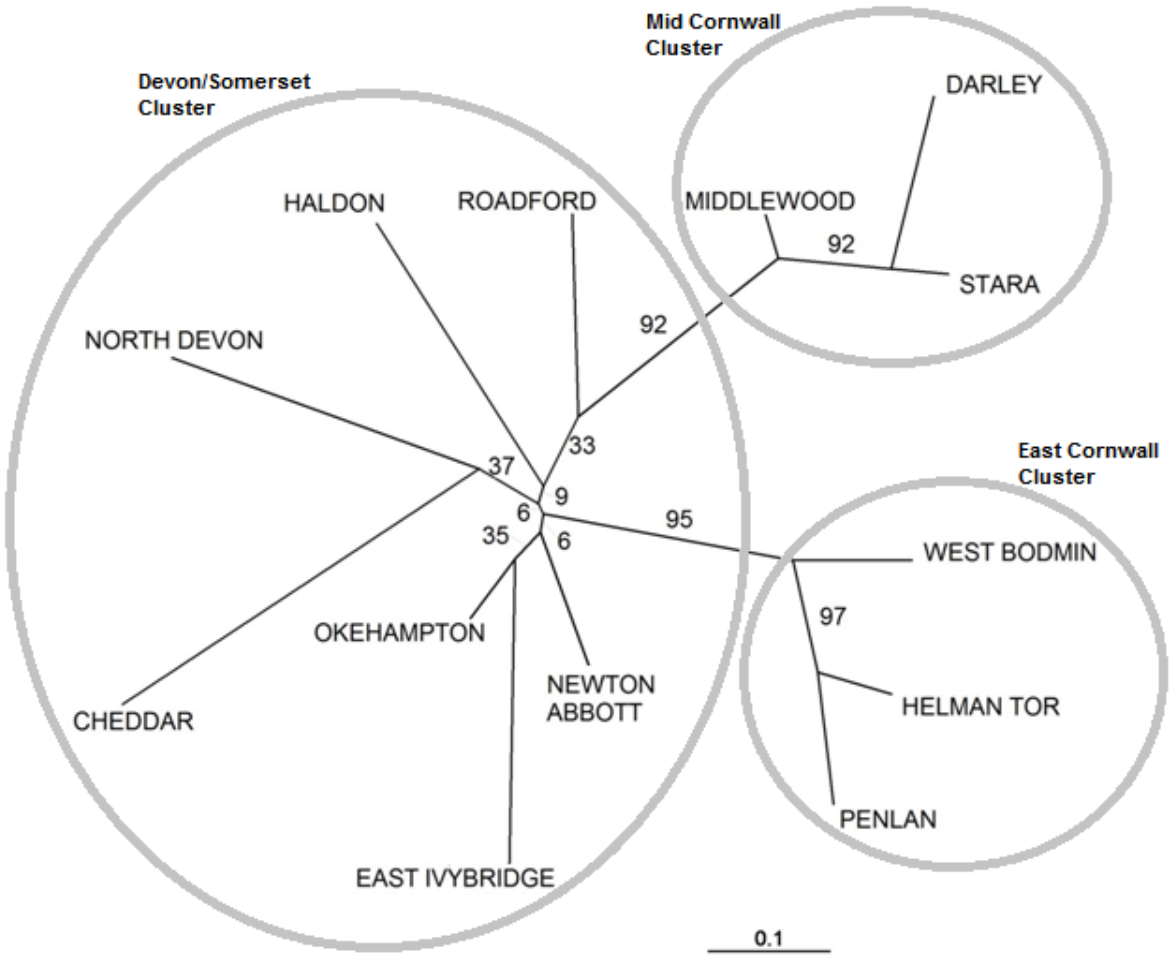

Figure 7. The neighbour-joining tree calculated using Nei's standard genetic distance (Nei 1972). Numbers indicate the percentage of bootstraps where the next cluster up the tree branch was included. Grey circles indicate the three highest hierarchical clusters as defined by STRCUTURE analysis, which demonstrates that both analyses are in concurrence.

Table 1. Pairwise F_{ST} for the 13 populations. All values were significant - all pairwise tests *p*-values were >0.025, after Bonferroni correction.

Region	**Mid Cornwall**			**East Cornwall**			**Devon and West Somerset**						
Site	HelmanTor	Penlan	Cabilla	Middlewood	Darley	StaraWood	Roadford	NorthDevon	Okehampton	EastIvybridge	NewtonAbbott	Haldon	Cheddar
HelmanTor	0	0.21	0.22	0.31	0.43	0.40	0.31	0.41	0.24	0.35	0.25	0.34	0.37
Penlan	0.21	0	0.26	0.35	0.48	0.46	0.35	0.45	0.26	0.38	0.30	0.36	0.44
Cabilla	0.22	0.26	0	0.25	0.33	0.31	0.27	0.27	0.18	0.29	0.21	0.29	0.32
Middlewood	0.31	0.35	0.25	0	0.10	0.12	0.14	0.23	0.15	0.24	0.18	0.25	0.32
Darley	0.43	0.48	0.33	0.10	0	0.11	0.19	0.24	0.20	0.30	0.25	0.30	0.37
StaraWood	0.40	0.46	0.31	0.12	0.11	0	0.20	0.26	0.19	0.27	0.25	0.31	0.38
Roadford	0.31	0.35	0.27	0.14	0.19	0.20	0	0.14	0.09	0.17	0.14	0.16	0.21
NorthDevon	0.41	0.45	0.27	0.23	0.24	0.26	0.14	0	0.13	0.23	0.16	0.22	0.25
Okehampton	0.24	0.26	0.18	0.15	0.20	0.19	0.09	0.13	0	0.13	0.10	0.17	0.20
EastIvybridge	0.35	0.38	0.29	0.24	0.30	0.27	0.17	0.23	0.13	0	0.15	0.24	0.28
NewtonAbbott	0.25	0.30	0.21	0.18	0.25	0.25	0.14	0.16	0.10	0.15	0	0.16	0.21
Haldon	0.34	0.36	0.29	0.25	0.30	0.31	0.16	0.22	0.17	0.24	0.16	0	0.28
Cheddar	0.37	0.44	0.32	0.32	0.37	0.38	0.21	0.25	0.20	0.28	0.21	0.28	0

Table 2. Results of Analysis of Molecular Variance (AMOVAS). A comparison of the degree of population subdivision at several hierarchical levels . AMOVAs were compared for four different population structure models: a) 13 geographically defined populations, b) 13 geographically defined populations divided into the three genetically defined higher hierarchical regions, c) 13 genetically defined clusters grouped into the 3 higher hierarchical regions assigned by STRUCTURE and d) 12 genetically defined clusters assigned by BAPS. F_{ST} /F_{CT}/F_{SC} refers to the fixation index- a measure of genetic differentiation among subpopulations. F_{IS} is the inbreeding coefficient and F_{IT} is the overall fixation index of individuals relative to the total population. All data partitions were significant, *p*-value<0.0001.

	Sum of squares	Variance components	% variation	Fstat	
a) Geographical populations					
Among populations	762.74	1.85	23.08	0.23	F_{ST}
Among individuals within populations	1288.89	0.53	6.64	0.09	F_{IS}
Within individuals	1158.50	5.63	70.28	0.30	F_{IT}
b) Geographical populations in 3 regions					
Among regions	357.48	0.90	10.83	0.11	F_{CT}
Among populations within regions	405.26	1.21	14.64	0.16	F_{SC}
Among individuals within populations	1288.89	0.53	6.44	0.09	F_{IS}
Within individuals	1158.50	5.63	68.09	0.32	F_{IT}
c) Genetic populations - STRUCTURE					
Among regions	335.99	1.07	12.63	0.13	F_{CT}
Among populations within regions	475.72	1.16	13.63	0.16	F_{SC}
Among individuals within populations	1468.45	0.61	7.23	0.10	F_{IS}
Within individuals	1281.00	5.64	66.51	0.33	F_{IT}
d) Genetic populations - BAPS					
Among populations	760.53	1.70	21.10	0.21	F_{ST}
Among individuals within populations	1519.64	0.72	8.91	0.11	F_{IS}
Within individuals	1281.00	5.64	69.99	0.30	F_{IT}

Isolation by distance

There is highly significant evidence of IBD across the study area, where there was an increase in genetic differentiation with increasing distance (R^2=0.26, *p*-value<0.0001, figure 8). Correlations between genetic distance and spatial distance remained significantly positive when regions were analysed separately (Mantel tests, Devon, *p*=0.001; Cornwall, p=0.009, figure 9). The populations within Cornwall demonstrated a significantly stronger pattern of IBD than those in Devon (permutation tests of difference in slope between regions, *p*-value=0.028), however this was more apparent at larger spatial distances, as there was no significant difference between the intercepts of the IBD regressions for the two regions (permutation tests, *p*-value=0.95). There was also no significant difference in the goodness-of-fit of the IBD correlations (permutation tests, *p*-value=0.83).

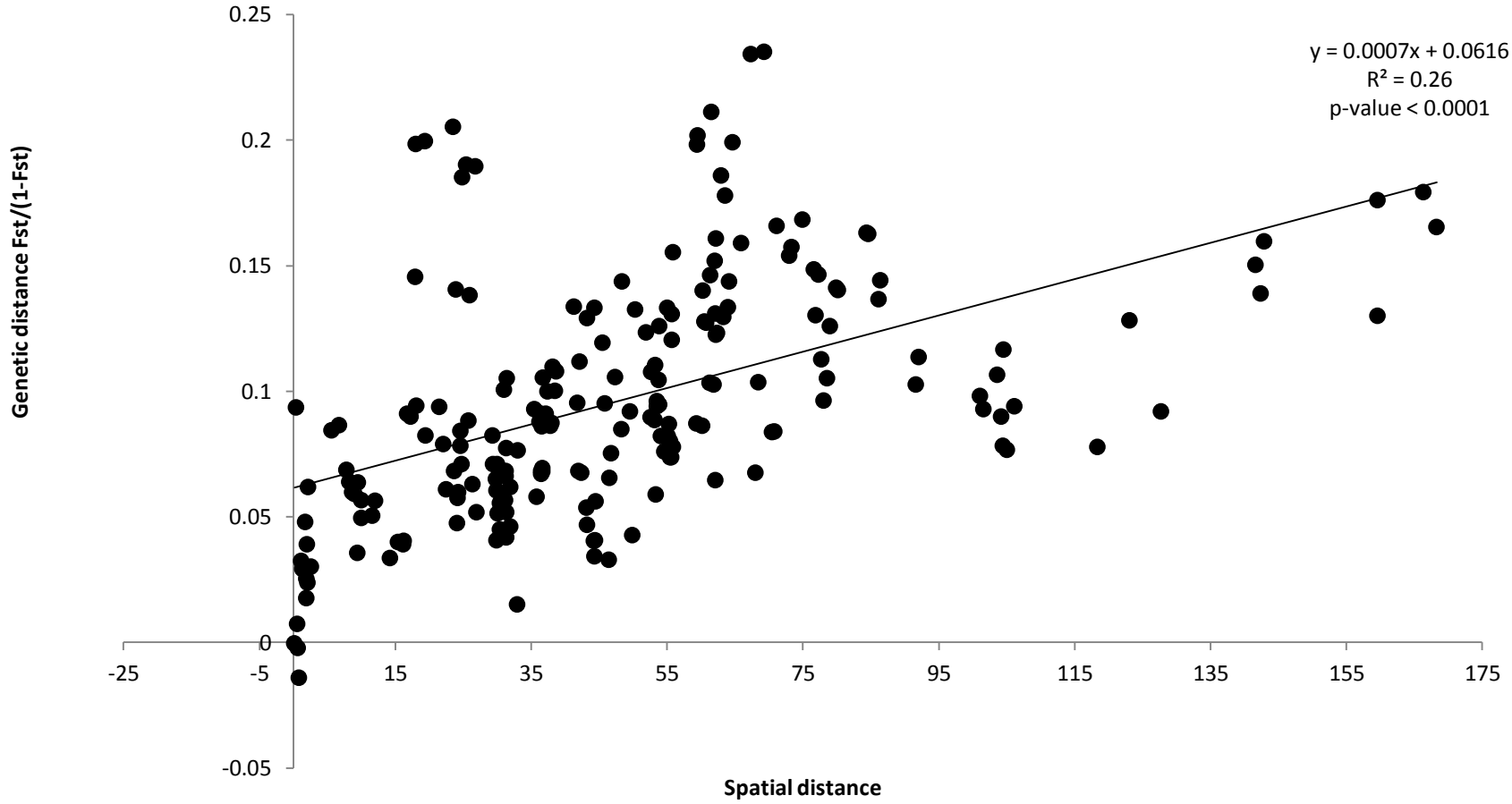

Figure 8. Plot of IBD analysis, with spatial distance against genetic distance, showing significant evidence for a pattern of IBD. Samples n ≥ 5 per site, across all south west survey area (21 woodland sites)

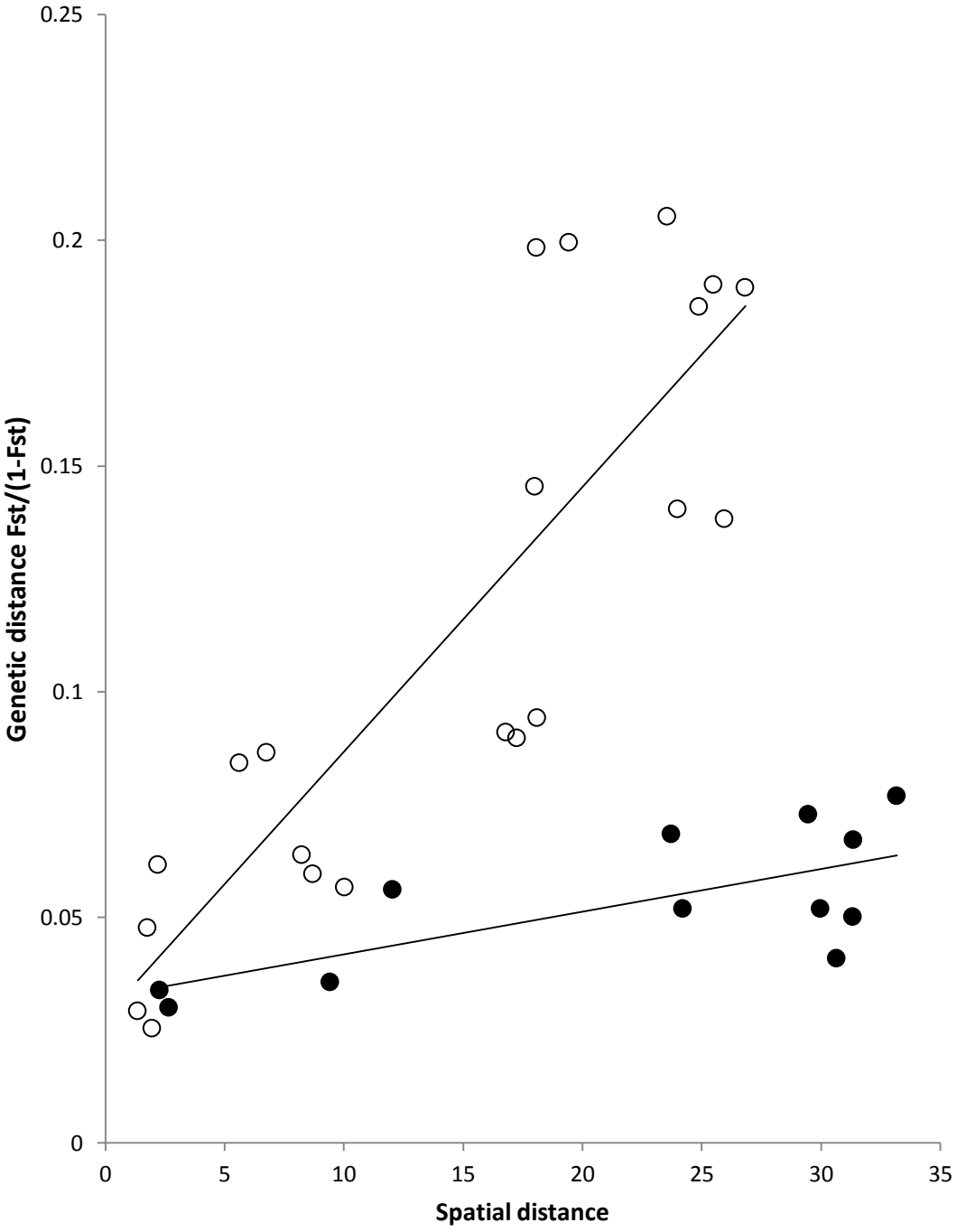

Figure 9. Comparison of the patterns of IBD, showing a plot of spatial distance against genetic distance, in Cornwall (open circles) and Devon (solid circles), proxies for peripheral and core areas respectively.

Regional and longitudinal genetic diversity

The frequency of private alleles was significantly different amongst regions ($F_{1,9}$=8.01, *p*-value=0.02, figure 10a). There was also evidence for a clinal pattern, where the frequency of private alleles declined with longitude from east to west ($F_{2,9}$=9.53, *p*-value=0.006, figure 10a). Region also demonstrated a significant effect on allelic richness ($F_{2,10}$=10.49, *p*-value=0.004, figure 10b), however, there was no significant longitudinal effect and hence no evidence of a clinal pattern ($F_{1,9}$=3.34, *p*-value=0.101, figure 10b). The inbreeding coefficient, F_{IS}, showed no significant response to either regional ($F_{2,10}$=0.42, *p*-value=0.67, figure 10c) or longitudinal ($F_{1,11}$=3.44, *p*-value=0.09, figure 10c) effects. There was a highly significant difference between Mid Cornwall and Devon/Somerset, with the former region having lower values of frequency of private alleles and allelic richness (Tukey post-hoc testing, *p*-values=0.004 and 0.0007 respectively). Similarly, the East Cornwall region showed significantly lower levels of allelic richness and frequency of private alleles compared to the Devon/Somerset region (Tukey post-hoc testing, *p*-values=0.048 and 0.0003 respectively). However, there was no significant difference between Mid Cornwall and East Cornwall regions for both measures of genetic diversity. There was no significant interaction between the two explanatory variables, region and longitude, on allelic richness ($F_{2,7}$=0.67, *p*-value=0.54) or the frequency of private alleles ($F_{2,7}$=0.04, *p*-value=0.96).

Summary Statistics

The number of individuals sampled per population with n>5 ranged from 6 to 38, with an average of 16.5 (SD=10.3) individuals. Across loci and populations the mean number of alleles was 3.84 (SD=0.89); mean observed heterozygosity 0.53 (SD=0.09) compared to 0.57 (SD=0.09) for the expected heterozygosity; mean F_{IS} inbreeding coefficient 0.07 (SD=0.17); mean allelic richness 3.12 (SD=0.58) and the mean frequency of private alleles 0.11 (SD=0.08) (data shown in table 3).

Eight of thirteen populations showed deviations from Hardy-Weinberg due to heterozygosity deficiency (Helman Tor, West Bodmin, Penlan, Middlewood, Roadford, Okehampton, East Ivybridge and Newton Abbott). Across the 13 populations, no locus

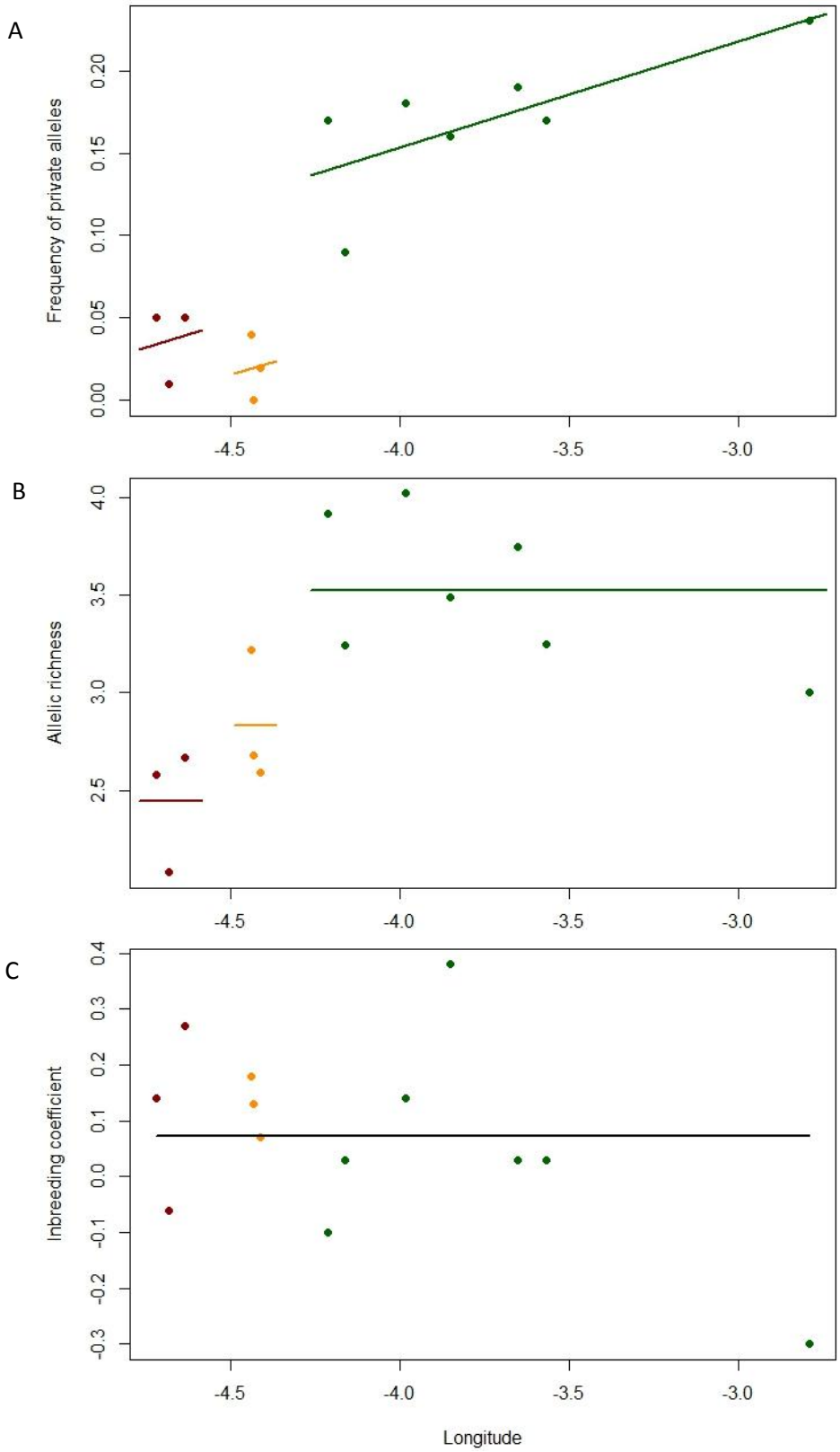

Figure 10. Plots showing the model outcomes of the effect of region and longitude on: a) frequency of private alleles; b) allelic richness; and c) inbreeding coefficient. Longitude is plotted against the population genetic parameter, and regions are denoted by different colour points: red-Mid Cornwall, orange-East Cornwall and green-Devon/Somerset.

consistently indicated a high frequency of null alleles or significant deviations from Hardy-Weinberg equilibrium or, after correction for multiple tests, evidence of linkage disequilibrium (supplementary table S2). Therefore, the population deviations appear to be due to ecological mechanisms, such as sub-structuring (i.e. the Wahlund effect (Hartl & Clark 2007)), rather than loci not adhering to the assumptions of the model. The results of our population genetic analyses, however, should be interpreted with some caution, as many of the analyses are based on the assumption of Hardy-Weinberg equilibrium. However, this is often unavoidable as some wild populations, especially those of conservation concern, simply may not confer to these assumptions (Pearse & Crandall 2004). The allele frequencies for the 22 loci and all 237 samples are provided in supplementary table S3.

Bottleneck

Test results for bottleneck events in the 13 populations are provided in table 4. Across all populations and the two analysis methods there is no consistent evidence for bottlenecks in any of the populations surveyed. The Wilcoxon sign-rank test indicated that three populations exhibited a significant departure from the expected heterozygosity for populations in mutation-drift equilibrium. Two populations (Darley and Roadford) showed a higher expected heterozygosity than would be predicted from the observed allele number, which indicates that these populations have suffered a recent bottleneck event. The third population (Helman Tor) showed heterozygosity deficiency, which may be due to the Wahlund effect, whereby sub-structuring within the population leads to a reduction in the average expected heterozygosity (Hartl & Clark 2007). However, for all populations the Garza-Williamson index was above the suggested threshold of M=0.68 (Garza & Williamson 2001), suggesting no populations have experienced a bottleneck.

Table 3. Summary statistics for the 13 populations; number of dormice sampled (N), mean and standard deviation of number of alleles (Na (SD)), observed heterozygosity (Ho (SD)) and expected heterozygosity (He (SD)), and mean and standard error of allelic richness (Ar (SE)) and private alleles (Ap (SE)), inbreeding coefficient (F_{IS}), *p*-value for Hardy-Weinberg test for heterozygosity deficiency, (HetDef), with significant *p*-values in bold.

Population	N	Na (SD)	Ho (SD)	He (SD)	Ar (SE)	Ap (SE)	Fis	Het Def
Helman Tor	17	3.52 (1.72)	0.36 (0.29)	0.40 (0.25)	2.58 (0.24)	0.05 (0.02)	0.14	**0.00**
West Bodmin	18	3.48 (1.17)	0.44 (0.16)	0.51 (0.16)	2.67 (0.18)	0.05 (0.03)	0.27	**0.00**
Penlan	13	2.88 (0.81)	0.35 (0.14)	0.42 (0.18)	2.08 (0.19)	0.01 (0.01)	-0.06	**0.00**
Middlewood	11	3.67 (1.43)	0.56 (0.20)	0.60 (0.15)	3.22 (0.26)	0.04 (0.02)	0.18	**0.00**
Darley	10	2.95 (0.74)	0.58 (0.20)	0.56 (0.11)	2.68 (0.15)	0.00 (0.00)	0.13	0.68
Stara	9	2.73 (0.55)	0.50 (0.17)	0.50 (0.15)	2.59 (0.11)	0.02 (0.01)	0.07	0.67
North Devon	6	3.36 (1.00)	0.64 (0.28)	0.61 (0.17)	3.24 (0.20)	0.09 (0.05)	0.03	0.72
Roadford	8	4.18 (1.37)	0.58 (0.19)	0.70 (0.12)	3.92 (0.25)	0.17 (0.06)	-0.10	**0.00**
Okehampton	38	5.46 (2.18)	0.60 (0.17)	0.66 (0.17)	4.02 (0.27)	0.18 (0.06)	0.14	**0.00**
East Ivybridge	19	4.62 (1.69)	0.51 (0.21)	0.61 (0.20)	3.49 (0.27)	0.16 (0.06)	0.38	**0.00**
Newton Abbott	37	5.38 (1.80)	0.60 (0.16)	0.66 (0.16)	3.75 (0.25)	0.19 (0.05)	0.03	**0.00**
Haldon	19	4.29 (1.71)	0.56 (0.17)	0.59 (0.15)	3.25 (0.24)	0.17 (0.06)	0.03	0.24
Cheddar	10	3.38 (1.02)	0.58 (0.22)	0.54 (0.18)	3.00 (0.22)	0.23 (0.14)	-0.30	0.87

Table 4. Results of tests for bottleneck events in the 13 populations, from BOTTLENECK and using the Garza-Williamson Index.

		Wilcoxon test				Garza-Williamson	
Population	**N**	**One tail Hdef**	**One tail Hex**	**Two tail Hdef or Hex**	**Sign test**	**Index**	**SD**
Helman Tor	17	0.00	1.00	0.00	0.00	0.85	0.18
West Bodmin	18	0.49	0.53	0.97	0.57	0.85	0.19
Penlan	13	0.19	0.83	0.38	0.24	0.80	0.22
Middlewood	11	0.93	0.07	0.15	0.45	0.83	0.17
Darley	10	0.99	0.01	0.02	0.03	0.85	0.20
Stara	9	0.78	0.23	0.46	0.30	0.79	0.24
North Devon	6	0.84	0.17	0.34	0.49	0.87	0.16
Roadford	8	1.00	0.00	0.00	0.01	0.86	0.13
Okehampton	38	0.95	0.05	0.10	0.14	0.94	0.10
East Ivybridge	19	0.34	0.67	0.68	0.21	0.86	0.17
Newton Abbott	37	0.79	0.22	0.43	0.29	0.89	0.16
Haldon	19	0.50	0.51	1.00	0.48	0.83	0.18
Cheddar	10	0.53	0.49	0.97	0.41	0.87	0.17

Discussion

This study describes the genetic population structuring and isolation by distance patterns, differentiation, diversity and inbreeding amongst hazel dormouse populations from the core and periphery of their range in southwest England. Our results clearly reveal significant genetic differentiation across all levels of population structure and variation in genetic diversity between regions.

The strong genetic differentiation and structuring amongst all populations at the landscape scale concurs with our predictions and the findings of Md. Naim *et al*. (2012). Since dormice are habitat specialists, with low dispersal rates, reproductive potential and population densities (Bright 1993, Bright & Morris 1996), we expect this species to form small, isolated and genetically differentiated populations within their preferred habitat matrix. This lends further support to the premise that dormice are particularly vulnerable to habitat loss and fragmentation. Furthermore, the evidence for a pattern of IBD is expected for a species with low dispersal ability (Trizio *et al*. 2005). The IBD effect appears to be stronger in the Cornwall populations, compared to those found in Devon. Due to small sample size and uneven distribution of samples within the Cornwall sites, it is difficult to determine whether the observed pattern can be attributed to a stronger clinal IBD effect *per se*, due to differences in dispersal behaviour, or to the Cornish populations being more isolated by landscape barriers and thus corresponding higher differentiation (Guillot *et al*. 2009). Also of note, is the sub-structuring by STRUCTURE of the Okehampton population, which may be due to an anthropogenic dispersal barrier. However, the BAPS analysis did not subdivide this population and therefore further research is required to determine the extent to which landscape features, such as railways and roads confer dispersal barriers to dormice.

Lower allelic richness in the Mid Cornwall and East Cornwall regions compared to Devon/Somerset, but with no effect of longitude, are likely due to limited dispersal between Cornwall and Devon/Somerset. Natural and man-made landscape barriers to dispersal reduce gene flow, leading to small, isolated, more differentiated populations that experience elevated genetic drift and lower genetic diversity (Goossens *et al*. 2005, Miller & Waits 2003, Slatkin 1987). In our study, the large river Tamar runs along the border between

Cornwall and Devon and may create a dispersal barrier to dormice, especially in the south where it is at its widest (figure 3). In the north this watercourse is narrower and likely to be more permeable to dormouse dispersal, where the tree canopy connects above the waterway, however, generally there is less woodland cover, with habitat being dominated by moorland (Bodmin Moor, figure 3).

However, both region and longitude showed significant correlations with the frequency of private alleles. Therefore, in addition to the barrier effects of the river Tamar and Bodmin Moor, there is strong evidence for a decline in this measure of genetic diversity from the core to the periphery of the dormouse range sampled along the southwest England peninsula. This concurs with our expectations, based on the central-periphery hypothesis (Diniz-Filho *et al*. 2009, Lawton 1993, Vucetich & Waite 2003).

There was relatively lower population differentiation amongst Devon/Somerset populations compared to those within Mid Cornwall and there was a stronger IBD pattern amongst the populations found within Cornwall, compared to populations from the centre of Devon. Peripheral populations are likely to be more isolated and patchily distributed, with reduced population sizes and more variable densities (Lawton 1993, Vucetich & Waite 2003). Such restricted gene flow between habitat patches, compared to within the core range where there may be a more continuous distribution, will lead to increased genetic differentiation (Keyghobadi *et al*. 2005, Slatkin 1987). Additionally, isolated, small populations are subject to elevated genetic drift, further contributing to genetic differentiation amongst peripheral populations, as well as reducing genetic diversity in comparison to larger populations (Frankham *et al*. 2002).

However, the edge-of-range patterns do not imply causation, and alternative mechanisms may be driving the variation in genetic diversity between populations. Contemporary explanations are confounded by historical variables, such as past barriers to gene flow, habitat configurations and climatic changes that have driven range expansions and contractions (Hampe & Petit 2005, Slatkin 1987). Our observed pattern of genetic diversity may be explained by residual effects of post-glacial range expansion (Garner *et al*. 2004). It is assumed terrestrial mammals migrated from mainland Europe into eastern England across the land-bridge, Doggerland, which concurs with genetic patterns described

in other small mammals (Searle *et al*. 2009). Colonisation tends to take place in a series of waves of founder events. Each founder event includes only a subset of core genetic diversity. This leads to a gradient of reduced diversity along the colonisation route away from the core (Hewitt 1999).

It is likely that a combination of historical and contemporary ecological mechanisms will be acting on the population genetics of these populations (Slatkin 1987). To tease apart these mechanisms, wider sampling and further analyses are required, such as approximate Bayesian computation and coalescent modelling (Cornuet *et al.* 2008). In addition, the integration of phylogeographic information may allow us to distinguish between contemporary and historical influences and quantitative genetics may provide insights into adaptive processes (Eckert *et al.* 2008). To more vigorously test the periphery-core hypothesis, analyses such as ours are required across the entire range of dormice. Additional landscape analyses may highlight the relative importance of natural and anthropogenic features which hinder dispersal. Such studies would require a more continuous sampling scheme across the landscape (Manel *et al.* 2003).

With these caveats in mind, we can still attempt to tease apart the mechanisms driving population genetic parameters at the edge of the dormouse's range. Lower genetic diversity at edge-of-range could be due to: a reduction in resource quality or availability; a reduction in the optimality of climatic conditions; historical colonisation processes; or recent major population-wide disturbances. We propose that changing climatic conditions would impose clines in diversity rather than our observed abrupt changes, while recent disturbances would reveal the signal of genetic bottlenecks, which we do not observe. This leaves two rival mechanisms for the difference between core and peripheral populations: an abrupt change in resources, or colonisation limitation between the regions. In fact these mechanisms are likely to be coupled: smaller patches of woodland in Cornwall, which occur in steep-sided valleys separated by moorland on its granite backbone, may cause increased population subdivision and isolation. This effect may then be exaggerated by dispersal barriers between the regions: a strong candidate barrier is the river Tamar.

Implications

Irrespective of underlying mechanisms, it is of concern that populations in Cornwall are less genetically diverse as they are suffering from a small effective population size and genetic drift (Frankham 1996). Such populations are vulnerable to inbreeding depression, which may be more influential in populations that are under more stress (Armbruster & Reed 2005), such as peripheral populations that are characterised as having lower quality habitats (Brown 1984, Vucetich & Waite 2003). As such, these populations in Mid and East Cornwall regions may be at a higher risk of extinction (Frankham 1996, Frankham 2005, Saccheri *et al.* 1998). However, we did not find a significant effect of either region or longitude on the inbreeding coefficient, which suggests dormouse behaviour is facilitating the avoidance of inbreeding even in the least genetically diverse populations. For example, polyandry may increase the effective population size, as discussed in Chapter 6. Whilst there are records of hazel dormice in west Cornwall (Environmental Records Centre for Cornwall and the Isles of Scilly 2012) our 2008 survey across west Cornwall failed to detect any dormice here, despite conducting seven month-long surveys at 20 sites, following the recommended guidelines (Bright *et al.* 2006). This does, indirectly, suggest a range contraction from the western tip of Cornwall.

Whilst we have highlighted variation in population genetics parameters, it remains open to debate how best to use this information to conserve hazel dormice in southwest England. It is argued that peripheral populations warrant conservation consideration as, due to differences in natural selection, they may have local adaptations that are advantageous to the evolution of future populations (Lesica & Allendorf 1995). Significantly distinct populations, or "Evolutionarily Significant Units", are considered of conservation priority (Moritz 1994). The Cornish populations are well differentiated from dormice further west in Devon, presumably due to the river Tamar and Bodmin Moor. The larger, source population in Devon, therefore cannot flood these peripheral populations with genotypes that are not adapted to local conditions, leaving these populations an increased chance to adapt (Eckert *et al.* 2008). Range contractions should be of concern for the entire population as a whole, as models predict a positive correlation between regional occupancy and local abundance (Lawton 1993, Zuckerberg *et al.* 2009) and the loss of peripheral populations may have a knock-on negative effect on core population abundance. Channell & Lomolino (2000) found

that range contractions do not always occur from edge towards core and therefore support conservation of edge populations, especially for species where the core has been heavily impacted upon by human actions. Therefore there is a strong argument for the conservation of Cornish dormouse populations. Conversely, it is argued that as core populations are more stable and contain higher levels of genetic diversity it is more efficient to focus limited conservation resources, in such core areas (Petit *et al.* 1998). This promotes the alternative argument for prioritising conservation of Devon populations. Whichever approach is taken, links between habitat suitability and wider landscape permeability must be considered.

In the light that Cornish hazel dormouse populations are likely to be genetically distinct, it would be unadvisable to recommend landscape-scale management plans that connect Cornish and Devon populations, or to translocate animals from other locations into unoccupied Cornish woodlands. Such actions may prove to diminish the genetic distinctiveness of Cornish populations and lead to a loss of important adaptations that allow them to survive on the edge of this species range. Clearly this supposition warrants further investigation, however, in the meantime we recommend that conservation managers concentrate on the habitat management of Cornish woodlands that ensure the persistence of existing dormice populations across Cornwall. In addition, we advocate the linking of populations with hedgerows and other suitable habitat corridors within the Cornish region, to encourage recolonisation of woodland patches were dormice appear to have become extinct. Ultimately these actions should aim to increase the population size and halt further loss of Cornish hazel dormouse genetic diversity.

Conclusions

Our study provides evidence that a combination of dispersal barriers and core-periphery effects influence key population genetics parameters in south-western UK edge of range of the hazel dormouse. Several parameters tested revealed regional changes, moving from the eastern core to the western periphery: namely genetic diversity, differentiation, structure and isolation by distance. However, we found no evidence for changes in levels of inbreeding or frequencies of bottleneck events at the range edge. Further studies are required to determine if these patterns apply throughout the entire range of hazel dormice. Such data is vital in order to identify and define conservation priorities. Additionally, it

allows the elucidation of regional effects on population parameters in a species vulnerable to habitat loss and fragmentation.

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Supplementary Table S1. Error checking results for the 28 genotyped loci, shaded entries denote values that justified the dropping of that locus.

Locus	% Amp Failed	Error rate - Alleles	Error rate - Individuals	Allelic dropout	False alleles
Mav021	14.60	0.01	0.03	0.00	0.02
Mav038	7.12	0.01	0.02	0.00	0.01
Mav043	4.56	0.01	0.02	0.00	0.00
Mav044	5.11	0.03	0.05	0.02	0.00
MavSF10	4.74	0.01	0.02	0.03	0.00
Mav011	2.74	0.01	0.03	0.03	0.00
Mav053	1.09	0.00	0.00	0.00	0.00
Mav015	8.21	0.03	0.04	0.05	0.00
Mav032	11.86	0.01	0.02	0.00	0.00
Mav051	2.55	0.02	0.03	0.00	0.00
MavSH3	5.84	0.00	0.01	0.03	0.00
Mav033	2.19	0.01	0.02	0.51	0.00
MavSG3	2.19	0.02	0.04	0.00	0.01
Mav005	16.24	0.02	0.03	0.02	0.00
Mav017	5.11	0.01	0.01	0.02	0.00
Mav030	11.86	0.01	0.02	0.01	0.00
Mav036	8.94	0.01	0.01	0.00	0.00
Mav040	4.93	0.00	0.00	0.00	0.00
Mav048	2.55	0.03	0.04	0.02	0.00
MavA5	11.50	0.01	0.02	0.02	0.00
MavSB5	31.39	0.06	0.06	0.03	0.05
MavSG6	2.74	0.01	0.02	0.00	0.00
Mav034	2.92	0.01	0.01	0.00	0.00
Mav047	1.64	0.01	0.01	0.70	0.00
Mav003	6.39	0.02	0.03	0.02	0.00
MavSG9	3.10	0.03	0.06	0.02	0.06
Mav049	5.47	0.01	0.01	0.03	0.00
MavSF12	24.27	0.02	0.05	0.08	0.00

Supplementary Table S2. Summary for 22 loci used in genetics analysis of 13 dormouse populations. Size range of alleles across populations in bp, K= mean number of alleles across populations, n=number of samples genotyped per locus per population, Null = estimated frequency of null alleles, HW= *p*-value for test of deviation from Hardy Weinberg equilibrium prior to multiple test correction, bold highlights values significant after correction for multiple tests, using the False Discovery Rate.

			West Bodmin			Cheddar			Darley			East Ivybridge			Haldon			Helman Tor			Middlewood			Newton Abbott			North Devon			Penlan			Roadford			Stara			Okehampton		
Locus	Size range (bp)	K	n	Null	HW	n	Null	HW	n	Null	HW	n	Null	HW	N	Null	HW	n	Null	HW	n	Null	HW	n	Null	HW	n	Null	HW	n	Null	HW	n	Null	HW	n	Null	HW	n	Null	HW
Mav021	139-201	4.7	17	0.15	0.09	10	-0.20	1.00	10	0.05	1.00	19	0.21	0.01	18	-0.03	0.83	14	0.05	0.74	10	0.01	0.54	35	0.01	0.89	5	-	0.90	10	-0.05	1.00	8	-	0.41	6	-	1.00	33	0.07	0.16
Mav038	259-269	3.0	18	0.00	1.00	10	-0.07	0.56	10	0.21	0.48	19	0.15	0.01	18	0.22	0.08	14	-0.01	-	10	0.09	0.57	37	0.04	0.60	6	-	0.39	13	-	-	8	-	0.38	7	-	1.00	36	-0.01	0.81
Mav043	160-184	3.6	18	0.10	0.35	9	-	0.63	10	-0.01	0.62	19	0.08	0.42	18	0.05	0.41	17	0.78	0.03	10	-	-	37	0.00	0.10	6	-	0.48	13	-0.04	1.00	8	-	0.03	7	-	1.00	37	0.11	0.37
Mav044	126-160	4.7	18	-0.02	0.48	9	-	0.80	10	-0.17	0.52	19	-0.10	0.07	19	-0.04	0.46	17	0.18	0.07	10	-0.03	1.00	37	0.05	0.00	6	-	-	13	0.09	0.12	8	-	0.08	9	-	0.51	37	0.00	0.55
MavSF10	210-238	3.8	18	0.01	0.22	10	-0.05	1.00	10	-0.11	1.00	18	0.09	0.01	19	-0.04	1.00	17	0.11	0.13	10	-0.11	1.00	37	0.00	0.60	6	-	0.09	13	-0.13	1.00	8	-	0.17	9	-	1.00	37	0.04	0.14
Mav011	169-214	5.5	18	0.08	0.01	10	-0.06	1.00	10	-0.14	0.73	17	0.02	0.75	19	0.02	0.33	16	0.03	0.78	11	0.09	0.01	36	0.17	0.05	6	-	0.87	12	0.11	0.28	8	-	0.33	8	-	1.00	37	0.14	0.01
Mav053	215-227	3.5	18	0.02	1.00	10	-0.11	1.00	10	0.25	0.31	19	0.01	0.40	19	-0.01	1.00	17	0.06	0.26	11	0.68	**0.00**	36	0.04	0.68	6	-	0.27	13	0.16	0.23	8	-	0.06	9	-	1.00	37	0.05	0.37
Mav015	243-251	2.9	18	-0.06	0.56	8	-	1.00	10	0.14	0.10	17	0.14	0.26	18	0.09	0.47	16	0.34	0.00	11	0.25	0.27	37	-0.02	0.26	6	-	1.00	12	0.00	1.00	8	-	0.08	9	-	1.00	35	0.20	0.03
Mav032	124-158	3.2	18	0.06	0.66	8	-	0.53	10	-0.14	1.00	16	-0.16	0.31	17	0.06	0.60	17	-0.04	1.00	11	0.14	0.09	37	0.11	0.01	6	-	1.00	13	0.13	0.09	8	-	0.74	9	-	0.53	30	-0.07	0.43
Mav051	229-244	4.0	18	-0.06	1.00	9	-	1.00	10	-0.07	0.66	17	-0.02	0.87	19	-0.03	0.39	16	-0.03	0.41	11	0.01	0.76	37	0.01	0.63	6	-	1.00	13	-0.03	0.30	8	-	0.71	8	-	1.00	36	0.14	0.02
MavSH3	203-217	3.5	18	-0.05	1.00	10	-0.13	0.44	10	0.19	0.02	18	0.21	0.06	18	0.01	0.74	15	-0.01	-	11	0.08	0.76	36	0.21	0.03	6	-	0.53	13	0.16	0.39	8	-	1.00	8	-	0.01	37	0.04	0.49
MavSG3	123-158	5.0	17	-0.02	1.00	8	-	0.82	10	-0.11	0.20	18	-0.05	0.83	18	0.04	0.57	17	0.11	0.11	11	-0.05	1.00	37	0.06	0.69	6	-	0.74	13	-0.08	1.00	8	-	0.46	9	-	0.34	38	0.00	0.26
Mav017	239-267	4.0	18	0.06	0.73	10	0.12	0.06	10	-0.16	0.86	19	0.06	0.09	17	-0.06	0.82	16	-0.14	0.05	10	-0.10	0.15	37	0.02	0.70	6	-	0.12	13	0.23	0.24	8	-	0.71	8	-	1.00	37	-0.09	0.15
Mav030	122-176	5.4	17	0.07	0.03	10	0.01	0.44	10	0.03	0.19	14	0.16	0.01	15	0.01	0.26	16	-0.07	0.05	9	-	0.52	35	0.04	0.08	6	-	1.00	13	0.08	0.52	7	-	0.11	8	-	0.39	37	0.05	0.66
Mav036	203-209	2.2	17	0.20	0.26	10	-0.07	1.00	10	0.09	0.57	19	-	-	16	0.06	0.44	17	-0.01	-	10	-0.14	1.00	37	0.11	0.30	6	-	0.23	13	-	-	7	-	0.63	9	-	-	31	0.16	0.03
Mav040	176-180	1.9	18	0.06	0.51	10	-0.21	0.22	9	-	-	18	-0.01	-	18	-0.05	1.00	17	-	-	9	-	1.00	36	-0.01	1.00	6	-	1.00	13	-	-	8	-	0.12	7	-	1.00	37	-0.05	0.63
Mav048	201-237	4.2	18	0.05	0.32	10	0.05	0.46	10	0.13	0.47	19	0.07	0.21	19	0.08	0.49	17	-0.21	0.17	11	0.06	0.30	36	-0.03	0.86	6	-	0.27	13	0.15	0.25	8	-	0.38	8	-	0.60	38	0.01	0.08
MavA5	236-254	4.6	18	0.21	0.07	9	-	0.52	9	-	1.00	15	-0.02	0.18	16	-0.03	0.94	15	0.44	**0.00**	11	-0.05	0.67	30	-0.07	0.77	5	-	0.65	9	-	0.18	8	-	1.00	7	-	0.18	34	0.15	0.03
MavSG6	171-190	3.5	18	0.10	0.13	10	-0.05	1.00	10	-0.14	0.73	18	-0.05	0.08	18	0.06	0.83	17	0.17	0.29	11	-0.02	0.36	37	0.03	0.68	6	-	1.00	13	-0.01	-	8	-	0.59	9	-	0.63	36	0.09	0.09
Mav034	229-247	2.2	18	-	-	9	-	0.22	10	-0.05	1.00	19	-0.02	1.00	19	-	-	16	-0.01	-	11	-0.19	0.48	37	-	-	5	-	1.00	12	-	-	8	-	1.00	9	-	1.00	37	0.09	0.35
Mav003	222-237	2.8	18	0.10	0.04	10	-	-	10	-0.13	0.52	15	0.38	0.00	19	0.01	1.00	17	0.45	0.03	11	0.00	0.61	35	0.03	0.31	6	-	1.00	11	-	-	8	-	0.02	9	-	1.00	37	-0.02	0.59
Mav049	204-220	3.5	18	0.22	0.16	10	-0.07	1.00	10	-0.16	0.16	16	0.45	0.00	18	0.02	0.91	17	-0.12	0.42	11	-0.03	0.56	35	0.04	0.30	6	-	1.00	10	-	-	8	-	0.30	9	-	0.37	36	0.03	0.34

Supplementary Table S3. Allele frequencies across all populations (Total) and for each population for 22 microsatellite loci used for population genetics analysis.

	Total													
Mav021	**139**	**161**	**165**	**169**	**173**	**177**	**181**	**185**	**189**	**193**	**197**	**201**		
	0.00	0.03	0.07	0.14	0.22	0.08	0.12	0.12	0.06	0.10	0.05	0.00		
Mav038	**259**	**261**	**263**	**265**	**267**	**269**								
	0.00	0.09	0.44	0.26	0.18	0.01								
Mav043	**160**	**164**	**168**	**172**	**176**	**180**	**184**							
	0.01	0.13	0.46	0.18	0.08	0.11	0.03							
Mav044	**126**	**130**	**134**	**138**	**142**	**145**	**148**	**150**	**152**	**156**	**160**			
	0.02	0.04	0.17	0.17	0.35	0.00	0.09	0.01	0.14	0.02	0.00			
MavSF10	**210**	**218**	**224**	**226**	**228**	**230**	**232**	**234**	**236**	**238**				
	0.01	0.00	0.24	0.17	0.27	0.11	0.10	0.08	0.02	0.01				
Mav011	**169**	**174**	**178**	**182**	**186**	**190**	**194**	**198**	**200**	**202**	**206**	**210**	**214**	
	0.03	0.12	0.02	0.02	0.09	0.25	0.14	0.10	0.01	0.07	0.12	0.03	0.02	
Mav053	**215**	**217**	**219**	**221**	**223**	**225**	**227**							
	0.09	0.17	0.39	0.28	0.04	0.03	0.01							
Mav015	**243**	**245**	**247**	**249**	**251**									
	0.12	0.10	0.38	0.39	0.02									
Mav032	**124**	**137**	**141**	**145**	**149**	**153**	**158**							
	0.03	0.04	0.12	0.39	0.40	0.03	0.00							
Mav051	**229**	**231**	**233**	**235**	**237**	**239**	**241**	**242**	**244**					
	0.06	0.05	0.08	0.39	0.03	0.18	0.15	0.03	0.03					
MavSH3	**203**	**205**	**207**	**209**	**211**	**213**	**217**							
	0.00	0.31	0.12	0.43	0.13	0.00	0.00							
MavSG3	**123**	**131**	**133**	**135**	**138**	**140**	**142**	**145**	**147**	**149**	**151**	**154**	**156**	**158**
	0.07	0.08	0.02	0.01	0.03	0.13	0.16	0.26	0.13	0.08	0.01	0.02	0.00	0.02
Mav017	**239**	**243**	**247**	**251**	**255**	**259**	**267**							
	0.17	0.24	0.35	0.11	0.11	0.02	0.00							
Mav030	**122**	**138**	**142**	**148**	**152**	**156**	**160**	**164**	**168**	**172**	**176**			
	0.00	0.07	0.05	0.01	0.15	0.23	0.21	0.12	0.11	0.05	0.01			
Mav036	**203**	**205**	**207**	**209**										
	0.17	0.76	0.06	0.01										
Mav040	**176**	**178**	**180**											
	0.71	0.27	0.02											
Mav048	**201**	**209**	**213**	**217**	**221**	**225**	**229**	**233**	**237**					
	0.01	0.02	0.20	0.11	0.30	0.23	0.10	0.02	0.02					
MavA5	**236**	**238**	**240**	**242**	**244**	**246**	**248**	**250**	**252**	**254**				
	0.04	0.16	0.29	0.16	0.16	0.03	0.10	0.05	0.01	0.00				
MavSG6	**171**	**173**	**175**	**177**	**179**	**181**	**183**	**185**	**188**	**190**				
	0.02	0.07	0.44	0.08	0.09	0.01	0.09	0.10	0.08	0.02				
Mav034	**229**	**230**	**237**	**239**	**241**	**243**	**247**							
	0.00	0.01	0.01	0.85	0.10	0.01	0.01							
Mav003	**222**	**225**	**228**	**231**	**234**	**237**								
	0.03	0.18	0.56	0.12	0.05	0.06								
Mav049	**204**	**208**	**212**	**216**	**220**									
	0.08	0.25	0.34	0.24	0.09									

	Cabilla						Cheddar					Darley				EastIvybridge							
Mav021	**139**	**161**	**165**	**169**	**173**		**173**	**177**	**181**	**185**	**193**	**169**	**181**	**185**		**173**	**177**	**181**	**185**	**189**	**193**		
	0.03	0.09	0.32	0.41	0.15		0.10	0.60	0.10	0.05	0.15	0.60	0.10	0.30		0.11	0.11	0.13	0.24	0.18	0.24		
Mav038	**263**	**267**					**261**	**263**	**265**	**267**		**265**	**267**			**259**	**261**	**263**	**265**	**267**	**269**		
	0.67	0.33					0.05	0.65	0.25	0.05		0.35	0.65			0.03	0.50	0.11	0.16	0.08	0.13		
Mav043	**164**	**168**	**172**	**176**	**180**		**160**	**164**	**168**	**172**		**164**	**168**	**172**	**184**	**168**	**172**	**176**	**180**	**184**			
	0.03	0.33	0.53	0.08	0.03		0.17	0.22	0.44	0.17		0.10	0.45	0.25	0.20	0.24	0.05	0.18	0.50	0.03			
Mav044	**130**	**134**	**138**	**142**			**134**	**138**	**142**	**148**		**134**	**138**			**126**	**130**	**138**	**142**	**148**	**152**		
	0.06	0.36	0.06	0.53			0.11	0.06	0.61	0.22		0.55	0.45			0.03	0.03	0.05	0.37	0.05	0.47		
MavSF10	**224**	**228**	**230**	**234**			**230**	**232**				**224**	**226**			**228**	**230**	**232**	**234**				
	0.56	0.28	0.14	0.03			0.10	0.90				0.60	0.40			0.42	0.06	0.19	0.33				
Mav011	**190**	**194**	**198**	**210**	**214**		**190**	**194**	**206**			**186**	**190**	**198**		**178**	**182**	**186**	**190**	**194**	**198**		
	0.58	0.22	0.03	0.03	0.14		0.25	0.55	0.20			0.05	0.55	0.40		0.09	0.09	0.18	0.50	0.03	0.12		
Mav053	**217**	**219**	**221**	**223**			**219**	**221**	**223**	**225**		**219**	**221**	**223**		**215**	**219**	**221**	**223**	**227**			
	0.03	0.36	0.58	0.03			0.10	0.25	0.05	0.60		0.80	0.15	0.05		0.03	0.37	0.37	0.13	0.11			
Mav015	**243**	**247**	**249**				**247**	**251**				**245**	**247**	**249**		**245**	**247**	**249**					
	0.42	0.06	0.53				0.56	0.44				0.10	0.20	0.70		0.35	0.44	0.21					
Mav032	**145**	**149**					**141**	**149**				**141**	**145**			**145**	**149**						
	0.50	0.50					0.44	0.56				0.25	0.75			0.53	0.47						
Mav051	**231**	**233**	**235**	**239**			**235**	**237**	**244**			**235**	**239**	**241**	**244**	**233**	**235**	**237**	**239**	**241**	**244**		
	0.03	0.56	0.28	0.14			0.72	0.22	0.06			0.45	0.05	0.40	0.10	0.03	0.56	0.06	0.12	0.21	0.03		
MavSH3	**205**	**207**	**209**				**205**	**207**	**209**	**211**		**205**	**209**	**211**		**205**	**207**	**209**					
	0.08	0.03	0.89				0.10	0.40	0.05	0.45		0.50	0.30	0.20		0.61	0.31	0.08					
MavSG3	**131**	**135**	**145**				**140**	**142**	**145**	**147**		**140**	**145**	**151**	**154**	**135**	**138**	**140**	**142**	**147**			
	0.94	0.03	0.03				0.25	0.06	0.13	0.56		0.60	0.25	0.05	0.10	0.08	0.03	0.03	0.72	0.14			
Mav017	**239**	**243**	**247**	**251**	**255**	**259**	**243**	**247**	**251**			**239**	**243**	**247**	**255**	**243**	**247**	**251**	**255**				
	0.03	0.36	0.22	0.06	0.25	0.08	0.30	0.60	0.10			0.45	0.20	0.30	0.05	0.03	0.16	0.63	0.18				
Mav030	**138**	**152**	**156**	**160**			**160**	**164**	**168**	**172**		**156**	**160**	**164**		**138**	**142**	**152**	**156**	**160**	**164**	**168**	**172**
	0.44	0.12	0.38	0.06			0.20	0.10	0.40	0.30		0.30	0.40	0.30		0.04	0.04	0.11	0.25	0.18	0.29	0.04	0.07
Mav036	**205**	**207**					**203**	**205**	**207**			**203**	**205**			**205**							
	0.68	0.32					0.05	0.85	0.10			0.40	0.60			1.00							
Mav040	**176**	**178**					**176**	**178**				**178**				**176**	**180**						
	0.81	0.19					0.65	0.35				1.00				0.97	0.03						
Mav048	**221**	**225**	**229**				**201**	**213**	**217**	**221**	**229**	**221**	**225**	**229**		**209**	**213**	**217**	**221**	**225**	**233**		
	0.44	0.42	0.14				0.20	0.15	0.20	0.35	0.10	0.75	0.20	0.05		0.13	0.24	0.13	0.34	0.13	0.03		
MavA5	**238**	**240**	**244**	**250**			**238**	**240**	**244**			**236**	**242**	**244**		**236**	**238**	**240**	**242**	**244**	**246**	**250**	
	0.28	0.67	0.03	0.03			0.28	0.61	0.11			0.28	0.11	0.61		0.03	0.17	0.13	0.47	0.07	0.03	0.10	
MavSG6	**175**	**177**	**179**				**175**	**177**				**173**	**185**	**188**		**171**	**179**	**183**	**185**				
	0.75	0.22	0.03				0.90	0.10				0.40	0.05	0.55		0.06	0.42	0.31	0.22				
Mav034	**239**						**230**	**237**	**239**	**241**	**247**	**239**	**241**			**229**	**239**	**241**					
	1.00						0.28	0.17	0.22	0.11	0.22	0.35	0.65			0.03	0.95	0.03					
Mav003	**222**	**228**	**231**				**225**					**222**	**225**	**228**	**231**	**225**	**228**	**237**					
	0.06	0.72	0.22				1.00					0.20	0.10	0.40	0.30	0.23	0.20	0.57					
Mav049	**208**	**212**					**208**	**212**	**216**			**212**	**216**	**220**		**208**	**212**	**216**					
	0.22	0.78					0.10	0.85	0.05			0.10	0.40	0.50		0.22	0.13	0.66					

	Haldon								HelmanTor									Middlewood						
Mav021	**173**	**177**	**181**	**193**					**161**	**165**	**169**							**165**	**169**	**173**	**185**	**189**	**193**	
	0.72	0.06	0.17	0.06					0.32	0.46	0.21							0.11	0.11	0.39	0.11	0.22	0.06	
Mav038	**263**	**265**	**267**						**263**	**267**								**265**	**267**					
	0.58	0.39	0.03						0.96	0.04								0.40	0.60					
Mav043	**164**	**168**	**172**						**164**	**168**								**168**						
	0.03	0.33	0.64						0.06	0.94								1.00						
Mav044	**126**	**130**	**138**	**142**	**148**	**150**	**152**	**156**	**130**	**134**	**138**	**142**	**152**					**134**	**138**	**142**				
	0.13	0.11	0.05	0.13	0.26	0.05	0.24	0.03	0.06	0.03	0.15	0.68	0.09					0.35	0.35	0.30				
MavSF10	**226**	**228**	**230**	**232**	**234**				**224**	**226**	**234**							**224**	**226**	**232**	**238**			
	0.26	0.53	0.13	0.03	0.05				0.85	0.12	0.03							0.60	0.05	0.25	0.10			
Mav011	**169**	**174**	**178**	**190**	**198**	**206**	**210**	**214**	**194**	**198**	**202**	**206**	**210**					**182**	**186**	**190**	**194**	**200**	**202**	**206**
	0.03	0.58	0.03	0.13	0.13	0.03	0.05	0.03	0.19	0.09	0.13	0.53	0.06					0.05	0.05	0.36	0.09	0.09	0.23	0.14
Mav053	**217**	**219**	**221**						**217**	**219**	**221**							**217**	**219**	**221**				
	0.66	0.32	0.03						0.26	0.21	0.53							0.18	0.68	0.14				
Mav015	**247**	**249**	**251**						**243**	**247**	**249**	**251**						**245**	**247**	**249**				
	0.53	0.44	0.03						0.66	0.03	0.28	0.03						0.05	0.14	0.82				
Mav032	**124**	**141**	**145**	**149**	**153**				**145**	**149**								**137**	**145**	**149**				
	0.09	0.18	0.15	0.47	0.12				0.09	0.91								0.14	0.77	0.09				
Mav051	**229**	**231**	**235**	**241**	**242**				**233**	**235**	**239**							**235**	**239**	**244**				
	0.29	0.34	0.18	0.13	0.05				0.22	0.16	0.63							0.55	0.36	0.09				
MavSH3	**205**	**207**	**209**	**211**	**213**				**209**	**211**								**205**	**209**	**211**				
	0.08	0.47	0.28	0.14	0.03				0.97	0.03								0.32	0.45	0.23				
MavSG3	**123**	**140**	**142**	**145**	**149**	**151**			**135**	**140**	**142**	**145**	**147**					**140**	**145**	**147**				
	0.03	0.03	0.53	0.36	0.03	0.03			0.03	0.18	0.03	0.71	0.06					0.45	0.45	0.09				
Mav017	**239**	**243**	**247**	**251**					**239**	**243**	**247**	**255**	**259**					**239**	**243**	**247**	**255**	**259**		
	0.12	0.15	0.59	0.15					0.03	0.28	0.53	0.03	0.13					0.25	0.10	0.25	0.30	0.10		
Mav030	**122**	**152**	**160**	**164**	**168**	**172**			**138**	**148**	**152**	**156**	**160**	**164**	**168**	**172**	**176**	**156**	**160**	**164**	**172**			
	0.03	0.53	0.07	0.07	0.27	0.03			0.34	0.03	0.03	0.03	0.25	0.13	0.13	0.03	0.03	0.56	0.17	0.17	0.11			
Mav036	**203**	**205**	**209**						**205**	**207**								**203**	**205**					
	0.09	0.81	0.09						0.97	0.03								0.25	0.75					
Mav040	**176**	**178**							**176**									**176**	**178**					
	0.11	0.89							1.00									0.44	0.56					
Mav048	**213**	**217**	**225**						**213**	**221**	**225**							**213**	**221**	**225**	**229**			
	0.26	0.50	0.24						0.15	0.62	0.24							0.05	0.32	0.14	0.50			
MavA5	**238**	**244**	**248**	**250**					**238**	**240**	**242**	**254**						**236**	**238**	**240**	**242**	**244**	**248**	
	0.13	0.28	0.41	0.19					0.70	0.23	0.03	0.03						0.23	0.09	0.05	0.09	0.18	0.36	
MavSG6	**171**	**175**	**179**	**181**	**185**				**175**	**177**								**173**	**175**	**185**	**188**	**190**		
	0.14	0.25	0.22	0.03	0.36				0.85	0.15								0.14	0.09	0.05	0.55	0.18		
Mav034	**239**								**239**	**241**								**239**	**241**					
	1.00								0.97	0.03								0.68	0.32					
Mav003	**225**	**228**							**225**	**228**	**231**							**222**	**228**	**231**				
	0.32	0.68							0.03	0.94	0.03							0.09	0.73	0.18				
Mav049	**208**	**212**	**216**						**204**	**208**	**212**	**216**	**220**					**208**	**212**	**216**	**220**			
	0.44	0.39	0.17						0.24	0.56	0.15	0.03	0.03					0.23	0.05	0.36	0.36			

	NewtonAbbott								NorthDevon					Okehampton								
Mav021	**169**	**173**	**177**	**185**	**193**	**197**	**201**		**173**	**177**	**181**	**185**	**189**	**173**	**177**	**181**	**185**	**189**	**193**	**197**		
	0.19	0.24	0.04	0.19	0.21	0.11	0.01		0.10	0.10	0.40	0.20	0.20	0.05	0.14	0.35	0.14	0.06	0.14	0.14		
Mav038	**261**	**263**	**265**	**267**					**263**	**265**	**267**			**259**	**261**	**263**	**265**	**267**				
	0.08	0.24	0.53	0.15					0.50	0.42	0.08			0.01	0.15	0.57	0.15	0.11				
Mav043	**164**	**168**	**172**	**176**	**180**				**164**	**172**	**176**	**180**		**164**	**168**	**172**	**176**	**180**				
	0.03	0.65	0.07	0.18	0.08				0.17	0.25	0.42	0.17		0.26	0.27	0.18	0.05	0.24				
Mav044	**126**	**130**	**138**	**142**	**148**	**152**	**156**	**160**	**134**	**142**				**130**	**134**	**138**	**142**	**145**	**148**	**150**	**152**	**156**
	0.01	0.03	0.01	0.50	0.12	0.22	0.09	0.01	0.92	0.08				0.04	0.11	0.30	0.22	0.01	0.16	0.01	0.12	0.03
MavSF10	**218**	**226**	**228**	**230**	**232**	**234**			**228**	**230**				**210**	**224**	**226**	**228**	**230**	**232**	**234**	**236**	
	0.01	0.20	0.32	0.35	0.01	0.09			0.83	0.17				0.05	0.03	0.19	0.34	0.05	0.12	0.14	0.08	
Mav011	**169**	**174**	**186**	**190**	**194**	**198**	**206**		**186**	**190**	**194**	**198**	**202**	**186**	**190**	**194**	**198**	**202**	**206**	**210**		
	0.14	0.38	0.03	0.08	0.22	0.10	0.06		0.08	0.50	0.17	0.17	0.08	0.32	0.19	0.09	0.09	0.20	0.05	0.04		
Mav053	**215**	**217**	**219**	**221**	**223**				**219**	**223**				**215**	**219**	**221**	**223**					
	0.13	0.42	0.42	0.01	0.03				0.75	0.25				0.39	0.36	0.23	0.01					
Mav015	**245**	**247**	**249**						**245**	**247**				**245**	**247**	**249**						
	0.15	0.38	0.47						0.25	0.75				0.01	0.66	0.33						
Mav032	**124**	**137**	**141**	**145**	**149**				**141**	**145**	**149**	**153**		**141**	**145**	**149**						
	0.11	0.04	0.26	0.28	0.31				0.17	0.58	0.08	0.17		0.13	0.53	0.33						
Mav051	**229**	**235**	**239**	**241**	**242**				**235**	**237**	**241**			**229**	**231**	**235**	**237**	**239**	**241**			
	0.11	0.42	0.01	0.34	0.12				0.17	0.25	0.58			0.10	0.07	0.58	0.03	0.10	0.13			
MavSH3	**205**	**207**	**209**	**211**					**205**	**207**	**209**	**211**		**203**	**205**	**209**	**211**	**217**				
	0.38	0.01	0.56	0.06					0.33	0.08	0.25	0.33		0.01	0.59	0.24	0.12	0.03				
MavSG3	**123**	**138**	**140**	**142**	**145**	**147**	**149**	**158**	**140**	**147**	**149**	**151**		**123**	**133**	**138**	**140**	**142**	**145**	**147**	**149**	**156**	**158**
	0.24	0.04	0.01	0.20	0.12	0.20	0.16	0.01	0.08	0.58	0.25	0.08		0.14	0.09	0.11	0.03	0.01	0.22	0.14	0.21	0.01	0.03
Mav017	**239**	**243**	**247**	**251**	**255**				**239**	**243**	**247**	**267**		**239**	**243**	**247**						
	0.36	0.16	0.27	0.18	0.03				0.33	0.17	0.42	0.08		0.18	0.45	0.38						
Mav030	**148**	**152**	**156**	**160**	**164**	**168**	**172**		**152**	**160**	**164**	**168**	**172**	**142**	**152**	**156**	**160**	**164**	**168**	**172**	**176**	
	0.03	0.29	0.11	0.24	0.14	0.16	0.03		0.25	0.25	0.08	0.08	0.33	0.23	0.11	0.28	0.20	0.08	0.07	0.01	0.01	
Mav036	**203**	**205**							**203**	**205**	**207**			**203**	**205**	**207**						
	0.38	0.62							0.50	0.42	0.08			0.16	0.68	0.16						
Mav040	**176**	**178**							**176**	**178**				**176**	**178**	**180**						
	0.96	0.04							0.42	0.58				0.80	0.15	0.05						
Mav048	**209**	**213**	**217**	**221**	**225**	**229**	**237**		**217**	**221**	**225**	**229**		**213**	**217**	**221**	**225**	**229**	**233**			
	0.03	0.60	0.07	0.07	0.07	0.07	0.10		0.17	0.08	0.17	0.58		0.13	0.12	0.21	0.36	0.08	0.11			
MavA5	**238**	**240**	**242**	**244**	**246**	**248**	**250**	**252**	**238**	**240**	**244**	**246**		**236**	**238**	**240**	**242**	**244**	**246**	**248**	**250**	
	0.05	0.55	0.20	0.05	0.02	0.05	0.02	0.07	0.30	0.30	0.10	0.30		0.01	0.06	0.16	0.37	0.19	0.06	0.13	0.01	
MavSG6	**175**	**177**	**181**	**185**	**190**				**175**	**177**	**183**			**173**	**175**	**177**	**179**	**183**				
	0.49	0.15	0.03	0.27	0.07				0.33	0.58	0.08			0.15	0.43	0.01	0.10	0.31				
Mav034	**239**								**239**	**241**	**243**			**239**	**241**	**243**						
	1.00								0.60	0.10	0.30			0.89	0.09	0.01						
Mav003	**225**	**228**	**231**	**234**	**237**				**222**	**225**	**228**			**225**	**228**	**231**	**234**					
	0.29	0.33	0.07	0.21	0.10				0.17	0.33	0.50			0.07	0.57	0.30	0.07					
Mav049	**204**	**208**	**212**	**216**	**220**				**208**	**212**	**216**			**204**	**208**	**212**	**216**	**220**				
	0.17	0.04	0.41	0.27	0.10				0.08	0.67	0.25			0.17	0.15	0.36	0.21	0.11				

	Penlan				Roadford								StaraWood			
Mav021	**169**	**173**			**173**	**181**	**185**	**189**	**197**				**169**	**181**	**185**	
	0.10	0.90			0.13	0.13	0.13	0.38	0.25				0.42	0.25	0.33	
Mav038	**263**				**261**	**263**	**265**						**265**	**267**		
	1.00				0.06	0.19	0.75						0.14	0.86		
Mav043	**164**	**168**			**160**	**164**	**168**	**172**	**184**				**168**	**184**		
	0.69	0.31			0.06	0.31	0.25	0.19	0.19				0.71	0.29		
Mav044	**130**	**134**	**142**	**152**	**138**	**142**	**148**						**134**	**138**	**142**	
	0.08	0.38	0.38	0.15	0.63	0.31	0.06						0.44	0.50	0.06	
MavSF10	**224**	**228**			**226**	**228**	**234**	**236**	**238**				**224**	**226**		
	0.77	0.23			0.44	0.25	0.13	0.06	0.13				0.33	0.67		
Mav011	**194**	**206**	**210**	**214**	**178**	**186**	**190**	**194**	**198**	**200**	**202**	**206**	**182**	**190**	**198**	
	0.08	0.54	0.21	0.17	0.19	0.06	0.06	0.19	0.13	0.06	0.19	0.13	0.19	0.69	0.13	
Mav053	**217**	**219**	**221**		**217**	**219**	**221**	**225**					**219**	**221**	**223**	
	0.04	0.12	0.85		0.13	0.38	0.44	0.06					0.61	0.33	0.06	
Mav015	**243**	**249**			**243**	**245**	**247**	**249**					**245**	**247**	**249**	
	0.50	0.50			0.06	0.50	0.31	0.13					0.06	0.72	0.22	
Mav032	**137**	**145**	**149**	**153**	**137**	**145**	**149**	**158**					**137**	**145**	**149**	
	0.08	0.12	0.65	0.15	0.19	0.31	0.44	0.06					0.17	0.72	0.11	
Mav051	**229**	**233**	**239**		**235**	**239**	**241**	**242**					**235**	**239**	**244**	
	0.04	0.23	0.73		0.56	0.19	0.19	0.06					0.19	0.38	0.44	
MavSH3	**205**	**207**	**209**		**205**	**207**	**211**						**205**	**209**	**211**	
	0.04	0.15	0.81		0.38	0.31	0.31						0.13	0.38	0.50	
MavSG3	**131**	**140**	**142**	**145**	**140**	**142**	**145**	**147**	**156**	**158**			**140**	**151**	**154**	
	0.04	0.04	0.08	0.85	0.19	0.06	0.31	0.13	0.06	0.25			0.67	0.06	0.28	
Mav017	**243**	**247**	**255**		**243**	**247**	**255**						**239**	**243**	**255**	
	0.15	0.46	0.38		0.31	0.44	0.25						0.38	0.19	0.44	
Mav030	**152**	**156**	**160**		**156**	**160**	**164**	**168**	**172**	**176**			**156**	**160**	**164**	
	0.19	0.77	0.04		0.07	0.29	0.21	0.29	0.07	0.07			0.19	0.75	0.06	
Mav036	**205**				**203**	**205**	**207**						**203**	**205**		
	1.00				0.36	0.57	0.07						0.06	0.94		
Mav040	**176**				**176**	**178**	**180**						**176**	**178**		
	1.00				0.25	0.44	0.31						0.43	0.57		
Mav048	**221**	**225**	**229**		**213**	**217**	**221**	**225**	**229**				**221**	**225**	**229**	
	0.58	0.38	0.04		0.31	0.06	0.13	0.31	0.19				0.69	0.19	0.13	
MavA5	**238**	**240**			**244**	**246**	**248**	**250**					**236**	**242**	**244**	
	0.17	0.83			0.25	0.06	0.31	0.38					0.29	0.14	0.57	
MavSG6	**173**	**175**			**175**	**177**	**179**	**183**					**173**	**185**	**188**	
	0.04	0.96			0.38	0.06	0.44	0.13					0.28	0.06	0.67	
Mav034	**239**				**239**	**241**							**239**	**241**		
	1.00				0.56	0.44							0.72	0.28		
Mav003	**228**				**225**	**228**	**231**						**222**	**228**		
	1.00				0.31	0.56	0.13						0.11	0.89		
Mav049	**208**				**204**	**208**	**212**	**216**	**220**				**208**	**212**	**216**	**220**
	1.00				0.06	0.44	0.13	0.25	0.13				0.17	0.22	0.56	0.06

Genetics analyses glossary

GENEMAPPER v3.7 software (Applied Biosystems)

This software facilitates the scoring of alleles from raw genotype data. The processed data can then be exported to a spreadsheet in a format for down-stream analysis.

MICROSATELLITE TOOLKIT (Park 2001)

This is an add-in utility for Excel, which we used to identify and rectify any incorrectly scored alleles. Preliminary error checking can be performed, such as to detect invalid allele sizes and population/sample names and missing genotypes through typing errors. This toolkit also can calculate some basic summary statistics and convert genotype data into formats for use in many population genetics software programs.

PEDANT v1.0 (Johnson & Haydon 2007)

We used this program to calculate the allelic dropout and false allele error rate per microsatellite loci, comparing the original scored genotypes to re-runs for 16% of our samples. Pedant allows the estimation of the maximum likelihood of error rates from duplicate genotypes when reference genotypes are not available, for which it also provides confidence intervals.

LOSITAN (Beaumont *et al*. 1996, Antao *et al*. 2008)

We employed LOSITAN to test the assumption that the microsatellite loci used in our analyses were not under selection and hence neutral. The software LOSITAN uses the Fst outlier method to detect loci under selection.

ML-RELATE (Kalinowski *et al.* 2006)

This program was used to calculate a maximum likelihood pair-wise relatedness coefficients for samples within each population using our genotype data. This was carried-out in order to identify genetic relationships in our samples. For relationships with a relatedness coefficient above 0.4 we removed one of the samples, hence removing close relatives, which may bias population genetics analyses.

CERVUS v3.0 (Kalinowski *et al*. 2007)

This software package uses allele frequencies for likelihood parentage testing. The preliminary analysis produces a range of summary statistics that includes the estimated frequency of null alleles, which we used to check and select out all loci per population, for further downstream

population genetics analyses. CERVUS also converts genotype data into several formats for use in other statistical packages, such as GENEPOP.

GENEPOP v4.0.10 (Raymond & Rousset 1995, Rousset 2008)

This program is available for use both on the internet, and to download to a PC, and performs a variety of population genetics analyses. We used the program to test if our loci adhered to the Hardy-Weinberg genotypic equilibrium and linkage disequilibrium for each population. These are important, underlying assumptions required for many population genetics analyses. We also performed a global multi-sample score test across loci for each population, to identify deviations from Hardy-Weinberg due to heterozygosity deficiency. Finally we calculated the inbreeding coefficient (F_{IS}) using Weir & Cockerham's (1984) method.

ADZE v1.0 (Szpiech *et al.* 2008)

Here, the rarefaction method is employed in order to correct for variation in sample size between populations, and therefore more accurately calculate the frequency of private alleles and allelic richness for each analysed population. This methodology is important as these measures of genetic diversity are sensitive to sample size.

ARLEQUIN v3.5 (Excoffier & Lischer 2010)

This statistical package provides the user with a wide range of population genetics tests, from simple intra-population summary statistics to inter-population analyses. We used this program to calculate the mean and standard deviation for the number of alleles, and observed and expected heterozygosities. We also used performed several locus-by-locus AMOVAs in order to evaluate the amount of genetic differentiation in our data (Excoffier *et al.* 1992). Additionally, Garza-Williamson ratio (Garza & Williamson 2001) was calculated using this software. This parameter is based on the number of alleles relative to allele size range. After a bottleneck, increased genetic drift will reduce the number of alleles in a population, however only the loss of the largest and/or smallest alleles will affect allele size range. Therefore the number of alleles is likely to be reduced more rapidly than allelic range, resulting in a smaller ratio of allelic number to size range in a population which has experienced a bottleneck and thus not in equilibrium. This method is more sensitive than the method of Cornuet & Luikart (1997) at detecting bottlenecks that have occurred further back in time (Garza & Williamson 2001).

BOTTLENECK v1.2.02 (Cornuet & Luikart 1997)

This program has been designed to identify recent bottlenecks in populations using genotype data. The methodology uses a comparison of heterozygosity and allelic diversity parameters, as the latter is lost more rapidly after a bottleneck event. Populations that have experienced a recent bottleneck will exhibit an observed heterozygosity excess compared to the expected heterozygosity from the population's observed number of alleles, assuming the loci are at mutation-drift equilibrium. We used two-phase mutational model, as it has been suggested to provide the most accurate characterisation of the microsatellite mutation process (Di Rienzo *et al*. 1994).

STRUCTURE v2.3.3 (Pritchard *et al*. 2000, Falush *et al*. 2003)

STRUCTURE uses Bayesian clustering to assign individuals to populations based solely on genotype data with no *a priori* information on sample location Since the true number of populations (*K*), in the study area is unknown separate models are run for a range of hypothesised *K* values. For each *K* run a Markov Chain Monte Carlo approach is used to assign each individual to one or more of the *K* clusters, which are characterised by allele frequencies. The assignments maximise clusters adherence to Hardy-Weinberg and linkage equilibrium. Individuals are given a membership coefficient (*Q*) for each cluster, which is a probabilistic value of the individual's ancestry for that cluster. Where individuals are admixed they may be assigned to two or more clusters. Each *K* model should be run independently several times to check for consistent estimates. Each *K* model is given an estimated log probability of data value, which can be used to determine which is the most appropriate number of *K*.The log-probability of data can indicate which value of *K* is most likely, depending on the highest value and where data points for independent runs for the same *K* converge. As the interpretation of these plots can be challenging Evanno *et al*. (2005) suggest using the delta *K* statistic, which uses the rate of change in log-probability of data between successive *K* values to determine the most likely *K* value. The bar plots produced in STRUCTURE are also vital in the interpretation of the data, where *Q* for each individual for all clusters is displayed, with each vertical bar corresponding to an individual and each cluster represented by a different colour.

STRUCTURE HARVESTER v0.6.92 (Earl & vonHoldt 2011)

This online tool allows the rapid processing and visualisation of STRUCTURE results, by summarising and plotting Ln probability of data, and Delta K against K, as per Evanno *et al*. (2005). Output files for use the software CLUMMP are also generated.

CLUMMP v1.1.2 (Jakobsson & Rosenberg 2007)

CLUMMP summarises repeat STRUCTURE output runs for a given K. The choice of combination of algorithm parameters is likely to be driven by computation time. The output file can then be adjusted for use in DISTRUCT.

DISTRUCT v1.1 (Rosenberg 2004)

This software package is used to construct and edit graphical displays of labelled bar plots from STRUCTURE analyses, which show the membership coefficient (*Q)* for each individual for all clusters, for a particular K.

BAPS (Corander *et al*. 2008)

Using Bayesian algorithms, BAPS characterises individuals into population genetic clusters using allele frequencies. BAPS allows the incorporation of spatial data into the model, which, assuming that clusters are somewhat spatially smooth, increases the power of the model to correctly define the genetic population structure.

POPULATIONS v1.2.32 (Langella 1999)

We used this software to plot a neighbour-joining tree with genetic distances, using the bootstrapping option, which calculates the support for the tree nodes. We used Nei's standard genetic distance, which measures the proportion of alleles that are different between pairwise comparisons of populations (Nei 1972). The output file was then used in TREEVIEW to generate a graphic.

TREEVIEW v1.6.6 (Page 1996).

This is a tree-plotting software package, which allows the editing of genetic tree data, such as those generated by analysis in POPULATIONS.

MICROSATELLITE ANALYSER v4.05 (Dieringer & Schlötterer 2003)

This package allows the generation of a variety of microsatellite summary statistics and converts genotype datasets into several formats for use in genetic analysis software programs. We used this program to generate the FST matrix, which allows interpretations to be made concerning the extent of genetic differentiation amongst sampled populations. We used the Weir & Cockerham (1984) method, which was preferable it as takes sample size and uneven sample sizes into account. Wright's (1978) F_{ST} interpretation guidelines state that values

ranging from 0.05-0.15 indicate moderate, 0.15-0.25 great and above 0.25 very great, genetic differentiation.

SPAGeDi v1.3 (Hardy & Vekemans 2002)

SPAGeDI, or Spatial Pattern Analysis of Genetic Diversity, uses spatial coordinates and genotype data to perform spatial genetic analyses. We used this software to investigate the presence of isolation by distance (IBD) in our samples. IBD refers to the propensity of individuals to reproduce with others that are geographically close. This results in a clinal distribution of genetic differentiation in relation to spatial distance across a species range, with populations that are geographically more distant also being more genetically differentiated and is due to limited dispersal and continuous distribution (Wright 1943). In our analysis employed permutation tests, which is equivalent to a Mantel test.

Chapter 6: Multiple paternity in the hazel dormouse:

not so promiscuous after all?

Abstract

Polyandry, leading to multiple paternity within litters, has been demonstrated across a range of animal taxa and therefore is an important phenomenon in the mating systems of many species. Complex interactions between life history, ecological and evolutionary mechanisms drive the prevalence of multiple paternity, which in turn will impact upon population parameters such as effective population size and genetic diversity. As such, information on reproductive behaviour will better inform habitat management, mitigation and captive breeding programmes for species of conservation concern.

Here, we utilise 22 microsatellite markers to investigate the occurrence and frequency of multiple paternity in 12 litters of the hazel dormouse, Muscardinus avellanarius*, from populations in southwest England. We use group-wide maximum likelihood and probability-based pairwise sibship analyses in the program COLONY, which are based on allele-frequencies.*

We detected multiple paternity in 50% of litters, which is significantly lower than previously described for hazel dormouse populations investigated in Wales. Of the six litters that exhibited multiple paternity, analyses inferred that four litters were sired by two males, one by three males and one by four males.

It is unclear what is responsible for the conflicting results between the previous study and our work. Therefore we discuss the technical, ecological and evolutionary effects that may be driving the variation in multiple paternity rates described by the two different studies and highlight important areas of further research.

Introduction

Studies pertaining to natural mating systems not only contribute to the growing knowledge of life histories, but also have important implications for populations of conservation concern. This includes how mating systems influence effective population size and genetic diversity, and informing captive breeding programmes (Martinez *et al*. 2000, Moore *et al*. 2008, Sugg & Chesser 1994). Clearly, it is important that information on natural behaviour is incorporated into conservation management plans (Anthony & Blumstein 2000).

Increasingly, genetic markers have been employed to infer relatedness and the nature of mating systems (Jones & Ardren 2003, Walling *et al*. 2010), often yielding results that vary greatly from studies based on behavioural observations (Hughes 1998). For example, the prevalence of multiple mating by females, has proven to be much more common than previously recognised (Griffith *et al*. 2002, Zeh & Zeh 2001). Polyandry has now been demonstrated across many animal taxa, including insects (Arnqvist & Nilsson 2000), reptiles (Uller & Olsson 2008), birds (Griffith *et al*. 2002) and mammals (Soulsbury 2010). Assuming females produce two or more offspring, and more than one mated male is successful at fertilising ova, such behaviour leads to multiple paternity within a litter (Dean *et al*. 2006).

Despite the acceptance that polyandry is prevalent, the drivers of such mechanisms continue to be debated. Breeding behaviour is associated with costs, including time spent searching for a mate, antagonistic interactions and increased risk of mortality through predation and disease transmission (Daly 1978). Subsequently, the traditional assumption was that females are passive and should not actively exhibit polyandry, as their optimal reproductive success generally requires just one mating event (Bateman 1948, Uller & Olsson 2008). Multiple mating by females may occur through sexual conflict, for example for cost avoidance of resisting male harassment (Arnqvist 1989, Clutton-Brock & Parker 1995). However, these assumptions have since been contested, hypothesising that females actively seek multiple mates, as this behaviour is adaptive, conferring increased lifetime and reproductive fitness (Arnqvist & Nilsson 2000, Birkhead & Møller 1998, Hosken & Stockley 2003). Berteaux *et al*. (1999) revealed, through the use of tethered male meadow voles, *Microtus*

pennsylvanicus, that females actively choose to mate with multiple males. However, other studies find evidence lacking for many of the suggested benefits to females and maintain polyandry can be explained by sexual conflict interactions (Akçkay & Roughgarden 2007, Westneat & Stewart 2003).

Furthermore, the nature of how multiple mating may increase female fitness continues to be debated (Jennions & Petrie 2000, Stockley 2003). Where males provide direct benefits such as nuptial gifts and parental care, multiple-mates may confer additional benefits to the female, assuming it does not lead to divorce (Arnqvist & Nilsson 2000, Hosken & Stockley 2003). If infanticide by males is prevalent, uncertainty in paternity may reduce such behaviour (Hrdy 1979, Wolff & Macdonald 2004). Bet-hedging through insemination from multiple males will confer fertilisation insurance (Hoogland 1998). Polyandry may also be driven by indirect genetic benefits, such as reduced inbreeding, increased genetic diversity of offspring and the increased likelihood of compatibility of genotypes between parents (Colegrave *et al*. 2002, Mays & Hill 2004, Tregenza & Wedell 2002, Zeh & Zeh 2001). Indeed, polyandry has been associated with reduced early embryonic failure (Stockley 2003). At the population level, multiple paternity may increase effective population size, reducing inbreeding and loss of genetic diversity through genetic drift (Anthony & Blumstein 2000, Snugg & Chasser 1994, but see Karl 2008).

Here, we use microsatellites to focus on multiple paternity in the hazel dormouse, *Muscardinus avellanarius*. Relatively low population densities, reproductive potential and dispersal ability makes this small rodent especially vulnerable to habitat degradation, loss and fragmentation, mechanisms often driven by anthropogenic actions (Bright 1993, Bright & Morris 1996). The resultant small, isolated dormouse populations are prone to genetic drift and inbreeding (Frankham 1995). Mating systems will also affect these ecological and evolutionary parameters and therefore a better understanding of dormouse breeding behaviour is vital for its informed conservation. A captive breeding and reintroduction programme is underway in the UK, with the aim of reinstating dormice to parts of their historical range (Mitchell-Jones & White 2009). The management of this programme will benefit from knowledge of the natural mating systems and information on how genetic diversity is maintained in the wild (Moore *et al.* 2008).

We can make a prediction regarding the relative rate of multiple paternity in hazel dormice, based on comparative analyses with other rodents that occupy similar niches. Two species of small rodents found in England in similar habitats to hazel dormice are wood mice *Apodemus sylvaticus* and bank voles *Myodes glareolus.* Recorded rates of multiple paternity in litters of wood mice are moderately high at 50% to 68.2% (Baker *et al*. 1999, Bryja *et al*. 2008) and average at 35.5% for bank voles (Soulsbury 2010). Relative testes size correlates positively with rates of multiple paternity across rodent species, due to sperm competition (Ramm *et al*. 2005, Soulsbury 2010). Hazel dormice do not have prominent testes and therefore this morphological trait is not used to distinguish between sexes in the field, whereas reproductively mature wood mice and voles can be confidently sexed because males have very evident testes (Bright & Morris 2005). Indeed, wood mice are reported to have amongst the highest relative testes to body size ratio across the rodents (Moore *et al*. 2002). Based on this premise we would hypothesise levels of polyandry will be lower in hazel dormice compared to these other species. It should, however, be noted that quantitative data on relative dormouse testes size is not available and so we cannot rule out the possibility that hazel dormice possess large internal testes.

Further support for the hypothesis that hazel dormice would exhibit lower multiple paternity rates than other rodent species may come from a consideration of mating systems, although the information currently available is scarce. Male hazel dormice are territorial during the mating season and have larger home ranges than females, which overlap with several females (Bright & Morris 1991, Juškaitis 2008). Male dormice leave hibernation earlier than females, possibly to secure high quality home ranges in advance of the breeding season (Juškaitis 2008). This suggests that dominant male dormice monopolise access to females, which would reduce opportunities for subordinate males to mate with females and hence the prevalence of multiple paternity. In contrast, wood mice are thought to exhibit scramble competition in mate selection, whereby access to females is not monopolised by just a few males, leading to substantial promiscuity and subsequent high levels of multiple paternity (Moore *et al*. 2002). Similarly, in many rodent species that exhibit the highest levels of multiple paternity, mate chasing occurs, where several males mate with one female during the short period in which she is receptive (e.g. yellow-pine chipmunks, Tamias

amoenus (Schulte-Hostedde *et al*. 2004) and the North American red squirrel, *Tamiasciurus hudsonicus,* (McFarlane *et al*. 2010)). However, current information on mating systems in hazel dormice also suggests that the females are also territorial during the breeding season, presumably as females are likely to require high quality habitats with sufficient food supplies that are patchily distributed (Juškaitis 2008, Ściński & Borowski 2007). Such promiscuous mating systems where males have large home ranges and compete over receptive, territorial females have been observed in other arboreal rodents, such as the edible dormouse, *Glis glis,* (Ściński & Borowski 2007) and the red squirrel, *Sciurus vulgaris* (Lee 2001). Such behaviour would lead to intra-sexual male competition when males aggregate around a receptive female, and thus potentially relatively higher levels of polyandry. However, there is evidence that female hazel dormice are not, at least consistently, territorial, such as evidenced by the occasions when litters from two different females are amalgamated into a crèche (Bright et al. 2006).

However, a very high proportion (95%) of hazel dormouse litters were reported to show multiple paternity in populations in Wales (Md. Naim *et al*. 2011). This is amongst the highest reported in mammals, based on a meta-analysis study by Soulsbury (2010), where the mean rate of multiple paternity across mammals was 35.7% (range 0–92%). As such, hazel dormice lie above the range of multiple paternity frequency described in this meta-analysis. This discrepancy from our predictions may be due to other life history parameters confounding expected correlations between testes size and multiple paternity rate, such as length of breeding season (Ribble & Millar 1992, Soulsbury 2010). Alternatively, the high rate in multiple paternity revealed by Md. Naim *et al*. (2011) may be due to ecological or evolutionary mechanisms particular to the populations sampled. Indeed, great variation exists in multiple paternity rates amongst populations of other species (Dean *et al*. 2006, Petrie & Kempanaers 1998).

Therefore, further investigation of multiple paternity in alternative hazel dormice populations is warranted. This would allow an assessment of consistency between studies and populations and to confirm if hazel dormice are generally prone

to very high levels of polyandry. To this end, we employed polymorphic microsatellite markers to estimate the frequency of multiple paternity in litters of hazel dormice populations from south west England.

Methods

Sampling

Genetic samples suitable for our parentage analysis were obtained from five woodland sites in the counties of Cornwall and Devon which are located in the southwest of England. Samples were collected from April 2009 to October 2011, as part of a larger study on dormouse population genetics (Chapter 5). Access to wild dormice was possible through the routine monitoring of dedicated nest box schemes, which are part of a long-term national dormouse monitoring programme (Bright *et al*. 2006). Genetic material was taken by plucking 1-3 small clumps of fur from animals. Samples were taken under Natural England licence (numbers 20090841, 20100962 and 20111228). Data on sampling date, woodland site (including an OS grid reference), nest box number, sex, weight, age (juvenile or adult), reproductive condition, torpor state (torpid or active) and any distinguishing features (e.g. short/missing tail) were recorded for each dormouse. Hair samples were stored dry in micro centrifuge tubes at -20°C.

Genotyping

For all samples DNA was extracted using Chelex (Walsh *et al*. 1991). PCR conditions and profile were performed as in Chapter 4, using the 28 loci as previously described in Chapter 5 of this thesis. Amplified products were genotyped in an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems) and allele sizes determined using GENEMAPPER v3.7 software (Applied Biosystems). After the loci selection methods in Chapter 5, 22 loci were selected for parentage analysis.

Parentage analysis

Analysis was carried out in COLONY v2.0.1.9 (Wang 2004, Wang & Santure 2009). Compared to other parentage programs, COLONY utilises known information on relatedness, such as siblings from the same litter. This allows for more correct paternities to be assigned, and produces fewer incorrect assignments through the use of individual rather than population-level confidences (Walling *et al*. 2010). Parentage inferences were determined using group-wide maximum likelihoods, which incorporate prior family relationship data (i.e. known siblings, mothers and fathers) in order to estimate the best maximum likelihood relatedness configurations. These results were then used to determine the most likely number of fathers per litter and therefore ascertain if multiple paternity has occurred. Further, we contrasted these results to probability-based pairwise sibship relationships between litter mates, also using COLONY. Through the examination of the proportion of full and half siblings within a litter, this latter analysis allows us to infer if the mother of each litter mated - and successfully produced offspring - with multiple males. The maximum likelihood configuration methods are more vigorous as all related individuals in the cluster are used in the analysis, rather than just pair-wise comparisons when analysing sibship data. However, the latter analysis allows comparison of two different methodologies, which further ensures data is robust.

As COLONY analysis is based on allele frequencies, each population was run independently to ensure accurate paternity estimates. Different years were also run separately, so that between-generation relationships would not confound the results. The parameters used were as follows. We assumed both males and females exhibit polygamy, to allow for the presence of paternal or maternal half siblings. Inbreeding was allowed for, as this is possible in our study species which is insular and as our sample size was small computation time would not be constraining. We used a medium run length and the full likelihood method, which has been shown to be the most accurate (Wang 2004; Wang & Santure 2009). We did not employ updating of allele frequencies, as our family sizes were small and therefore our analysis would gain little benefit from this option. The microsatellite error rate, which is required by COLONY for each locus, was based on the genotyping error and allelic dropout rate determined from analyses in Chapter 5.

For each separate analysis, all dormouse samples that were successfully genotyped for that population and year were input into COLONY. This provided COLONY with a larger sample size in which to calculate the allele frequencies and therefore improve accuracy. Dormouse genotypes were classified either as: juveniles, adult males or adult females. This was based on the field data provided by experienced monitors in the field. Age classification was based on a combination of weight and fur colour, and sex was determined for adults by inspection of genitalia. Determining the sex of juveniles is often inconclusive and not necessary for our analyses and so was not incorporated. Any samples with the necessary field data missing were omitted from further analyses. All three sets of classified genotypes were then uploaded into COLONY, using an estimated probability of 0.4 that the mother and father were present in the sample genotypes.

Known relationships based on field data were also input into COLONY for analysis. We defined litters as groups of juveniles of the same age (i.e. similar weights), sharing the same dormouse nest box on the same date, with or without adults also in the same box. Using the field data, we selected litters that comprised of three or more juveniles because the confidence in detecting multiple paternity increases with number of juveniles sampled. In 11 of the 12 litters, one or more adults were found in the same box. Here the adult genotypes were manually compared to each of the juveniles to determine if the adult was likely to be a parent of the offspring. This was to confirm sibship, as there are reports of different females combining litters into a "crèche" (Bright *et al*. 2006). Adults in the same nest box were assumed to be the parent of a juvenile if, across all loci, at least one of the adult's alleles was present in each of the juvenile's locus genotypes. If there was only one genotype mismatch, from the 22 loci, between potential parent-offspring pairs, the genotype scoring was checked and if the disparity could be reliably attributed to genotyping error the conflicting loci genotypes were removed from further analysis from both the adult and juvenile. Where there were two or more mismatches we did not assume that the adult was the parent, a situation which occurred in two litters. In one litter, two adult females and one adult male were present and by using the above methodology, one female was confirmed as the mother and the other two adults were assumed not to be the parents as they had a total of 15 mismatches with the juveniles. Within the second

litter there was one adult female and male and, whilst the female was confirmed as the mother, the male demonstrated 7-8 mismatches with each juvenile and therefore was discounted as the father. The inferred parental relationships were input into COLONY, as known maternal and/or paternal sibling groups. For families with three or more juveniles sharing a nest box, but no adult present, these relationships were input into COLONY as known siblings, with no adults assigned as the parent. We also checked that no litters within the same site/year were repeat samples, by manually comparing individuals' genotypes.

Once computations were complete the output was summarised, using the maximum likelihood configuration results to determine the number of fathers per litter. Analyses were replicated three times to check for consistency between runs. Additionally, to assess concurrence with this configuration data, the pairwise sibship data was summarised, giving the number of full-sibling and half-sibling relationships present in each litter. Litters with at least one relationship of half-siblings, above the probability threshold of 0.70 were assumed to show reasonable evidence of multiple paternity.

A Chi-squared test was used to determine if there is a significant difference in rates of multiple paternity between this study and that conducted in Wales by Md. Naim *et al*. (2011). A Welch two sample t-test was used to detect any differences in the mean weights of the known mothers of the litter between those that displayed multiple paternity and those that did not. An ANOVA and F-test were performed to investigate if litter size was influenced by the weight of the mother. All tests were carried out in R v2.14.1 (R Foundation for Statistical Computing 2011).

Results

Sampling

A total of 216 dormouse samples were used in the analyses, (98 juvenile, 60 adult female and 58 adult male). We performed eight analyses over five sites from Cornwall and Devon, across the three-year period of 2009-2011, which comprised 12 litters (table 1). The mean number of juveniles per litter was 4.75 (SD = 1.42, range 3-

8). Table 1 summarises the number of genotypes included in each site/year analysis, for the three classified groups, juveniles, adult females and adult males, as well as the number of predefined sibship groups within each of these analyses.

Loci selection

All chosen loci had an amplification rate above 85% (for samples that were amplified in a minimum of 50% of the loci) and an error rate below 5%. Chosen loci showed no evidence of a consistently high frequency of null alleles or significant deviations from Hardy-Weinberg equilibrium or evidence of linkage disequilibrium across populations (after correction for multiple tests using the False Discovery Rate). Additionally all loci were not found to be under selection or sex-linked. See Chapter 5 for summary statistics for each locus and population.

Multiple paternity analysis

For 11 of the 12 litters a female adult that was present in the nest box was confirmed by our analyses to be the mother of all offspring in the litter. Additionally, for one litter a male present in the nest box was assigned as the father of all juveniles of that litter (Newton Abbott 2009 mother ID 15 father ID 14). Only one litter had no parent assigned to it.

The best maximum likelihood configuration data from COLONY indicate that six, (50 %), of the 12 analysed litters had more than one sire. For all but one litter a single female was confirmed as the mother to all juveniles, and therefore provides robust analysis. For the litter with no known maternal genotype, the analysis strongly suggests all offspring are half siblings, again indicating polyandry, although we cannot rule out the possibility that juveniles were from different mothers. All three re-runs were consistent, all providing the same familial configurations across the 12 analysed litters. Figure 1 shows the frequency of the number of inferred fathers per litter and table 2 summarises all COLONY output.

Table 1. Sample size summary for each site/year used in the COLONY analyses, whereby genotypes are classified into juveniles, adult males and adult females. The number of litters represents the number of known and predefined sibship groups, with three or more juveniles, within the samples.

Site	Year	No. juvenile genotypes	No. adult female genotypes	No. adult male genotypes	No. litters
Haldon	2009	16	5	3	2
	2011	5	1	8	1
Newton Abbott	2009	18	7	2	2
	2011	9	11	17	1
Okehampton	2009	10	8	7	1
Roadford	2010	8	1	0	1
West Bodmin	2010	20	8	5	2
	2011	12	19	16	2

Analysis of the sibship data concurs with the configuration results, where those litters with at least one half-sibling relationship, above the probability threshold of 0.70, further confirms evidence of dormice multiple paternity in the same litters (table 2). Only one litter (West Bodmin, 2011, mother ID 468) had slightly differing conclusions from the two analytical approaches, where the configuration infers multiple paternity but the sibship data were inconclusive. As explained in the methodology section, configuration analysis is more robust and therefore we are more confident in the conclusion that this litter does have multiple paternity.

Of the four litters that demonstrated multiple paternity and had more than three juveniles sampled, three showed a skew in the number of offspring sired by the different males. In the non-biased litter, four different males were inferred as having sired the four juveniles.

The rate of multiple paternity of 50% in this study of dormouse litters in south west England was significantly lower, compared to the study by Md. Naim *et al*. (2011), which was conducted in Wales and found multiple paternity in 95% of the 20 litters they analysed ($X^2_1 = 6.45$, $p = 0.011$).

There was no significant difference in maternal weight between monandrous and polyandrous litters ($t = -0.20$, $df = 7.8$, *p*-value = 0.85). Finally there was no correlation between litter size and weight of the mother ($F_{1,10} = 1.79$, *p*-value = 0.21). However, as the sample sizes were small, it is likely our data may not have the power to detect such signals.

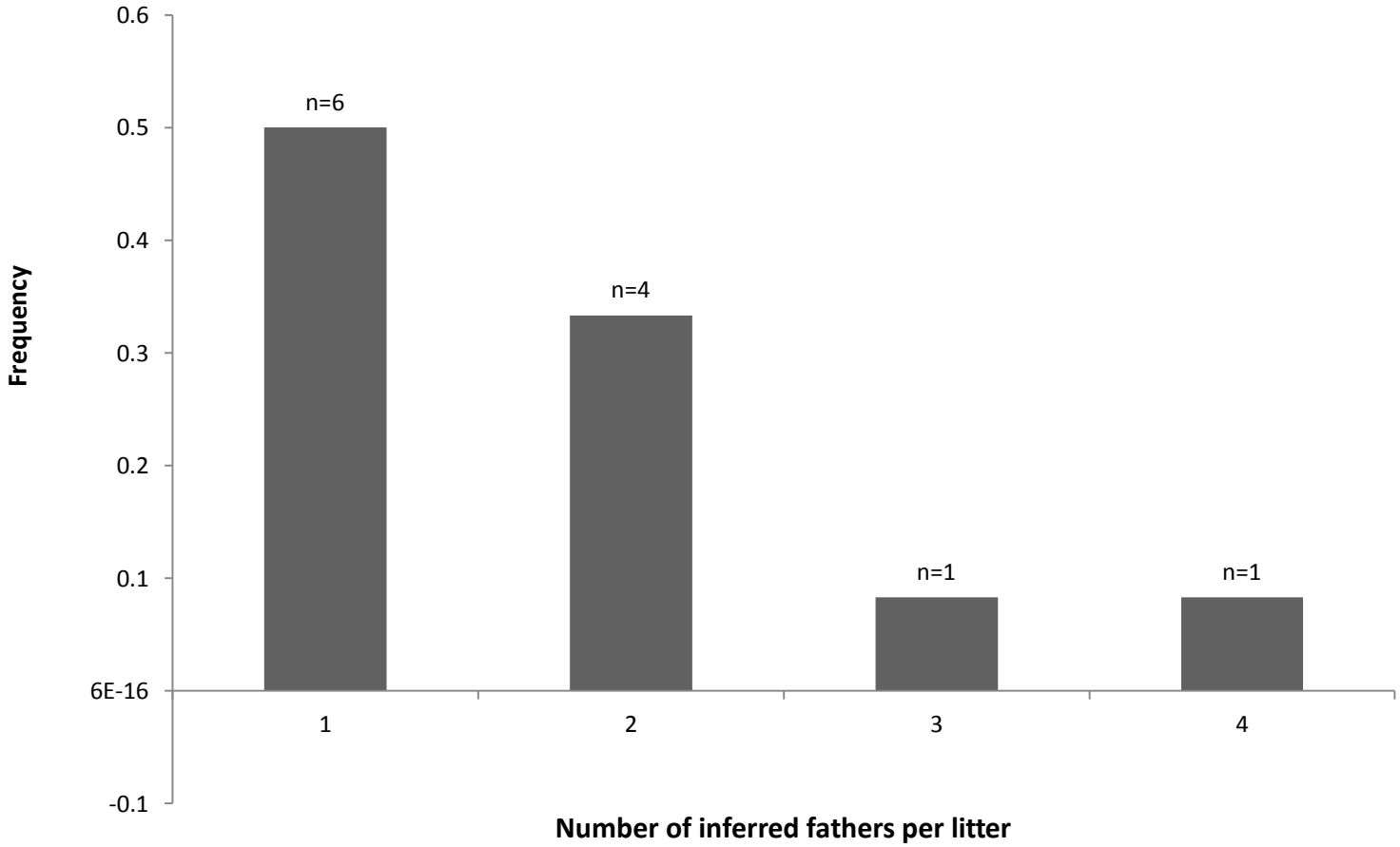

Figure 1. Frequency of number of fathers per litter across all populations and years, for the 13 litters sampled in Devon and Cornwall

Table 2. Parentage results summary from COLONY analysis for each of the 12 litters. Maximum likelihood configuration: Mother ID = identification number of the female assigned to the litter, No. juveniles = number of juveniles found in the litter, No. fathers = number of males that sired the litter, Ratio = ratio of juveniles sired by the different fathers for that litter. Sibship probability: the percentage of full, half sibling relationships within in each litter where probability is above 0.7, for pairwise comparisons below 0.7 the relatedness was classified as uncertain. Multiple paternity? =presence/absence of evidence for multiple paternity for each analysis method.

				Maximum Likelihood Configuration			Sibship Probability			
Site	**Year**	**Mother ID**	**No. juveniles**	**No. fathers**	**Ratio**	**Multiple paternity?**	**% full siblings**	**% half siblings**	**% uncertain sibship**	**Multiple paternity?**
Haldon	2009	95	6	1	1	No	100	0	0	No
	2009	96	6	3	4:1:1	Yes	40	60	0	Yes
	2011	No female in box	4	4	1:1:1:1	Yes	0	100	0	Yes
Newton Abbott	2009	15	5	1	1	No	100	0	0	No
	2009	28	5	1	1	No	100	0	0	No
	2011	606	4	1	1	No	100	0	0	No
Okehampton	2009	115	4	1	1	No	100	0	0	No
Roadford	2010	59	8	2	5:3	Yes	14	54	32	Yes
West Bodmin	2010	256	5	1	1	No	100	0	0	No
	2010	299	3	2	2:1	Yes	33	67	0	Yes
	2011	381	3	2	2:1	Yes	0	33	67	Yes
	2011	468	4	2	3:1	Yes	50	0	50	Inconclusive

Discussion

Our findings have revealed a moderate rate of multiple paternity, at 50% of the 12 litters across sampled populations in the south west of England. This frequency of multiple paternity is substantially lower than the 95% previously reported for two hazel dormouse populations and 20 litters in north Wales (Md. Naim *et al*. 2011). Hence our findings concur more closely with the prediction that rates of multiple paternity will be related to testes size. However, our results do still indicate a frequency of multiple paternity that is above the mean across mammals, suggesting hazel dormice harbour life history traits that predispose the species to moderate to high levels of multiple paternity (Soulsbury 2010).

For example, it has been demonstrated that female-female competition for suitable nesting territories results in a relatively lower proportion of hazel dormouse females within the breeding population (Juškaitis 2008). Such female territoriality may lead to aggregations of males at locations of receptive females, promoting promiscuity, as shown in edible dormice (Ściński & Borowski 2007) and the red squirrel (Lee 2001). Such a mating system would likely result in a high frequency of multiple paternity, Further studies on spatial organisation and mating systems are required to clarify the mating systems of hazel dormice and other arboreal mammals.

It is not possible to provide direct evidence for the cause of the discrepancy between the two studies. However, it is unlikely that a single multiple paternity rate will be applicable across a range of populations (Dean *et al*. 2006). Indeed, great variation in mating behaviour has been identified both amongst and within species, due to the complex interplay of ecological and evolutionary mechanisms that drive costs, benefits and constraints on reproductive behaviour (Petrie & Kempanaers 1998). Population density will affect mate encounter rates, which in turn determines the relative trade-offs between time searching for males versus any conferred benefits of polyandry to reproductive success (Dean *et al*. 2006, Uller & Olsson 2008). Female-female competition may be prevalent if males or the resources they provide are limited, suppressing any benefits that may be realised through polyandry (Berglund *et al*. 1993). Genetic benefits are likely to be better realised, and hence multiple paternity higher, in populations where there is greater genetic variation between males (Petrie

& Kempanaers 1998), although if polyandry is employed to reduce inbreeding this may be the reverse (Brooker *et al*. 1990). Evolutionary arms-races driven by sexual selection, which arise due to the fact that the evolutionary interests of males and females vary, will also dictate the dynamics of mating systems. Polyandry may be countered, and hence benefits reduced, by male strategies such as mate guarding, repetitive copulation and sperm competition (Long & Montgomerie 2006, Storey *et al*. 1995).

However, as methodology could not be fully controlled between the two studies and we do not have environmental variables to correlate with the rates of multiple paternity between the populations, it is not possible to be confident that the differences were due to variation between populations, rather than analyses. Variation in type and frequency of genotyping errors in either study, due to incorrect scoring, allelic dropout, false alleles and null alleles, would provide erroneous and therefore conflicting results. We did not use two loci that were utilised by Md. Naim *et al*. (2009), because they gave high rates of error (MavSB5), and allelic dropout (MavSF12). However, it should be noted that whilst they are the same loci, the primers were different and so this does not explicitly indicate any error by Md. Naim *et al*. (2009).

Whilst it is possible that our lower rate of multiple paternity may be explained by our analyses missing multiple paternity we feel this is unlikely. Insufficient informative polymorphic loci can lead to analyses failing to identify some sires, however we used 22 loci, compared to 10 by Md. Naim *et al*. (2011). Secondly, if the entire litter is not sampled, for example if a juvenile leaves the nest box before its siblings, dies or escapes during monitoring, there is a possibility that multiple paternity will be missed. However, this is unlikely to be a serious bias across all the sampled litters, as the mean number of juveniles per litter was comparable between both the English (4.62 SD = 1.45) and Welsh studies (4.1 SD = 1.17) and for reported mean litter sizes from other monitoring studies (Juškaitis 2008).

Our findings highlight the need for further research, with larger samples sizes, to investigate variation in multiple paternity across populations, in relation to spatial and temporal environmental variables. Such parameters may include, but are not limited to, population density, habitat quality, genetic diversity, reproductive skew,

season and climate. The data would allow inferences to be made concerning the impact of environmental changes, driven by anthropogenic actions, on mating behaviour in wild populations and the ultimate consequences. For example, habitat loss and fragmentation has been associated with a decrease in rates of multiple paternity (Banks *et al*. 2005). Additionally, small populations may be subject to the Allee effect, whereby a negative population growth rate and eventual extinction may result following difficulties in locating a mate, increased inbreeding, genetic drift, and loss of integrity due to hybridisation (Stephens *et al*. 1999). Further, the effect of nest box provision on hazel dormouse mating systems warrants investigation. Boxes increase nesting resources in an area and have also been shown to increase population density (Morris *et al*. 1990). This is likely to have an effect on operative reproductive skew and competition levels amongst populations, which are likely to impact upon mating systems and the prevalence of polyandry and sperm competition (Emlen & Oring 1977).

In conclusion, we have shown that rates of multiple paternity, in the hazel dormouse, although high, are not as dramatically so, as indicated by the only previous study in this species. We recommend further study of multiple paternity in populations at the core and periphery of the hazel dormouse's range, and of behaviour and ecological mechanisms that drive levels of polyandry in this species. The investigation of mating systems in natural populations is vital in conservation planning, mitigation and for informing captive breeding programmes.

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Chapter 7: Patterns of hibernation in the hazel dormouse:

Intra-specific variation and the influence of diet

Abstract

Hibernation facilitates the survival of endothermic organisms during periods of reduced ambient temperature and food availability. The efficiency of hibernation behaviour is likely to determine rates of weight loss, reproductive success and mortality, and thus be a key driver of population dynamics. To test the hypothesis that hibernation behaviour is plastic in response to available diet quality, we studied 24 captive hazel dormice, Muscardinus avellanarius, *under natural temperature and photoperiod fluctuations, with access to high or low calorie diets.*

All dormice exhibited several periods of arousal and feeding behaviour during winter months. We observed natural variation along a continuum in the mean length of torpor periods among individuals. Quite intuitively, relatively long-torpor dormice spent the majority of winter in hibernation, made few visits to food and were least likely to feed during visits. Critically, these dormice demonstrated no plasticity in response to diet. In contrast, relatively short-torpor dormice adjusted their behaviour according to diet, such that dormice on a high calorie diet spent less time in hibernation, had fewer bouts of torpor, visited food more often and were more likely to feed during foraging trips, than dormice offered low calorie diet. This plasticity is consistent with theory regarding the balancing of energy budgets, in response to current energy reserves and hibernation costs and benefits. Additionally, the expression of torpor increased with decreasing ambient temperature and day length.

We discuss the implications of changes in winter climate and food availability for the behaviour and winter survival of hazel dormice, and propose that heterogeneity among individuals in hibernation behaviour could be maintained by fluctuations in ambient temperature, food abundance and food quality in nature.

Introduction

Endotherms have evolved elevated metabolic rates and a high, constant body temperature, allowing the exploitation of thermally-variable habitats (Angilletta *et al*. 2010, Ruben 1995). However, when increased thermoregulatory costs, associated with low ambient temperatures, coincide with diminished food availability, the endothermic strategy may become unsustainable (Geiser 2004, Heldmaier *et al*. 2004). Hence, amongst other strategies, many endothermic species dwelling in periodically cold environments have evolved heterothermy, that is torpor and hibernation behaviour, in order to balance energy budgets (Angilletta *et al*. 2010).

The precise distinction between torpor and hibernation is somewhat ambiguous. It is suggested that, rather than being discrete conditions, torpor and hibernation can be considered as degrees along a continuum, with short, shallow torpor and prolonged, deep hibernation at the opposite extremes (Feldhamer *et al*. 2007).

A hibernator's ability to balance its winter energy budget efficiently is critical to its overall fitness (Humphries *et al*. 2002). There are various environmental factors that may influence survival during hibernation periods, such as winter length and severity, hibernacula conditions and disturbance (Boyles & Brack 2009). Food availability will clearly affect body mass, the latter of which has been shown to be a predictor of hibernation survival (Schorr *et al*. 2009). Turbill *et al*. (2011) highlighted a positive correlation between hibernation and increased winter survival, most likely due to reduced predation risk. However, a key trade-off associated with slower life history is a reduction in annual reproductive output in comparison to non-hibernating species of similar body size. Additionally, body condition when emerging from hibernation influences an individual's immediate survival and onset of breeding, which in turn determines fecundity and offspring survival (Bieber *et al*. 2012).

Many mammals that exhibit hibernation are of conservation concern, including several species of bats (Boyles *et al*. 2007, Turbill 2008), rodents (Bright *et al*. 1996, Lehmer & Biggins 2005), marsupials (Kortner & Geiser 1998) and primates (Schmid & Ganzhorn 2009). Given global, anthropogenic climate change and habitat fragmentation and degradation, it is important that we gain a better understanding of

links between diet quality, environment and behaviour in endangered hibernating mammals.

The hazel dormouse, *M. avellanarius*, a fat-storing hibernator, is listed on Appendix III of the Bern Convention and Annex IV of the EU Habitats and Species Directive, having declined in many parts of its northern range (Amori *et al*. 2008). Hazel dormice are particularly sensitive to temporal and spatial variation in food availability and their reliance on high quality, arboreal food requires them to hibernate during the winter (Bright *et al*. 1996). However, very little is known about the cues and constraints associated with torpor and hibernation in hazel dormice. Here, we consider three critical gaps in our understanding, as outlined below.

First, we know little about natural, intra-specific variation in hibernation behaviour patterns, such as length of hibernation bouts, in hazel dormice (but see Vogel & Frey 1995). Research has highlighted the prevalence of periodic arousals in hibernators, contravening the assumption that they 'sleep through' the winter (Lyman & Chatfield 1955, Twente & Twente 1965). The function of these periodic arousals remains undetermined, however explanations include excretion of accumulated metabolites (Geiser & Kenagy 1988), relief from oxidative stress (Carey *et al*. 2000), sleep deprivation avoidance (Daan *et al*. 1991, Trachsel *et al*. 1991), neural structure recovery (Larkin & Heller 1999), evaporative water loss replacement (Thomas & Geiser 1997) and the initiation of immune responses (Prendergast *et al*. 2002). Intra-specific variation in arousal patterns has been identified in various species, including the arousal and feeding frequency in Japanese dormice, *Glirulus japonicus*, (Otsu & Kimura 1993) and length of hibernation period and inter-bout arousals in *Marmota monax* at varying latitudes (Zervanos *et al*. 2010). This plasticity suggests hibernators optimise torpor patterns, making trade-offs between the potential benefits and costs of hibernation. Hibernation is ecologically and physiologically costly and so theoretically should be avoided when energetically possible (Humphries *et al*. 2003b). However, periodic arousals are energetically expensive and responsible for the majority of energy use during winter (Karpovich *et al*. 2009). Trade-offs, therefore, are likely to have an important impact on winter survival and so is worthy of investigation in species of conservation concern such as the hazel dormouse. As such, we explore the variation in mean length of bouts of torpor between individual dormice over the study

period, and describe such patterns along a continuum from relatively short to long torpor bouts.

Second, we hypothesise that the availability and quality of winter food sources will influence the expression of torpor, but also that this could be mediated by underlying behavioural variation among individuals. Energetic models predict that if a hibernator has sufficient energy stores prior to hibernation or if sufficient food is available during the winter period, the animal should forego torpor expression (Humphries *et al*. 2003b). Reduced torpor in hibernators provided with excess food has been documented (French 2000, Humphries *et al*. 2003a, Munro *et al*. 2005, Pavey *et al*. 2009). However, plasticity has generally been identified in food-storing hibernators, rather than fat-storers such as hazel dormice (but see Otsu & Kimura 1993, Pulawa & Florant 2000). Due to physical limitations, the latter are likely to be more energetically constrained and so less able to demonstrate behavioural plasticity (French 2000, Humphries *et al*. 2003b). We aim to use an experimental approach to infer the effects of winter food availability on hazel dormouse hibernation patterns.

Third, there is some debate regarding the supposition that torpor is induced by, and will lengthen and deepen in response to, decreasing ambient temperatures. During hibernation periods body temperature closely follows ambient temperature (Geiser 2004) and increased body temperature should correlate with decreased torpor expression and increased metabolic rate (Geiser & Kenagy 1988). An inverse relationship between ambient temperature and torpor expression has been identified in many hibernating species, including ground squirrels (*Spermophilus spp.*) and tree-roosting bats (Geiser & Kenagy 1988; Turbill 2008, Twente & Twente 1965) but the correlation was found to be weak in chipmunks (*Eutamias spp.*; Kenagy 1981) and non-existent in Japanese dormice (Otsu & Kimura 1993). Therefore, we aim to determine how the hibernation behaviour of our species of interest, the hazel dormouse, is affected by ambient temperature and day length in comparison to other species.

Methods

We studied the hibernation behaviour of 24 captive dormice (males n=9 and females n=15) at Paignton Zoo Environmental Park, Devon, UK. The dormice were individually housed in metre-cubed cages, located in an outside area exposed to natural light and environmental conditions, but protected from precipitation and away from public access. Inside each cage the dormice were provided with a wooden nest box, bedding and branches for environmental enrichment.

Dormice were randomly divided into two treatment groups, low (n=12) and high (n=12) calorie diet throughout the hibernation study period. High calorie diet consisted of a mix of seeds, corn, dog biscuits, egg, crushed insects, fruit and carrot, whilst for lower calorie diet seeds, corn and fruit were omitted. Food and water were provided *ad libitum*. Dormouse cage number, diet, sex and pre-study weight were recorded. From an inspection of the food bowl, keepers kept a daily log of feeding behaviour of each dormouse for the preceding night. Due to time constraints, it was not possible to weigh the food each morning and so three behaviour categories were used; no evidence of feeding, evidence of disturbing food but not feeding, and definite evidence of feeding.

Dormouse hibernation behaviour was inferred by recording nest temperature over part of the winter hibernation period; 15th October 2008 to 21st January 2009. A temperature probe fixed to a data logger (ThermaData logger TD2F, Electronic Temperature Instruments, UK) was inserted through the side of each nest box and into the dormouse's nest chamber. Cables were protected from gnawing by copper piping. Data loggers positioned outside the cages recorded nest chamber temperature every 15 minutes. Data were downloaded approximately every 20 days, before logger memory was exceeded. The logger's position outside the cage allowed easy access without causing any disturbance to the dormice. Throughout the study period ambient temperature in the enclosure area was also recorded with an environmental data logger (ThermaData logger HTB, Electronic Temperature Instruments, UK) in order to compare nest and ambient temperatures.

Nest temperatures close to ambient suggested the dormouse was absent from the box or in a thermo-conforming, torpid state. Nest temperatures above ambient

indicated the animal was present in the box but homeothermic and therefore not in torpor. Hibernation behaviour was measured as a bout, which was a continuous period of time, measured in hours, where nest temperature was stable and close to ambient, indicating the animal was in thermo-conforming. A bout was terminated when nest temperature rapidly increased, due to the animal arousing from torpor, increasing it's metabolism and body temperature and consequently increasing nest chamber temperature. Hibernation bouts were inferred only when nest temperature equalled ambient temperature for 20 hours or more. Whilst shorter periods of cool nest temperature may have been due to the animal thermo-conforming, a distinction between hibernation and absence from the nest box was necessary. It was deemed unlikely that dormice would be out of the nest for longer than 20 hours, as during this study the longest night was 17 hours. Dormice are nocturnal and during active periods have been found to start activity just after sunset and return to nests before sunrise (Bright *et al*. 1996).

In five boxes, temperature logger data suggested no significant increase in temperature above ambient for the entire hibernation period, despite other evidence of dormouse activity in these boxes. This is most likely due to the temperature probes not being close enough to the resting chamber within the nest. Therefore, these data were rejected and nest temperature data sample sizes were reduced (low diet n=10 and high calorie diet n= 9).

Validation of nest temperature as an accurate method to infer hibernation behaviour was carried out using four activity tubes. These comprised a plastic tube with a floor switch at the entrance of each dormouse nest box. When the animal walked through the tube, the floor pushed down on a switch connected to a data logger (Hobo U11 3-state/1-event data logger, Onset, USA), which recorded the time of each passage through the tube. The data from the activity tubes were visually compared to the data for the corresponding animal's nest temperature and feeding behaviour logs. Long periods (>20 hours) of match between nest and ambient temperature, followed by an increase in nest temperature, were consistently followed by triggering of the activity tube, and commonly followed by food disturbance events. This justified confidence in the use of the nest temperature loggers to infer dormouse

hibernation behaviour. See Supplementary Table S1 for a summary of the raw data of inferred hibernation behaviour.

Hibernation behaviour data were analysed using generalised linear models (see Crawley 2008) in R version 2.10.0 (R Foundation for Statistical Computing 2010). These models allow for response variables that have a non-normal distribution, through the use of a link function. Such analyses were used to compare hibernation bout lengths (length of a bout measured in hours) among individuals that demonstrated true hibernation during the survey period. The effects of diet, sex and initial weight on mean hibernation bout length across the entire study period were investigated using a Gaussian error structure. The influence of sex, initial weight, diet and hibernation bout length (including two-way interactions) on the proportion of time spent in hibernation (i.e. total bout length as a proportion of the total study period) were modelled using quasibinomial error structure to account for overdispersed proportion data. Models relating the number of hibernation bouts throughout the study period, and the total number of visits to food (i.e. frequency of daily records across total study period where food was disturbed and/or eaten by animal) , to mean hibernation bout length and diet treatment, used quasipoisson error structures to account for over dispersed count data. The effect of diet and mean hibernation bout length on the proportion of visits where food was eaten, was investigated using a quasibinomial error structure. The relative influence of date, mean daily temperature and mean day length on the length of hibernation bouts through time was analysed using a linear mixed-effects model with Gaussian error structure, using dormouse identity as a random effect. A binomial GLM was used to assess the influence of date, day length and ambient temperature on the probability of dormice being in hibernation, through time.

Ethical Note

The animals were kept by Paignton Zoo as part of a conservation reintroduction scheme, licenced by Natural England and at all times acting within the laws of the UK and abiding by all ethical policies of the British and Irish Association of Zoos and Aquariums, the European Association of Zoos and Aquaria and the World Association

of Zoos and Aquaria. All installation of research equipment took place during normal husbandry practices, to ensure no additional disturbance to the animals occurred. Diets were carefully designed to ensure they were sufficient to prevent any nutritional deficiencies and not cause starvation, but still allow a comparison of food quality. Whilst less accurate than sub-dermal body temperature loggers, temperature probes in the nest chambers were significantly less invasive and so deemed more appropriate for this protected species.

Results

Dormice demonstrated highly significant intra-specific variation in mean duration of each period of torpor ($F_{17,570} = 3.14$, $P = 0.001$). This variation was not influenced by the main effects of diet, sex or initial weight (mean torpor period: sex, $F_{1,15} = 0.18$, $P = 0.68$; weight, $F_{1,15} = 0.40$, $P = 0.54$; diet, $F_{1,15} = 0.87$, $P = 0.37$; proportion of time during survey spent hibernating: sex, $F_{1,15} < 0.001$, $P = 0.98$; weight, $F_{1,15} = 0.11$, $P = 0.75$; diet, $F_{1,15} = 0.95$, $P = 0.34$). Hereafter we classify dormice along a continuum of mean torpor period, ranging from 'short torpor' to 'long torpor'. As would be expected, the proportion of time spent in hibernation increased with increasing mean torpor period. However the slope of the relationship between mean torpor period and proportion of time spent in hibernation was different between diets (interaction between mean torpor period and diet, $F_{1,15} = 8.22$, $P = 0.01$, figure 1). The net result of this interaction was that dormice with short torpor spent a reduced proportion of their time in hibernation when fed higher quality diet, compared to those fed low quality diet (comparison of predicted values at mean torpor period 25hrs, $F_{1,15} = 7.68$, $P = 0.01$). Dormice with long torpor did not differ in proportion of time spent hibernating between diet treatments (comparison of fitted values at mean torpor period 100hrs, $F_{1,15} = 4.41$, $P = 0.05$).

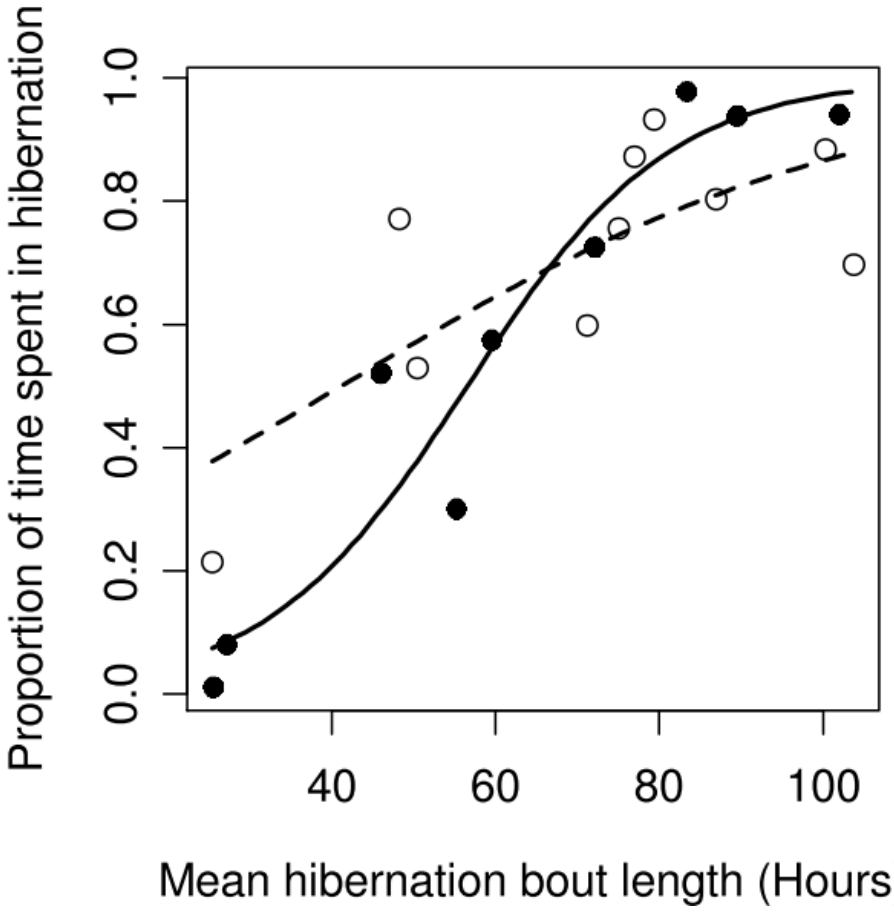

Figure 1. Proportion of total time spent in hibernation, throughout experiment for dormice on high (solid points and line) and low (open points and dashed line) quality diet treatments, showing variation in individual mean hibernation bout length in hours. Sigmoid fitted lines emerge from back-transformation from the logit link used to analyse binomial GLM.

Dormice from different diet treatments exhibited contrasting relationships between number of hibernation bouts and mean torpor period (interaction between diet and mean torpor period, $F_{1,15} = 6.95$, $P = 0.02$, figure 2). Dormice on high calorie diets decreased the total number of hibernation bouts with decreasing mean torpor period (test of slope > 0, $t_8 = 2.76$, $P = 0.01$, figure 2). Those dormice on low calorie diets showed no relationship between the total number of bouts and mean torpor period (test of slope < 0, $t_9 = 0.83$, $P = 0.42$, figure. 2). Hence the relationships between number of bouts and mean torpor period converged: dormice with short torpor entered hibernation more often when fed low, compared to high, quality diet (comparison of fitted values at mean torpor period 25hrs, $F_{1,15} = 9.45$, $P = 0.01$), but diet did not influence the number of hibernation bouts of dormice with long torpor (comparison of fitted values at mean torpor period 100hrs, $F_{1,15} = 1.14$, $P = 0.30$).

The food disturbance records demonstrated that dormice given a high calorie diet exhibited foraging behaviour on a significantly higher mean number of days than dormice on a low calorie diet throughout the experimental period ($F_{1,16} = 8.74$, $P = 0.01$, Fig. 3). The total number of visits to food decreased with increasing mean torpor period of each dormouse ($F_{1,16} = 36.94$, $P < 0.01$, figure 3). The slopes of this relationship for high and low quality diets are equivalent on a log scale (test of interaction, $F_{1,15} = 2.75$, $P = 0.12$)

When fed high quality diet, dormice with short torpor were highly likely to feed during food exploration visits, but this probability declined steeply with increasing mean torpor period (figure. 4). Dormice on low quality diet showed no such relationship between probability of feeding and mean torpor period (difference in slopes shown by interaction between mean torpor period and diet, $F_{1,15} = 13.63$, $P = 0.002$; test of slope < 0 for low quality diet, $t_9 = 0.76$, $P = 0.46$). Overall, the relationships on the two diets converged such that dormice with short torpor were more likely to eat high, compared to low, quality diet (comparison of fitted values at mean torpor period 25hrs, $F_{1,15} = 52.55$, $P < 0.001$), while dormice with long torpor fed on the two diet types with equal probability (comparison of fitted values at mean torpor period 100hrs, $F_{1,15} = 2.90$, $P = 0.11$).

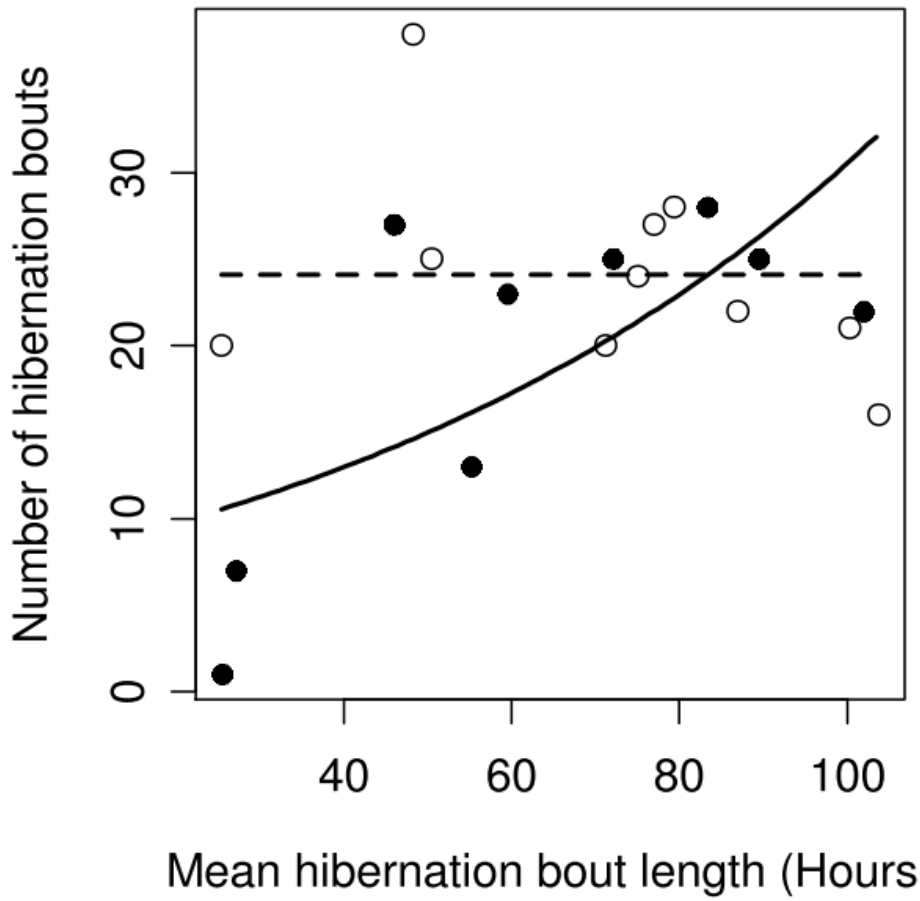

Figure 2. Number of hibernation bouts for dormice in high (solid points and line) and low (open points and dashed line) quality diet treatments, showing variation in individual mean hibernation bouts lengths.

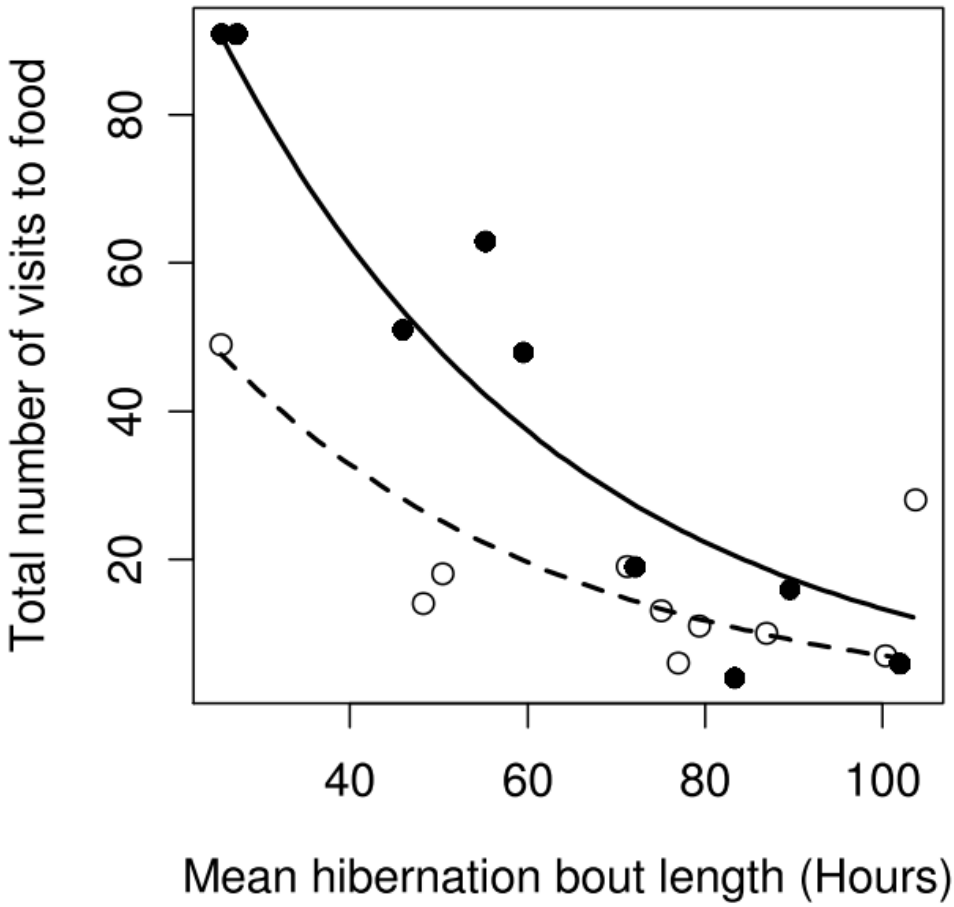

Figure 3. Total number of days dormice showed evidence of activity, through-out experiment for dormice on high (solid points and line) and low (open points and dashed line) quality diet treatments, showing variation in individual mean hibernation bout length in hours.

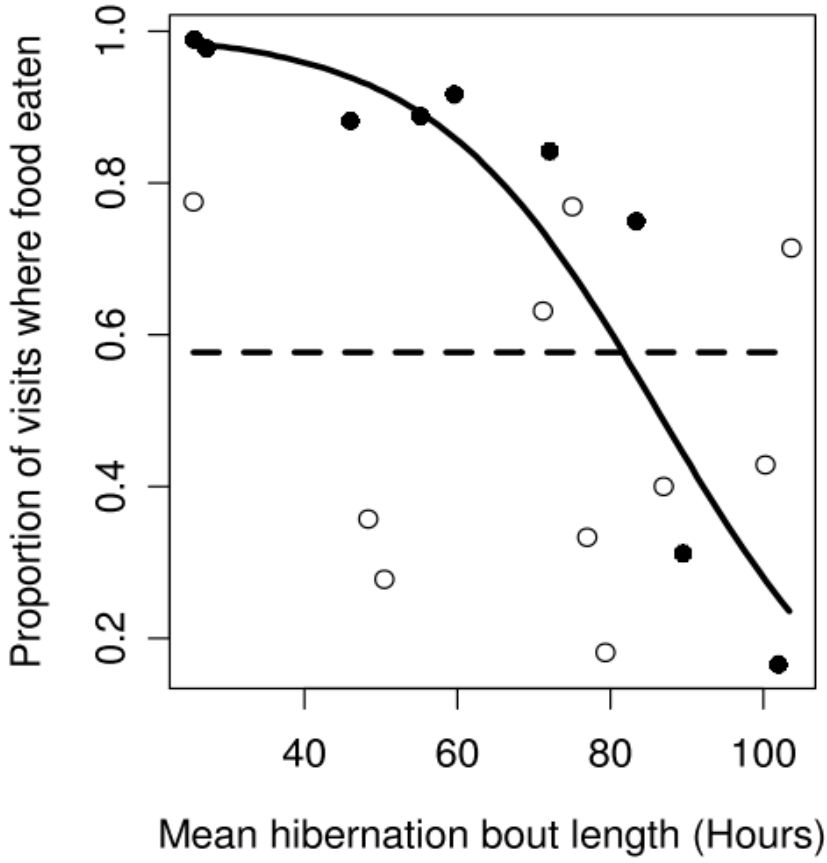

Figure 4. Proportion of visits to food where food was eaten, through-out experiment for dormice on high (solid points and line) and low (open points and dashed line) quality diet treatments, plotted against individual mean hibernation bout length in hours.

The length of hibernation bouts changed during the hibernation season. Mixed model regression and model simplification identified that this change appeared to be driven by an interaction between mean ambient temperature and mean day length (χ^2_1 =102.98 , $P < 0.001$, figure 5), with no extra contribution of date (χ^2_1 =0.20, $P = 0.66$).

Additionally, the hibernation season was summarised into three-day intervals, and the number of dormice that were in hibernation at least once in each interval, were counted. The probability of the dormice being in hibernation declined with increasing ambient temperatures (χ^2_1 =11.91, $P < 0.001$) and was not further influenced by date (χ^2_1 = 0.54, P =0.47) or mean day length (χ^2_1 = 0.1, P =0.75).

A summary of the raw data of inferred hibernation behaviour of 19 hazel dormice, through logging of nest chamber temperature throughout the winter study period is given in Supplementary Table S1. Plots testing the model assumptions of homoscedasticity and normal error structure, after implementation of the link function, are shown in Supplementary Table S2.

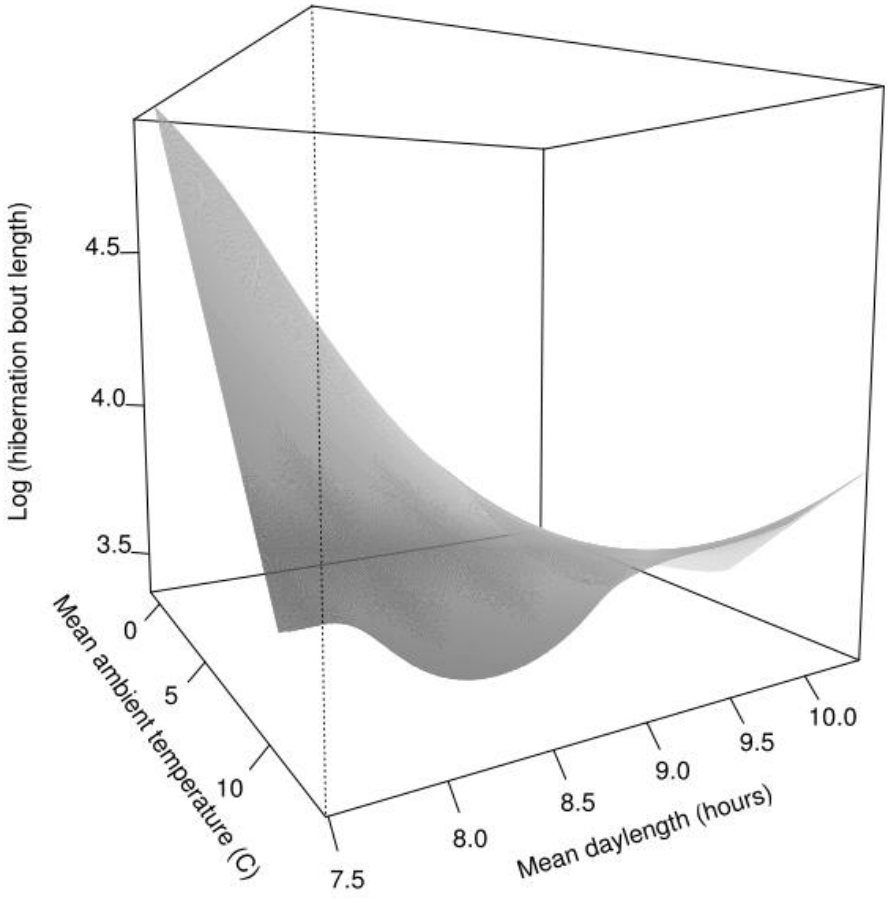

Figure 5. Three-dimensional plot showing the interaction between mean ambient temperature (degrees C) and mean day length (hours) on the length of the hibernation bout, for an 'average' dormouse. The missing data along the surface edges are due to limiting the temperature/day length to within where these measurements lay.

Discussion

We studied the intra-specific variation in hibernation patterns and the influence of diet quality, ambient temperature and day length on the hibernation behaviour of *M. avellanarius*.

Our results demonstrate variation in hibernation behaviour among individual hazel dormice, despite sharing similar environmental conditions. The proximate cause of the observed plasticity was not determined despite investigating the effect of diet, sex and weight. However, physiology, heritable traits or early life experience may be possible explanations for the observed intra-specific variation. A shortage of polyunsaturated fatty acids reduces torpor expression in other hibernating species (Frank 1992, Gesier & Kenagy 1987). Whilst this is unlikely to be the main mechanism driving variation in hibernation behaviour in our study, as all dormice were provided with the same diet prior to the manipulations, it exemplifies how underlying factors such as physiological condition prior to the hibernation period may be important.

Whatever factor pre-determined each dormouse's propensity to engage in long or short periods of torpor, its outcome shaped the subsequent relationship between hibernation behaviour and diet quality. The proportion of time spent in hibernation was not influenced by diet quality, sex or initial weight *per se*. Instead, the influence of diet was only revealed when its effect was mediated by each dormouse's position along the mean torpor period continuum. Long-torpor dormice spent a greater proportion of their time being torpid, but did not adjust the amount or frequency of torpor in response to diet quality. However, short-torpor dormice engaged in fewer bouts of torpor, and hence spent a lower proportion of their time in torpor when high quality diet was available. The plasticity in short-torpor dormice is consistent with the theory that hibernators should optimise torpor expression based on trade-offs between hibernation costs and benefits (Humphries *et al*. 2003b). The direct link to diet quality was evidenced by these dormice exploring high quality food more often, being more likely to consume high quality food, and adjusting their torpor patterns accordingly. This concurs with studies on other hibernating species, where higher energy diets reduce the need for hibernation (Humphries *et al*. 2003a, Munro *et al*.

2005, Pavey *et al*. 2009).These links between mean torpor length and the adjustment of hibernation behaviour in response to diet quality yield three important questions.

First, why did long-torpor dormice not adjust their behaviour according to diet? The simplest explanation might be that individuals that spend most of their time in a torpid state are time constrained and therefore simply unable to respond behaviourally to dietary cues. Alternatively, unresponsiveness may be due to a lack of appetite during hibernation. Garden dormice, *Eliomys quercinus,* lose their appetite prior to the hibernation period (Montoya *et al*. 1979). This may be explained by gut atrophy (Carey *et al*. 2003), which improves energy conservation, but is energetically costly and it is slow to resume digestion (Hume *et al*. 2002, Humphries *et al*. 2003b). Relatively more energetically constrained individuals risk wasting valuable energy for digestion if food supply is unpredictable. Long-torpor dormice may leave their hibernacula to excrete waste products and replace water due to evaporative loss (Thomas & Geiser 1997), but not to forage.

Second, what are the implications of variation in diet quality for short-torpor dormice? When higher levels of food are available, short torpor dormice are able to adjust their behaviour by reducing the number of hibernation bouts, and consequently lessen hibernation costs. Therefore, when food availability is relatively high during the winter, short torpor dormice may benefit from increased winter survival. However, they may simultaneously suffer from higher predation risk. In contrast, we suggest that short-torpor dormice may suffer a relatively higher risk of winter mortality when food is scarce or of poor quality. This is a key avenue for future research and will be driven by the ultimate cause(s) that govern an individual's trade-offs between hibernation and foraging costs and benefits.

Third, could the costs and benefits of long and short torpor, combined with natural unpredictability of food availability and quality, actually maintain variation in torpor duration? While we are unable to ascribe the observed variation in torpor duration to genetic, developmental, physiological or environmental causes, it remains possible that behavioural heterogeneity among hibernators could be maintained by a behaviour-by-environment interaction. When food is scarce or poor, long-torpor individuals may have the highest rates of winter survival. When food is abundant or of

high quality, relative survival may be highest among individuals that arouse regularly to feed. Spatial or temporal fluctuations in food distribution could then create a mosaic in which the expected fitness of long- or short-torpor strategies is equalised. This hypothesis is speculative, but deserves testing in natural populations of hibernators, particularly dormice.

The finding that increased ambient temperature negatively influences dormouse torpor expression agrees with findings for other hibernating species (Geiser & Kenagy 1988; Turbill 2008). Various attempts have been made to explain these findings and can be categorised into temperature-dependent metabolic, chemical, neural and physical explanations (Thomas & Geiser 1997). Day length has also been demonstrated to influence hibernation behaviour, with longer photoperiods decreasing the expression of torpor (Kenagy 1981). Whilst hibernation is controlled to some extent by endogenous circannual rhythms, the additional effect of day length suggests this cue is used to synchronise the internal rhythm (Kenagy 1981). Among the dormice studied here, torpor expression increased with a combined decrease in temperature and day length, but relatively high values of either day length or temperature caused individuals to reduce the period of torpor. The probability of being in a torpid state increased only with decreasing ambient temperature.

Our results provide key insights into the plasticity and constraints associated with hibernation, in a species of conservation concern and yield predictions of hibernation success in wild dormouse populations. That the climate is warming rapidly (IPCC 2007) is likely to have implications for dormouse over-wintering survival. Inter-annual variation in abundance of wild populations of dormouse in the UK indicates that cold, dry winters are correlated with higher dormouse abundance the following summer, suggesting warmer winter temperatures decrease dormouse winter survival (Sanderson 2004). Our findings provide an explanation for this: increasing ambient winter temperatures result in a higher frequency of periodic arousals that would in turn increase individual energy requirements (Thomas *et al.* 1990). Such costs could be mediated by the greater availability of winter food, however, this will only be the case if the phenology of dormouse food sources keep pace with climatic change. Otherwise, warm ambient temperatures combined with scarce or low quality food could be extremely detrimental to the over-wintering survival of dormice. We recommend

further research into the frequency and duration of torpor, in relation to temperature and food, in wild dormouse populations.

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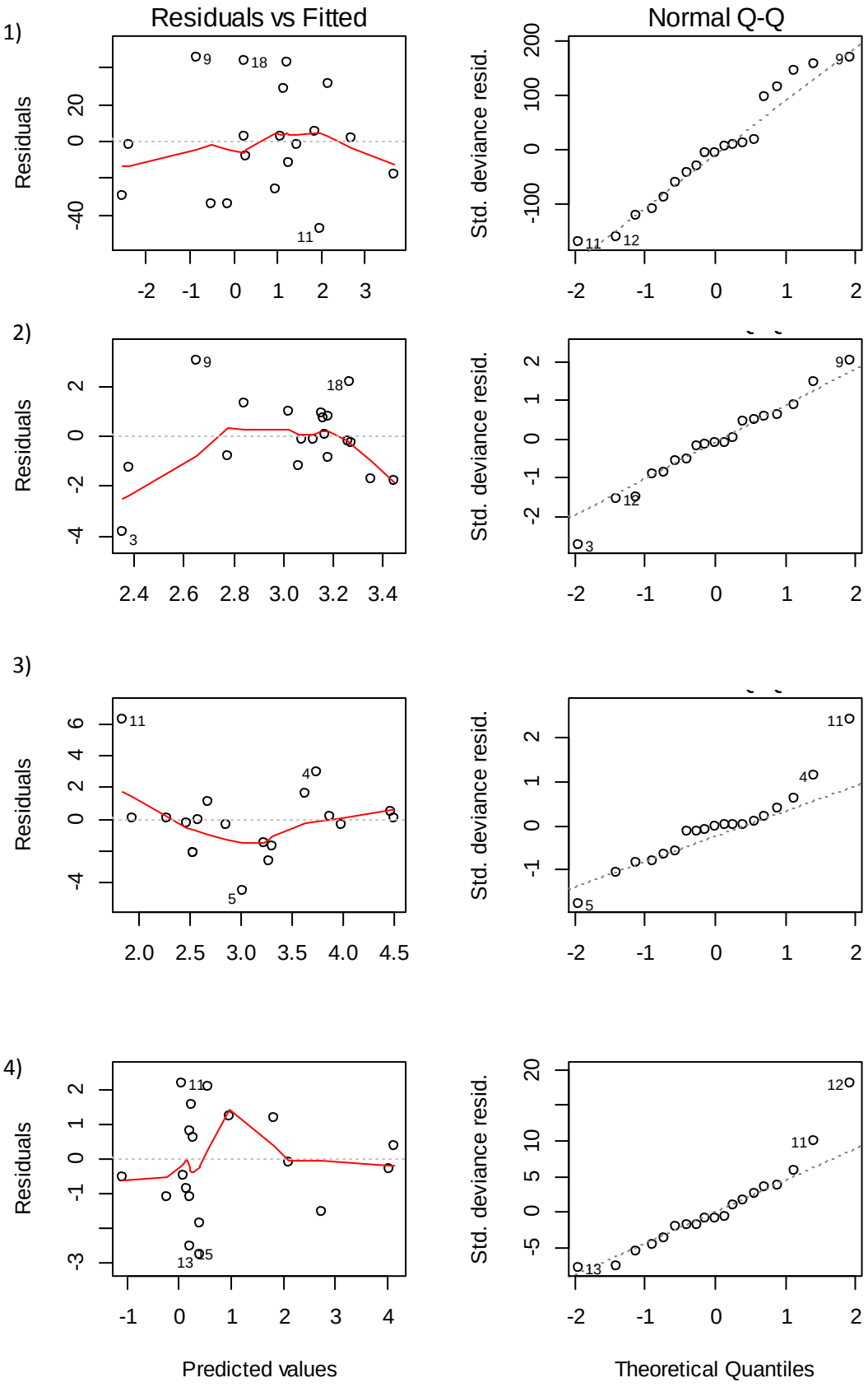
Supplementary Table S1. Summary of raw data of inferred hibernation behaviour of 19 hazel dormice, through logging of nest chamber temperature throughout the winter study period. Individuals were randomly allocated to a low or high quality diet and weighed prior to the start of monitoring. Time thermo-conforming indicates total time across study period individual dormice demonstrated bouts of 20 hours or longer, where nest chamber temperature was close to ambient, therefore inferred to be exhibiting hibernation behaviour. Time thermo-regulating is defined as total time monitored during study period less the time each individual was thermo-conforming, indicating periods where dormice had aroused from hibernation. The number of torpor bouts (time periods where nest temperature is close to ambient temperature for a minimum of 20 hours) and mean bout length across the study period is also shown. The daily food log data provides counts for the number of nights when provided food was a) disturbed (but no evidence of eaten) and b) eaten.

ID	Sex	Diet	Weight	Time thermoconforming (hours)	Time thermoregulating (hours)	Number of torpor bouts	Mean bout length	Daily food log	
								Disturbed food	Ate food
c1	F	High	31	2243	139.5	22	407.82	5	1
c11	F	Low	21	1835.75	546.75	38	193.24	9	5
c13	F	High	29	1240.5	1142	27	183.78	6	45
c2	M	Low	36	1424.5	958	20	284.90	7	12
c21	M	Low	26	1660	722.5	16	415.00	8	20
c22	F	Low	24	509	1873.5	20	101.80	11	38
c24	M	High	25	717.25	1665.25	13	220.69	7	56
c25	M	High	30	2237	145.5	25	357.92	11	5
c27	F	High	27	1369.25	1013.25	23	238.13	4	44
c28	F	Low	27	1801.5	581	24	300.25	3	10
c29	M	High	28	190.25	2192.25	7	108.71	2	89
c3	M	High	32	1729.25	653.25	25	288.21	3	16
c30	M	Low	29	2079.25	303.25	27	308.04	4	2
c4	F	High	30	25.5	2357	1	102.00	1	90
c5	F	Low	29	1261.5	1121	25	201.84	13	5
c6	F	Low	26	2106.75	275.75	21	401.29	4	3
c7	F	High	26	2332.75	49.75	28	333.25	1	3
c8	Low		28	2222.25	160.25	28	317.46	9	2
c9	Low		27	1913.25	469.25	22	347.86	6	4

Supplementary Table S2. Plots testing the model assumptions of homoscedasticity (residuals vs fitted) and normal error structure (normal Q-Q), after implementation of the link function, corresponding to figures 1-4.



Chapter 8: General discussion

This thesis has focussed on various aspects of the ecology and conservation of the hazel dormouse, *Muscardinus avellanarius*. Whilst single-species conservation may be limited, there are several important justifications for the interest in conserving hazel dormice and hence the work outlined within this thesis. Although dormice do not have a direct resource use, they provide many members of the public with the rare opportunity to interact with wild mammals through involvement in nest box surveys (Bright *et al*. 2006). It may be argued that this is an unnatural situation, reserved for the licensed few, however this point of view undervalues the impact such experiences have and how it influences attitudes towards nature (Guiney & Oberhauser 2009). Additionally, volunteers make important contributions to a long-term monitoring programme. Less hands-on, dormice are an endearing species that are often in the public eye through various forms of the media, and so the value of knowing the species exists should not be dismissed (Macguire & Justus 2008, Morris 2003). As such an iconic British mammal, they are also an important flagship species, fostering a general interest in nature conservation in human society (Caro & O'Doherty 1999).

Mitigation undertaken for dormice, such as habitat connectivity through hedgerow management and restoration, is likely to have positive effects on a range of other species and so promote persistence of biodiversity and ecosystem services (Morris 2003). Hazel dormice are scientifically interesting, due to their general ecology and life history which contrasts starkly to other small rodents. As dormice are sensitive to habitat fragmentation, they are important model species and hence may provide insights into landscape effects (Mortelliti *et al*. 2009). It is unknown to what extent, if at all, hazel dormice may contribute to ecosystem functioning. However, dormice have been suggested as a bioindicator species, in that their presence indicates a biologically diverse woodland (Morris 2003). There is also, the ethical consideration that dormice have an intrinsic right to existence. This, however, is being threatened by human action through increased conversion of natural habitat to agriculture, urbanisation and roads and therefore as the dominant species we have a responsibility to ensure our actions do not cause extinctions (Ehrlich & Ehrlich 1992).

Summary of thesis

In Chapter 2 alternative survey techniques, namely camera traps and footprint tracking, for detecting hazel dormouse presence were piloted. The results demonstrated that dormice and other small mammals would visit baited monitoring stations, which is vital if dormice are to be detected using these techniques. In addition, both methods successfully recorded the presence of small, arboreal mammals and the records were of sufficient quality to allow discrimination between hazel dormice and another sympatric species, the wood mouse *Apodemus sylvaticus*. A comparison between the camera trap and footprint tracking results showed that there was moderate to substantial agreement between the two survey methods, indicating their robustness. Feeding remains surveys, comprising hazel nut searches, are much less time dependant, but unlike camera trap and footprint tracking techniques, are only possible where hazel is present.

In Chapter 3, a simple algorithm based on Linear Discriminant Analysis (LDA) was developed for the objective species identification of small mammal footprints, as collected by the footprint tracking methodology outlined in Chapter 2. Analysis demonstrated that, through the use of several morphometric measures of footprints, it was possible to distinguish between footprints from hazel dormice and wood mice. The piloting of the algorithm by volunteers with no experience of footprint tracking allowed further refinement of the methodology. The final algorithm demonstrated that the volunteers were, with 95% confidence, able to correctly identify 94% of footprints that were sufficiently clear to allow an attempt at species identification. The LDA algorithm performance was compared to the results from manual identification by an ecologist familiar with small mammal footprint identification. Although the LDA algorithm was more conservative, where identification was attempted by eye and over 95% confidence was given by the LDA algorithm, agreement was 99%. Therefore, the algorithm provides an objective, accurate and precise technique for the discrimination between hazel dormouse and wood mouse footprints.

In chapter 4 the isolation and characterisation of new hazel dormouse microsatellite loci are described. Twenty-one markers were found to conform to the assumptions of the Hardy-Weinberg equilibrium and linkage disequilibrium, as well as

display an estimated null allele frequency <0.20, rendering them suitable for many molecular ecology applications. In addition, primers were redesigned from microsatellite loci that were reported in a previous primer notes publication by Md. Naim *et al*. (2009). The reasons for this was two-fold, firstly errors were identified in four of the ten reported primer sequences, which led to these markers failing to amplify, and secondly all primers were designed to be within a similar melting temperature range, which would provide more flexibility when multiplexing PCRs.

In chapter 5, the investigation of population genetics in hazel dormice in southwest England is presented. Here, significant genetic differentiation and structuring was identified, supporting the assumption that gene flow between populations of dormice is limited, due to their low dispersal ability, reproductive potential and population densities, as well as habitat specialism (Bright 1993, Bright & Morris 1996). Across the southwest region there was significant variation in genetic diversity. The two regions (as defined by population clustering analysis) further west, that are in Cornwall, show significantly lower levels of genetic diversity, compared to populations within the Devon/Somerset region. The cause of this is probably due to a combination of mechanisms, namely; edge-of-range effects, whereby isolated populations on the periphery tend to be smaller and so subject to genetic drift and inbreeding (Brown 1984, Lawton 1993, Vucetich & Waite 2003,); dispersal barriers due to the river Tamar and large areas of unsuitable dormouse habitat, such as Bodmin Moor (Slatkin 1987); and historical effects such as colonisation patterns creating a wave of founder events, reducing genetic diversity along the colonisation route (Hewitt 1999).

In Chapter 6 microsatellite genotype data was utilised in order to infer the rate of multiple paternity in litters sampled in southwest England through the investigation of relatedness of siblings. The results indicated a rate of multiple paternity significantly lower compared to a previous study conducted in Wales (Md. Naim *et al*. 2011). The reason for these contrasting results may be due to variation in ecological and evolutionary mechanisms in the different populations. For example, population densities may affect mate encounter rate and competition levels (Dean *et al*. 2006, Uller & Olsson 2008).

It is in Chapter 7, that an investigation of the effect of variation in diet quality and temperature on captive hazel dormice is described. The results indicated clear intra-specific variation in the propensity to hibernate, the underlying cause of which was undetermined and requires further research. However, this variation was an important consideration in how dormice responded to diet quality. Animals with long torpor bouts during the hibernation period did not significantly adjust their hibernation behaviour in response to the food provided. However, for animals that had relatively shorter torpor bouts, there was evidence that diet quality did influence their hibernation patterns. Specifically, if these dormice were given a relatively higher quality diet, they increased the time they spent out of torpor, whilst this was not the case for individuals that were provided with lower quality diet

Aims, objectives and conservation implications

It is anticipated that the findings outlined in this thesis will contribute to the monitoring and conservation planning that aim to protect the hazel dormouse. Furthermore, it is hoped that much of this research will have a broader relevance to biological knowledge and understanding, which will have relevance to broader conservation science. The work outlined in this thesis has significantly fulfilled the aims and objectives, as set out in the introduction and as such are briefly outlined below:

- *Carry out a pilot study to investigate the effectiveness of alternative techniques to determine the presence of hazel dormice.*

Whilst these techniques are not new *per se*, both have rarely been used in this context and therefore are currently not meeting their full potential. Both methods were successfully implemented and therefore we demonstrated their use as rapid, non-invasive and objective hazel dormouse survey techniques, suitable for a range of habitat types. The survey effort for these two methods can be measured in weeks, compared to existing nest box and nest tube survey methods that can extend over many months (Bright *et al*. 2006), although the former is more intensive. These more rapid tools are urgently required, as dormice presence may be missed if surveys are rushed, or development projects may be overly delayed whilst dormouse surveys are

underway. Increasingly, dormice are being found in non-typical habitats (Bright *et al*. 2006, Juškaitis 2008), and therefore less habitat dependant survey techniques are also required if these alternative habitats are to be monitored. The failure to detect hazel dormice at a site earmarked for development will lead to insufficient mitigation for the species, and hence potentially population loss. Developers may be frustrated and suffer economically if required to wait for long periods of time for survey results, increasing human-wildlife conflict. More broadly, both techniques are clearly not restricted to hazel dormice and it is hoped that they will be applied to ecological and conservation projects focusing on other small and/or arboreal species. The simple protocol followed to produce the LDA algorithm could be adapted for use in any community of animals, and therefore this study may be of interest to any ecologists investigating survey techniques. That small, nocturnal, arboreal mammals are amongst the most elusive of species demonstrates that the adoption of such techniques would be highly advantageous for use in these ecological niches.

- *Develop a suite of hazel dormouse microsatellite markers for use in molecular ecology analyses*

The novel hazel dormouse microsatellite markers we developed were essential for use in molecular analyses in Chapters 5 and 6, on population genetics and parentage respectively. The redesigning of incorrectly reported sequences will hopefully also help to avoid future researchers spending time trialling erroneous primers. The loci sequences and primers have been submitted to GenBank, a publically available genetic sequences database, and as such can be freely accessed by the research community for use in further molecular ecology studies on hazel dormice, and potentially other members of the Gliridae. Genetics has an important role to play in conservation, such as through the detection of populations that are genetically distinct or inbred, informing reintroduction programmes, and facilitating research that investigates ecology, evolution and behaviour of animals of conservation concern in order to better implement conservation actions (Frankham *et al.* 2002, Haig 1998).

- *Describe patterns of population genetics of hazel dormice at a regional scale.*

As far as we are aware, this thesis is the first description of patterns of population genetics of hazel dormice at a regional scale (Chapter 5). Cornish populations, on the edge of the hazel dormouse range, as hypothesised, demonstrated lower genetic diversity and higher genetic differentiation compared to those found in the core range, in Devon. The identification of this pattern of genetic diversity and differentiation has important implications for hazel dormouse conservation in the south west UK. In order to preserve the assumed Cornish hazel dormouse genetic distinctiveness, it would not be advisable to connect these to Devon populations on a large landscape scale, or to reintroduce individuals from outside Cornwall. Rather, within Cornwall, land managers would be advised to connect small woodland patches and conduct appropriate habitat management for this species, in order to ensure persistence of Cornish dormouse populations, and facilitate the recolonisation of woodland patches that currently do not have dormice present.

- *Quantify the rate of multiple paternity in litters sampled in southwest England and compare results to published data.*

The incongruence in frequency of multiple paternity between the previous study (Md. Naim *et al*. 2011), and the results in this thesis are important, as they highlight either an error in one of the studies, or an ecologically interesting variation in mating systems; either of which deserves further study. Rate of multiple paternity is a key parameter for consideration in species that exist in small populations, as it may influence effective population size, which in turn will influence genetic drift and inbreeding (Anthony & Blumstein 2000, Sugg & Chesser 1994). Natural behaviour should also be considered in captive breeding and reintroduction programmes (Md. Naim *et al*. 2011).

- *Investigate the effect of food availability and natural temperature fluctuations on the hibernation behaviour of hazel dormice.*

An experiment on the effect of food availability and natural temperature fluctuations on the hibernation behaviour of hazel dormice confirmed that there is significant inter-individual variation in bout length during hibernation (Chapter 7). As predicted, dormice that more frequently arouse from hibernation, and hence had relatively short bouts of torpor, reduced the extent to which they exhibit torpor where food availability is relatively higher. This is consistent with the theory that hibernating animals must make trade-offs between the energy conservation compared to the - presumably physiological - costs of torpor (Humphries *et al*. 2003). Additionally, as predicted, air temperature and day length both influenced dormouse hibernation behaviour. These results have important implications for hazel dormouse conservation, in regard to how winter food availability in combination with climatic conditions may affect dormouse trade-offs and hence survival. Such consequences may also be applicable to other hibernating species. In the light of the concerns regarding global climate change this information may be critical.

Further work

There remains much further research that could be carried out, both to continue the work in this thesis, as well as contribute to dormouse conservation throughout many other avenues. Such work would also likely inform broader conservation research and practices.

Concerning the camera trap and footprint dormouse-detection survey methods, further work is required to refine the methodologies, compare detection rates across different spatial and temporal variables and produce an explicit protocol. Further investigation into how dormice use their habitat may inform how best to employ the monitoring stations in regard to variables such as vegetation type and position in canopy. Detection rates could also be calibrated against robust measures of population size to determine if these techniques could be used as an index of abundance. This study may also encourage the use of these techniques for other arboreal small mammal species, in a variety of habitat types across the globe, and

hence assist with these methodologies meeting their full potential and aid the study of species that are particularly elusive.

Expansion of the footprint identification algorithm to incorporate more species would increase its utility to further habitats and ecosystems. Interdisciplinary research would allow for the development of more sophisticated footprint identification techniques, such as through the use of advanced statistical analyses or pattern-recognition analysis (Alibhai *et al.* 2008, Russell *et al.* 2009). Ultimately, a freely-available database of global species' footprints which allows researchers to access and share footprints, in conjunction with identification tools would have the potential to greatly expand tracking surveys, and make a significant contribution to the monitoring of species of conservation concern.

The population genetics study in Chapter 5 highlighted regional effects on dormouse genetic diversity, which are most likely driven by a combination of: edge-of-range effects due to sub-optimal habitat; an artefact of historical colonisation routes; and large landscape features that impose barriers to dormouse dispersal. Further analyses, such as approximate Bayesian computation, along with increased sample sizes may permit more detailed inferences to be made. It is an important task to evaluate the extent to which populations are influenced by edge-of-range effects across the entire distribution of hazel dormice. It is in their north-westerly range where population declines are occurring (Amori *et al*. 2008) and therefore there may be important core-periphery effects that are driving these population extinctions.

Other priorities should include assessment of the relative impact of different natural and anthropogenic landscape features on dormouse dispersal, and the importance of hedgerows in facilitating gene flow between habitat fragments. The combining of genetic data with GIS, would allow us to quantify landscape effects on dormouse population genetics and compare different dispersal barriers, to include natural and anthropogenic features. In the future, through the use of models that predict the effects of climate change, for example, on landscape variables, we may be able to make predictions of the vulnerability of existing populations.

By developing a better understanding of the mechanisms that drive current and future population genetic diversity and differentiation, it may be possible to identify

populations that are most vulnerable to extirpation, and inform conservation priorities. Such patterns may be applicable to other species that are also impacted upon by habitat loss and fragmentation. Indeed, the ecology of hazel dormice lends them well to research investigating the effects of habitat fragmentation.

The significantly lower rate of multiple paternity revealed in this thesis, compared to a previous report (Md. Naim *et al*. 2011), importantly highlights that not all hazel dormouse populations necessarily have such extreme levels of multiple paternity. The cause of the discrepancy remains unknown and therefore further investigation into the ecological and evolutionary effects on mating systems in dormice is required. More broadly, further research into the mechanisms that drive variation between populations would be of both academic and applied conservation interest, for many species that exhibit polyandry. Further studies should investigate variation in mating systems amongst dormouse populations and attempt to correlate this with environmental and population parameters. This would provide additional insights into the factors that drive dormouse behaviour, ecology, and evolution. Such data would further our understanding of the proximate and ultimate drivers of polyandry as well as highlight the consequences at a population level, such as effective population size and inbreeding. This information would also be of use to captive breeding and reintroduction projects.

The identification of intra-specific variation in hibernation patterns, and corresponding response to food availability, during the hibernation period, contributes to our understanding regarding the trade-offs dormice must make during winter. In the light of climate change more work is required to determine how this trade-off affects survival during winter, and how future climatic conditions may therefore affect dormouse abundance.

Conclusion

As stated in the general introduction in Chapter 1, conservation biologists are finding an increasing selection of tools at their disposal. This includes emerging practical field and laboratory techniques, theory, and sophisticated analytical methods,

from across the field of biology, but also other disciplines. These can provide alternative solutions, and encourage continuing innovative and adaptive thinking for conservation surveying, monitoring and planning. The results presented in this thesis, have benefited from many techniques that were not initially developed for conservation *per se*, such as molecular techniques, statistical analyses and technologies.

Additionally, the important contribution of various disciplines within biology to conservation is becoming ever more realised, and this is apparent in this thesis. There have been various debates on the relative importance of areas such as genetics and behaviour to conservation. Despite the contribution of molecular techniques to conservation having greatly increased in recent years, some question the relevance of genetics, and claim ecological and demographic parameters are of much greater importance (Frankham *et al.* 2002, Haig 1998). Whilst short-term goals may often take precedence, the maintenance of genetic diversity is vital (Haig 1998). Conservation genetics is useful in a variety of ways, such as, among many others; defining evolutionary significant units (Moritz 1994); determining migration patterns for species in fragmented populations (Goossens *et al*. 2005); understanding the significance of loss of evolutionary potential and inbreeding (Amos & Balmford 2001) and monitoring (Schwartz *et al.* 2006). As next-generation sequencing becomes more accessible, it is likely that the future will see conservationists utilise molecular techniques in increasingly novel ways. Likewise, the importance of behavioural research to conservation has also been questioned by many. However, Sutherland (1998) outlined in a review a plethora of broad areas that or concern to conservationist, in which behavioural studies may be relevant, such as small population extinctions, dispersal in fragmented populations and reducing predations. Indeed in this thesis we discuss the potential effect of behaviour and mating systems on effective population size (Anthony & Blumstein. 2000), and how plasticity in hibernation behaviour is influenced by ecological trade-offs (Humphries et al. 2003), both of which both have potentially important conservation implications.

As well as increased unified work with all biological disciplines, conservations must also be more proactive in bridging the gap between science and the general public. In order for biodiversity to be prominent on the political agenda peoples'

perception of nature and ecosystems need to change. This can be best achieved through the media and community involvement (Erhlich & Pringle 2008). The participation of the general public and volunteers is painfully undervalued, but actually mutualistically vital (Bell *et al*. 2008). Many crucial volunteer hours have contributed to this thesis, such as assistance in the collection of samples for genetic analysis and trials of footprint identification. In return it is hoped these volunteers have they also benefitted from contributing to science and that it advances their interest and concern for conservation, which they may pass onto others, including future generations.

The challenges that face future conservation biologists are monumental, and the successful preservation of biodiversity will require many approaches that include all facets of human society. As a flagship species, the hazel dormouse has a small, but nonetheless important role in this.

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