THE CONSERVATION AND LANDSCAPE GENETICS OF THE SAND LIZARD *Lacerta agilis*

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School of Life Sciences

University of Sussex

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…this 'aristocrat' needs our special care, a quiet large castle to live in and plenty of time to grow old. Only then can we preserve it for our children.

H. Strijbosch, Nijmegen, 1988

UNIVERSITY OF SUSSEX

Liam Russell **Doctor of Philosophy**

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SUMMARY

Lacerta agilis is a widespread lizard which reaches the western edge of its range in Britain where it is restricted to three geographically separated areas. Recent habitat loss and fragmentation have resulted in a significant decline and it is now a UK conservation priority. Sand lizards from across the Britain were genotyped at 15 microsatellite loci and the resulting dataset used to address questions regarding the conservation genetics, phylogeography and influence of landscape on patterns of genetic diversity.

Genetic diversity of Dorset populations compared favourably to European examples. However, diversity was significantly lower in Surrey and Merseyside. Significant genetic structuring occurred across small geographical distances even in relatively unfragmented landscapes. *Lacerta agilis* colonised Britain via a land bridge across the North Sea and reached the limits of its current distribution approximately 5,000 years BP. Subsequent climate cooling has resulted in a range contraction to areas where the habitat is suitable for the successful incubation of eggs.

A resistance surface was used to investigate the effect of landscape configuration on patterns of genetic diversity at multiple scales in Dorset. At a local scale, habitat type and rivers were the best predictors of genetic diversity. At a regional scale, rivers were most important, whereas habitat type and artificial barriers were less important. Artificial barriers may be more significant than the results suggest as their true effect has not yet been realised due to a genetic time-lag.

Male lizards from Merseyside exhibited significant differences in colour and pattern to the Dorset and Surrey populations. However, despite difference in colour, all populations were equally green, which is in keeping with the importance of 'greenness' as a sexual signal.

The implications of these findings for the conservation of *L. agilis* are discussed in the context of current challenges and predicted future global climate change.

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I hereby declare then this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree.

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My parents, who thought I had left home, gave me my old room back during the long periods in the lab and ate lots of ice cream to keep me supplied with tubs to put lizards in. But more importantly inspired in me a fascination for all that creeps, and I cannot thank them enough for their love and support.

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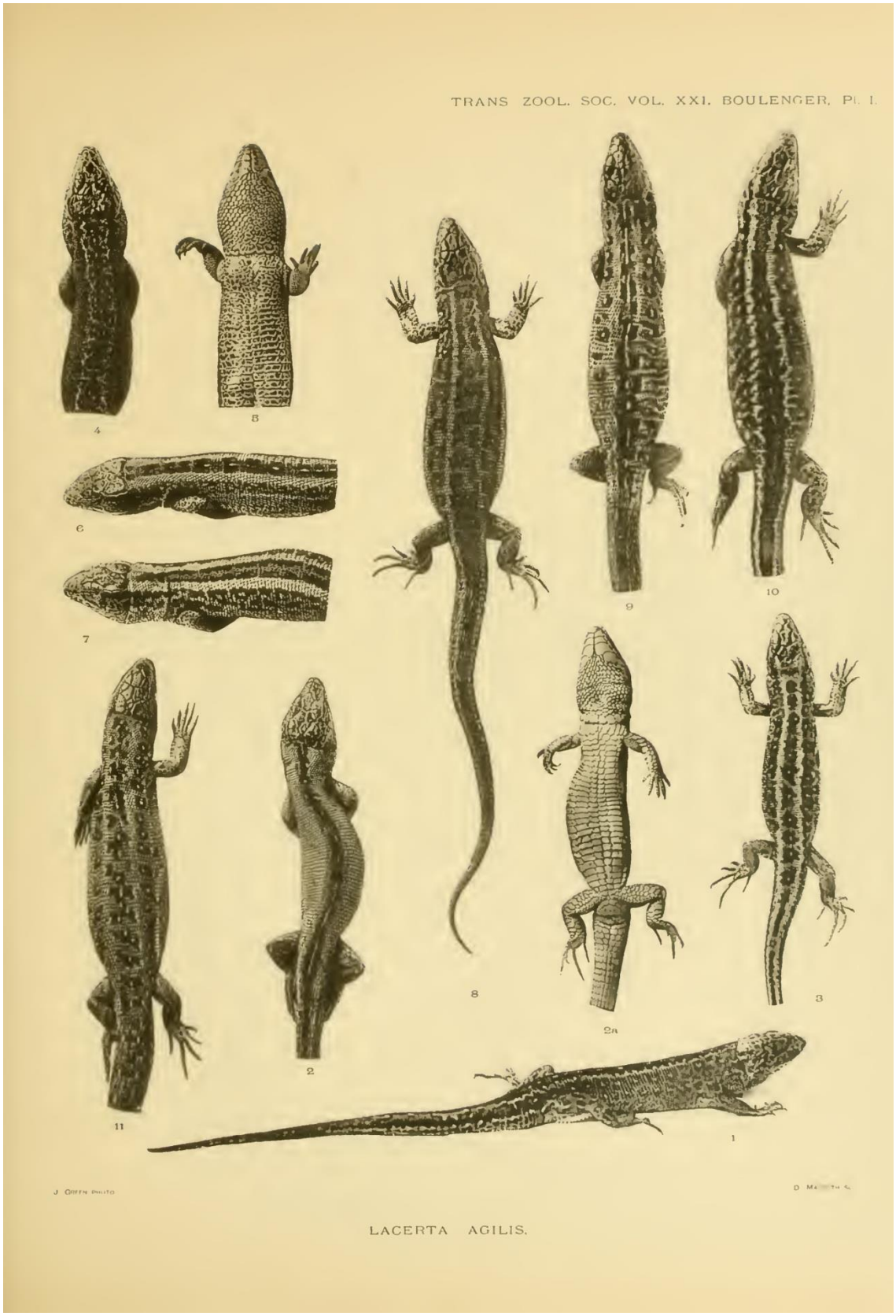
Overleaf: From G.A. Boulenger (1916): *On the Lizards allied to* Lacerta muralis*, with an Account of* Lacerta agilis *and* L. parva. Plate I. *Lacerta agilis*. 1. Male, from life, near Farnham, Surrey. 2. Male, Southport, Lancs, upper view. 2a. Same, lower view. 3. Male, Frencham [sic] Common, Surrey, upper view. 4. Male, Churt, Surrey, upper view. 5. Male, Freiburg, Baden, lower view. 6. Male, Kronstadt, Hungary, side view. 7. Male, Transylvania, side view. 8. Female, Tilford, Surrey, upper view. 9. Female, Berlin, upper view. 10. Female, Odensjö, Sweden, upper view. 11. Female, Ax-les-Thermes, Ariège, upper view.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LACERTA AGILIS.

1 INTRODUCTION

1.1 Introduction

Species are currently becoming extinct at a rate several times that of prior background levels due to an anthropogenic biodiversity crisis (Myers 1993; Pimm *et al.* 1995; Chapin *et al.* 2000; Woodruff 2001). The reasons for this are manifold: competition and predation by alien species (Mack *et al.* 2000; Clavero & Garcia-Berthou 2005); direct human exploitation (Rowcliffe *et al.* 2003; Brook & Sodhi 2006); global and local climate change (Thomas *et al.* 2004; Stork 2010); disease (Brito *et al.* 2012; McCallum 2012); and combinations of these (Kiesecker *et al.* 2001; Pounds *et al.* 2006), have all been implicated in contributing to extinctions of populations and species. However, habitat loss and fragmentation are considered to be leading causes in many habitats and species groups (Fahrig 2001; Brooks *et al.* 2002; Fahrig 2002, 2003; Cushman 2006).

1.2 Habitat Loss and Fragmentation

1.2.1 Introduction

Anthropogenic activity has increasingly modified natural landscapes creating small, isolated and fragmented populations in the remnants of previously extensive habitats. The effects of habitat loss and fragmentation, by which large areas of habitat or ecosystem are divided into smaller 'patches' surrounded by an inhospitable 'matrix' have been widely documented in many different parts of the world and in a wide variety of habitat types (Saunders *et al.* 1991; Debinski & Holt 2000; Hanski & Ovaskainen 2000; Fahrig 2002, 2003). Fragmented habitat also has a greater proportion of edges than a single large area of equivalent size, which leads to more 'edge effects' when edge habitat is less suitable than that nearer the centre of the patch (Ries *et al.* 2004).

As well as the direct impacts of habitat loss, fragmentation can also have a negative effect on the viability of a population by preventing migration between populations leading to inbreeding and genetic drift (Saunders *et al.* 1991; Mills & Allendorf 1996; Hanski 1998; Debinski & Holt 2000; Couvet 2002; Allendorf & Luikart 2007).

1.2.2 The Consequences of Isolation and Small Population Size

The size of a population of plants or animals can have significant implications for its long-term persistence. Small populations are at far greater risk of extinction than large ones as their long-term survival is dependent on the ability of relatively few individuals to survive and successfully reproduce. Isolation from other populations exacerbates this situation by preventing recruitment and the introduction of new genetic material from surrounding populations (Couvet 2002). This leaves them much more vulnerable to stochastic events, from which larger populations are better able to recover (Lande 1993). Stochastic events which affect population persistence fall into four categories (Shaffer 1981; Boyce 1992; Caughley 1994):

- *Genetic stochasticity* – changes in gene frequencies within a population brought about by processes such as genetic drift and inbreeding, which often result in reduced variability.

- *Demographic stochasticity* – changes in demographic parameters of a population such as random variation in survival and reproductive success between generations or random variation in sex ratios.

- *Environmental stochasticity* – changes in weather, climate, habitat and predation, parasitisation and disease, which affect survival and reproduction rates.

- *Natural (or anthropogenic) catastrophes* – such as fires, floods, etc. These are essentially an extreme form of environmental stochasticity that occur infrequently and affect a very high proportion of a population. This category includes anthropogenic habitat loss.

In wild populations stochastic events are often interlinked, for example, genetic stochasticity within a population may increase its vulnerability to environmental stochasticity by reducing its ability to adapt to changing environmental conditions.

It is widely recognised that small populations are vulnerable to genetic drift and inbreeding, which can lead to a reduction in genetic diversity, the fixing of deleterious mutations, inbreeding depression and reduced levels of fitness (Lynch & Gabriel 1990; Lande 1995; Frankham 1996; Reed & Frankham 2003; Allendorf & Luikart 2007), all of which have contributed to extinctions of wild populations (Frankham 1995; Saccheri *et al.* 1998). In wild populations, numerous measures of reduced fitness (weight at birth, survival rates,

reproduction and growth rates, resistance to predation, disease and environmental stress) have been linked to inbreeding depression (Keller & Waller 2002; Shikano & Taniguchi 2002).

Isolation from other populations exacerbates the effects of small population size as it restricts gene flow by preventing immigrants from introducing new genetic material (Saccheri *et al.* 1998; Higgins & Lynch 2001; Couvet 2002). Species which have specific habitat requirements and low vagility are of increased vulnerability to habitat fragmentation as individuals are often unable to disperse across the matrix. In this situation gene flow between different patches is prevented, effectively creating a number of small isolated populations from a previously large continuous one. Mills & Allendorf (1996) suggested that at least one immigrant per generation would be necessary to maintain genetic viability in most wild populations.

The potential impacts of isolation and small population size were graphically illustrated by a long-term study of a population of adders *Vipera berus* from Smygehuk in Sweden, which has provided compelling evidence of the detrimental effects of isolation due to habitat loss upon reptiles. The *V. berus* population at Smygehuk became isolated due to habitat loss and destruction and by the 1980s numbered approximately 30 animals, with an effective population size (the number of breeding adults required to maintain observed levels of genetic variability in a theoretical ideal population) of less than 15. At this time, genetic diversity measured using various genetic markers was significantly lower than in three other non-isolated populations (including one of a similar size), indicating high levels of inbreeding. This had a manifest effect on the reproductive success of the population; females there were producing significantly smaller broods, a high proportion of which were deformed or stillborn (Madsen *et al.* 1996). In an effort to prevent the probable extinction of the population, conservationists proposed a 'genetic restoration' of the population. In 1992, 20 adult male adults were captured from larger, more genetically variable populations and released in Smygehuk, with surviving individuals removed after four breeding seasons. By 1999 the number of males in the population (excluding the introduced snakes) had risen from less than five to nearly 35 with an associated increase in genetic variability, and the proportion of stillborn young fell rapidly (Madsen *et al.* 1999). By 2003, the number of males had risen to 39 (Madsen *et al.* 2004).

Related species can show markedly different response to fragmentation. Chiucchi & Gibbs (2010) investigated levels of historic and contemporary gene flow in the eastern Massasauga rattlesnake *Sistrurus catenatus* and found that recent fragmentation had had a limited effect

on the population genetics. This was attributed to the species existing in small isolated, yet inbreeding-resistant populations prior to anthropogenic influence on the landscape.

1.2.3 Natural Causes of Small Population Size and Low Genetic Diversity

Small, genetically impoverished populations can arise naturally as a result of biogeography. Populations on the edge of a species' range are often significantly less genetically diverse than those nearer the centre of the range as a result of population expansion patterns (Nichols & Hewitt 1994; Allendorf & Luikart 2007; Bohme *et al.* 2007; Eckert *et al.* 2008; Ramakrishnan *et al.* 2010). For example, the natterjack toad *Bufo calamita* reaches the western edge of its range in Britain and has low genetic diversity across the country (Hitchings & Beebee 1996; Rowe *et al.* 1999), with evidence that some populations have undergone genetic bottlenecks (Beebee & Rowe 2001a). Whilst the small population size and low genetic variability of some populations can be attributed to anthropogenic activities, even relatively large populations in extensive undisturbed areas of habitat exhibit low genetic diversity compared to similar European populations. Genetic diversity within European *B. calamita* populations is correlated with distance from an ice-age refugium in the Iberian Peninsula (Beebee & Rowe 2000) and the low diversity within Britain as a whole is in part attributable to biogeography and colonisation history. This pattern is reflected within Britain with populations near the edge of the range having low genetic diversity regardless of population size (Rowe *et al.* 1999). Whilst low genetic diversity across a country or region may be a result of colonisation history, differing levels of diversity between populations within these areas may be as result of anthropogenic activity. *B. calamita* in Denmark also have a low overall genetic diversity which can be attributed to its colonisation history, however subsequent habitat fragmentation has prevented gene flow between remaining populations resulting in high levels of differentiation (Allentoft *et al.* 2009).

Given the potential for different causes of low genetic variability and small population size, it is important to gain a clear understanding of the contemporary and historical reasons underlying observed patterns in genetic variability and population structure. False assumptions about the causes of levels of genetic variability and degree of structuring could result in the misallocation of resources for the conservation management of species. Although edge populations often show reduced genetic variation they can be particularly important for long-term species persistence as they often exist in extreme environmental conditions. This can mean they are better able to respond to environmental change and therefore may be particularly important with respect to the future evolution of species (Lesica & Allendorf 1995).

1.2.4 Habitat Fragmentation and Lizards

Lizards typically exhibit low vagility (Bennett 1983) and small body size, both of which decrease dispersal ability and therefore potentially increase vulnerability to adverse fragmentation effects (Gibbons *et al.* 2000; Jenkins *et al.* 2007). However, they have often been found to persist in small habitat patches for longer than other taxonomic groups (Burkey 1995; Prugh *et al.* 2008) provided the remaining habitat is of sufficient quality (Santos *et al.* 2008).

Habitat fragmentation can affect lizard populations via a number of different mechanisms. The size of a habitat patch can influence the likelihood of occupancy and density of individual lizard species, including *Lacerta viridis* (Maura *et al.* 2011), *Coleodactylus amazonicus* and *Gonatodes humeralis* (Carvalho *et al.* 2008), *Uma inornata* (Barrows & Allen 2007), *Psammodromus algirus* (Diaz *et al.* 2000) and *Gehyra variegata* (Sarre 1998), and the species-richness of the patch in lizard communities from a variety of habitats (Bell & Donnelly 2006; Michael *et al.* 2008; Watling & Donnelly 2008). However, the opposite effect has been recorded in *Podarcis siculus* as smaller habitat patches were less likely to support predatory snakes (Maura *et al.* 2011).

Patch size also has a demographic effect on lizard populations, for example survival rates in *Sceloporus woodi* (Hokit & Branch 2003a, b) and *Gnypetoscincus queenslandiae* (Sumner *et al.* 2004) were lower in smaller habitat patches. Reproductive output of *P. algirus* in the Mediterranean and *Tropidurus* spp. from the Amazon rainforest was correlated with patch size, with females from smaller habitat fragments producing fewer eggs and a smaller clutch mass (Vitt 1993; Diaz *et al.* 2005), a pattern which reflects that seen in naturally small island populations of lacertid lizards when compared to mainland populations (Siliceo & Diaz 2010). Smaller adult body size was observed in *G. queenslandiae* from small forest fragments, which also contained a smaller proportion of adults within the population (Sumner *et al.* 1999). The explanation for these patterns is not always clear, however prey availability and the loss of suitable microclimates due to edge effects have been suggested (Sumner *et al.* 1999).

Edge effects due to fragmentation resulted in an increase in predation on Australian skinks as the greater proportion of edge habitat within the landscape meant that the skinks more frequently came into contact with predatory bird species not found nearer to the centre of habitat patches (Anderson & Burgin 2008). In addition to the risk of predation, lizards in a habitat where the number of predators was artificially increased became less mobile and showed a dietary shift to smaller prey items which had a lower handling time (Hawlena & Perez-Mellado 2009).

Demographic impacts can occur when dispersal between habitat patches is prevented. Dispersal in viviparous lizards *Lacerta* (*Zootoca*) *vivipara* is density-dependent; when population density reaches a critical level, juvenile lizards will disperse to other neighbouring populations. Using experimental manipulation of connectivity between populations, Lecomte *et al.* (2004) observed that the size of connected populations became increasingly homogenised over time. By contrast, isolated populations typically underwent a population explosion followed by a rapid decline. Juvenile lizards which were 'forced' to disperse from lower connectivity patches had a lower likelihood of surviving hibernation than those from connected habitat (Boudjemadi *et al.* 1999).

The genetic implications of habitat fragmentation have been demonstrated in a number of species of lizards. Berry *et al.* (2005) and Levy *et al.* (2010) investigated the fragmentation effects caused by agricultural land use in the grand skink *Oligosoma grande* from New Zealand and the agamid *Ctenophorus ornatus* in Australia respectively. Both studies showed that populations from fragmented agricultural landscapes were less genetically diverse and more highly structured than those from areas with natural vegetation. A similar effect was also noted in fragmented *Lacerta agilis* populations from Sweden (Gullberg *et al.* 1998).

Many studies have demonstrated reduced variability and greater genetic structuring in lizards from fragmented habitats. However, a number of other studies have found no apparent effect. Habitat fragmentation in the form of deforestation inhibited dispersal in the skink *Egernia cunninghami*, resulting in higher levels of genetic similarity within clusters within deforested areas compared to adjacent naturally vegetated areas (Stow *et al.* 2001). However, this did not result in increased levels of inbreeding as the species was able to avoid mating with close kin and therefore levels of genetic variability were maintained (Stow & Sunnucks 2004). Similar apparent resistance to inbreeding and loss of genetic diversity has been recorded in a number of lizard species (Sumner *et al.* 2004; Smith *et al.* 2009; McCoy *et al.* 2010; Maldonado *et al.* 2012; Remon *et al.* 2012). This has been attributed to relatively long generation times causing a time-lag before genetic effects are observable (Richmond *et al.* 2009; McCoy *et al.* 2010), or to reproductive strategies which avoid inbreeding and the resultant loss of genetic diversity due to preferential selection of genetically different (Olsson *et al.* 1999; Olsson *et al.* 2003; Stow & Sunnucks 2004) or genetically diverse mates (Laloi *et al.* 2011).

1.3 Landscape Genetics

1.3.1 Introduction

Landscape genetics is a relatively new field which combines genetic and spatial data to investigate how the landscape affects genetic processes such as gene flow and genetic drift (Manel *et al.* 2003; Holderegger & Wagner 2008). In a landscape where populations of an organism exist as 'islands' in a matrix of permeable habitat, one would expect to see a simple linear relationship between geographic and genetic distance, with genetic distance between populations increasing proportionately to geographic distance. This effect is commonly known as Isolation by Distance (IBD) (Wright 1943; Kimura & Weiss 1964) and occurs when genetic drift and gene flow are in equilibrium. In more complex landscapes, IBD effects can be obscured due to the differences in permeability of the matrix to migrants. This prevents gene flow between populations and therefore disrupts the equilibrium between gene flow and genetic drift. For example two populations separated by a barrier which prevented gene flow such as a river or a road would become genetically differentiated due to genetic drift despite a small geographic distance. Conversely there is likely to be a degree of gene flow between two populations separated by a relatively permeable habitat and therefore these are likely to be less genetically different over a larger geographic distance (Vignieri 2005).

Hutchison & Templeton (1999) investigated the effects of landscape history and configuration in the population genetics of the eastern collared lizard *Crotaphytus collaris*. As expected, in an unfragmented landscape with long-established lizard occupancy, they found gene flow and genetic drift to be in equilibrium, producing a linear relationship between genetic and geographic distance (Figure 1.1a). In an unfragmented landscape which had been relatively recently colonised by the lizards, gene flow was found to be dominant over genetic drift with low variance in genetic differentiation (Figure 1.1b). This was due to insufficient time for significant genetic drift to affect individual populations, which remained relatively similar to each other. In a highly fragmented landscape with lizard populations separated by large geographic distances, no IBD relationship was observed and there was high variance in genetic differentiation (Figure 1.1c). The high degree of long-established fragmentation in this landscape prevented migration between populations and therefore gene flow, meaning that genetic drift dominated. In a more recently fragmented landscape there was some correlation between genetic and geographic distance when geographic distance was small, whereas at larger distances this relationship was lost and variance of genetic differentiation increased (Figure 1.1d). This pattern of equilibrium between gene flow and genetic drift at a small local scale and dominant genetic drift at a larger regional scale was interpreted as a result of recent

fragmentation gradually reversing a regional equilibrium by preventing migration between populations. In time, this region is likely to develop a pattern seen in the highly fragmented landscape.

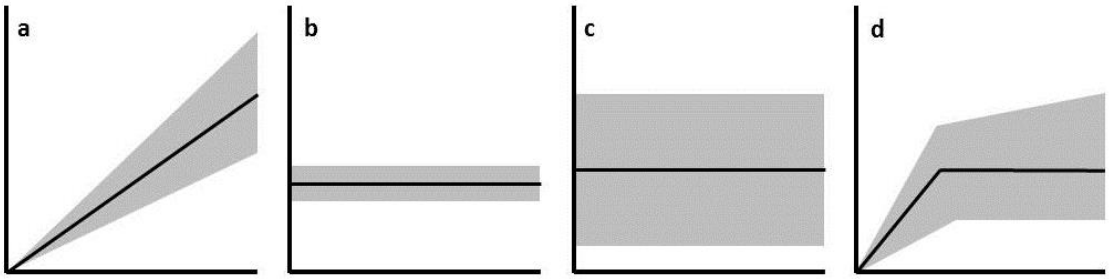


Figure 1.1. Relationship between geographic distance (X axis) and genetic distance (Y axis) in the eastern collared lizard *Crotaphytus collaris* in different landscape configurations. The black line is the hypothetical line of best fit; the shaded area denotes the spread of the data. a) Gene flow and genetic drift are in equilibrium in an unfragmented landscape. b) Gene flow is dominant but genetic differentiation is low in a recently colonised unfragmented landscape. c) Genetic drift is dominant and genetic differentiation is high in a highly fragmented landscape. d) Gene flow and genetic drift are in equilibrium over short geographic distances whereas genetic drift is dominant at large genetic distances due to increasing fragmentation disrupting equilibrium. After Hutchison & Templeton (1999).

Understanding the effect of the landscape on genetic processes can be extremely useful for conservation practitioners. Straightforward applications of landscape genetics may include the identification of natural discontinuities in gene flow patterns (Millions & Swanson 2007; Neaves *et al.* 2009; Quemere *et al.* 2010) as well as anthropogenic barriers (Liu *et al.* 2009; Clark *et al.* 2010; Davis *et al.* 2010), or revealing cryptic spatial genetic structuring (Latch *et al.* 2011). It can also be used to investigate factors which facilitate migration in particular species, such as correlating patterns in genetic differentiation with woodland corridors in roe deer *Capreolus capreolus* (Coulon *et al.* 2004), riparian corridors in the Pacific jumping mouse *Zapus trinotatus* (Vignieri 2005) and percentage of canopy cover in green spaces between populations of white-footed mice *Peromyscus leucopus* in New York City (Munshi-South 2012).

Landscape genetics can also be used to investigate distribution patterns of species, for example Giordano *et al.* (2007) found a negative correlation between genetic variation and altitude in the long-toed salamander *Ambystoma macrodactulym*, which shed light on the factors which limit its range, whilst Selkoe *et al.* (2010) found that kelp bed distribution accurately predicted genetic patterns in one fish and two marine invertebrate species. Angelone & Holderegger (2009) used a landscape genetics approach to assess the

effectiveness of measures to improve connectivity of European tree frog *Hyla arborea* habitat in Switzerland and found that these were facilitating migration between sites.

1.3.2 Resistance Surfaces

In complex landscapes, the IBD relationship between genetic and geographic distance is limited in its usefulness for describing population structure and genetic processes. In order to investigate such scenarios, many studies have used a resistance surface which quantifies the 'effective distance' between two populations to test the effect of landscape on the movement and migration of individuals and gene flow between populations (Adriaensen *et al.* 2003; Sutcliffe *et al.* 2003; Spear *et al.* 2010; Zeller *et al.* 2012). A resistance surface is a hypothetical representation of a landscape divided into individual cells or pixels, each of which has a cost (a resistance value) assigned to it which reflects how difficult it is for an organism to disperse through it. Resistance values can be assigned to each pixel according to many different geographical or environmental factors which affect an organism's dispersal (see Sawyer *et al.* (2011) and Zeller *et al.* (2012) for a review). Resistance surfaces can provide answers to a variety of landscape genetics questions such as the identification of landscape features which particularly influence gene flow, the identification of barriers to gene flow, the identification and designation of movement corridors for conservation purposes and the prediction of species' reactions to environmental change (Spear *et al.* 2010).

There are two commonly used methods of calculating effective distance of an organism moving through a resistance surface: Least Cost Path (LCP) and Isolation by Resistance (IBR). The LCP (Adriaensen *et al.* 2003) is the path between two points on the resistance surface which has the least cumulative resistance value of all the pixels through which it passes (Figure 1.2). IBR (McRae 2006; McRae *et al.* 2008) is based on the theory of electrical circuits where gene flow is analogous to an electrical current (McRae & Beier 2007). The current flows across all of the pixels of the resistance surface and is able to flow more easily across wide areas of low resistance and less easily across areas of high resistance (Figure 1.3). The distance metric is the total resistance value across the entire surface. IBR offers advantages over LCP because the current follows many routes across the surface, whereas LCP assumes that any organism moving across a landscape has prior knowledge of the optimal path. This is unlikely to reflect true between-population migration patterns as gene flow is unlikely to follow a single linear path and therefore IBR is more likely to reflect patterns of gene flow across a landscape.

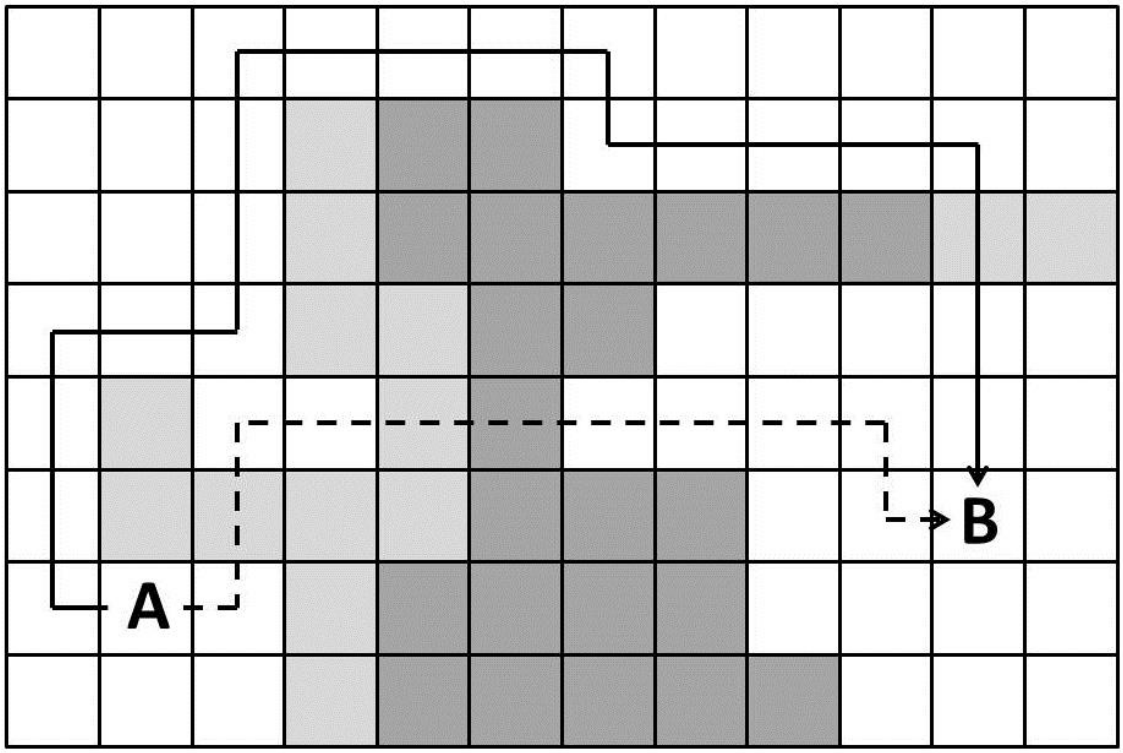


Figure 1.2. Example of Least Cost Path (LCP) on a hypothetical resistance surface. In this example, if the resistance of the white cells = 1, the light grey cells = 2 and the dark grey cells = 5 the dashed line represents the LCP with a total cost of 18, compared to 23 of the solid line. If the resistance values are changed so that the white cells = 1, the light grey cells = 4 and the dark grey cells = 10, the solid line becomes the LCP with a cumulative cost of 25, whist the dashed line has a cumulative cost of 27

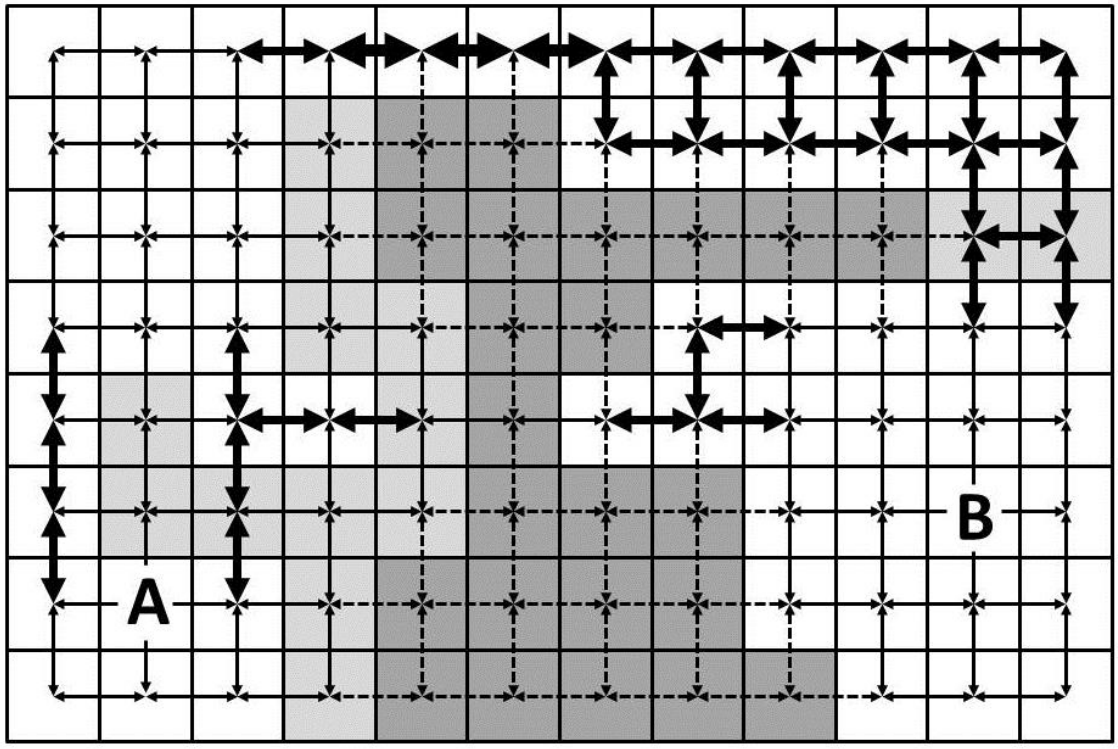


Figure 1.3. Example of Isolation by Resistance (IBR) on a hypothetical resistance surface. Darker colours are cells with higher resistance; the strength of the arrows represents the approximate relative importance of the pathway.

Accurately parameterising a resistance surface is vital if it is to provide useful estimates of species movement and gene flow across a landscape (Koen *et al.* 2012). There are a number of different approaches to quantifying resistance (see Spear *et al.* (2010) and Zeller *et al.* (2012) for reviews). Many studies use 'expert opinion' to parameterise the resistance surface and this technique remains the most commonly used (Zeller *et al.* 2012). Whilst this can prove accurate for species which are well studied or with which the investigator has extensive experience, it has obvious limitations (Murray *et al.* 2009; Sawyer *et al.* 2011) as knowledge of a species' ecology may not translate to information about gene flow.

Resistance surfaces can be parameterised using non-genetic field data which inform the investigator about the dispersal and migration patterns of the species. This may include data from radiotracking (Cushman & Lewis 2010), mark-recapture or satellite telemetry (Shafer *et al.* 2012) which track the movements of individual organisms. Stevens *et al.* (2006a) conducted behavioural experiments to assess the permeability of various habitat types and barriers to *B. calamita.* The results were later used to quantify a resistance surface which found that LCP better explained dispersal patterns than Euclidean distances (Stevens *et al.* 2006b). Such data can be difficult to obtain for many species, particularly those with cryptic behaviour, and the movements of individual organisms may not necessarily reflect gene flow. Resistance surfaces can also be parameterised on the basis of habitat and environmental suitability for a particular species. Ecological Niche Factor Analysis (Hirzel *et al.* 2002) can be used to determine the suitability of pixels on a resistance surface, to which a resistance value can be assigned, on the basis of occurrence records of the study species (Wang *et al.* 2008; Row *et al.* 2010).

A common approach is to construct several resistance surfaces with different resistance values for different features within a surface and then select the surface which best fits the genetic data. This approach identified sea lochs a significant barrier to gene flow and river corridors and woodland as facilitators of gene flow in red deer *Cervus elaphus* in Scotland (Perez-Espona *et al.* 2008).

Genetic data can be used to parameterise resistances surfaces. For example Garroway *et al.* (2011) used genetic data to parameterise a resistance surface for fishers *Martes pennanti* by creating a network of genetic connectivity and then fitting this to a habitat resistance surface which found that roads, rivers and deep snow impeded gene flow.

1.3.3 Examples of use of Resistance Surfaces

Amphibians have frequently been studied using resistance surfaces (Zeller *et al.* 2012) as they typically have specific environmental and habitat requirements, such as the need for ponds for

breeding, and therefore often exist in metapopulations (Gill 1978; Marsh & Trenham 2001). Goldberg & Waits (2010) used a resistance surface parameterised by habitat type and environmental gradients to investigate the population genetics of two pond-breeding amphibians: Columbia spotted frogs *Rana luteiventris* and long-toed salamanders *Ambystoma macrodactylum*. Developed land had the highest resistance for both species whilst agricultural and scrub habitat was the least resistant for *R. luteiventris*, and *A. macrodactylum* followed a moisture gradient where forest was the least resistant habitat. Spear *et al.* (2005) found an IBD pattern of genetic differentiation in blotched tiger salamanders *Ambystoma tigrinum*, when open shrub habitat and stream crossings occurred between populations, genetic differentiation between them was decreased, and where the populations were separated by areas of higher elevation, genetic differentiation was increased. Chaparral habitat was found to have the lowest resistance for dispersing California tiger salamanders *Ambystoma californiense* compared to grassland and oak woodland (Wang *et al.* 2009). Landscape resistance was used to investigate population differentiation in the Moor frog *Rana arvalis* and showed that roads were a significant barrier to dispersal between ponds, whereas habitat type had a relatively limited effect (Arens *et al.* 2007). The habitat of the Red Hills salamander *Phaeognathus hubrichti* has been highly fragmented by agriculture and forestry. Using habitat resistance, Apodaca *et al.* (2012) found that fragmentation was limiting present-day gene flow when compared to historical levels.

Gene flow across a landscape can be affected by different features at different scales. Murphy *et al.* (2010b) found that a variety of environmental and habitat conditions including precipitation during growth, habitat affected by previous fires, availability of cover, temperature, roads and development, and topographic complexity all influence connectivity in western toad *Bufo boreas* populations in Yellowstone National Park. However, different variables had a varying effect at different scales: habitat permeability was most important at a local scale, whilst temperature and moisture were more important across multiple scales. Different landscape features were demonstrated to affect gene flow at different spatial scales in *H. arborea*. At distances < 2 km large rivers were a significant barrier to gene flow, at distances > 2 km roads and forests were significant barriers whilst hedgerows and other landscape features with suitable structure facilitated gene flow (Angelone *et al.* 2011).

Timber rattlesnakes *Crotalus horridus* hibernate communally and individuals typically remain faithful to a hibernation site for life with offspring showing a high degree of philopatry (Brown *et al.* 2007). Inbreeding is avoided by matings during seasonal migration in the summer

months (Brown 1993). Clark *et al.* (2008) found that LCP, defined by the availability of basking habitat was highly correlated with genetic differentiation between hibernacula.

Environmental gradients may be less apparent than physical geography or habitat type, however they can influence the resistance of an environment to an organism and therefore have a significant influence on patterns of gene flow. For example, Jorgensen *et al.* (2005) showed that genetic differentiation patterns of herrings *Clupea harengus* in the North Sea were significantly associated with salinity and sea surface temperature. Dispersal routes of the wolverine *Gulo gulo* as defined by LCP based on spring snow cover was found to explain patterns in genetic differentiation (Schwartz *et al.* 2009).

Resistance surfaces can be applied to the practical conservation management of species, for example by identifying migration corridors. Epps *et al.* (2007) used a resistance surface to elucidate migration routes of bighorn sheep *Ovis canadensis* and identify where these were affected by anthropogenic barriers such as roads. This enabled the effectiveness of proposed mitigation measures such as translocation to be evaluated. A similar approach investigated dispersal pathways of the tiger *Panthera tigris* in India and identified areas where sub-optimal habitat could be improved (Rathore *et al.* 2012).

Reptiles are underrepresented in landscapes genetics studies. In a recent review, Zeller *et al.* (2012) identified only eight attempts to produce a resistance surface for reptiles, compared to 83 for mammals, 17 for amphibians, 16 for birds and 10 for invertebrates; only fish had fewer studies with one resistance surface produced.

1.3.4 Data Requirements for use of a Resistance Surface

The ability of any landscape genetics study to accurately predict the effect of landscape on genetic processes is limited by the strength and depth of the genetic data used to test the hypotheses. The number and variability of the genetic markers used has a significant influence on the power of any analysis undertaken; using a greater number of markers with higher variability will give more statistical power than increasing sample size (Landguth *et al.* 2012). Neutral genetic markers (i.e. those which are not under direct selection pressure) are ideal for landscape genetics studies as they allow the investigator to directly test the influence of various landscape features on processes such as gene flow, migration and dispersal (Holderegger *et al.* 2006). Microsatellites are highly variable neutral genetic markers commonly used in population genetics studies (Hedrick 1999; Zane *et al.* 2002; Ellegren 2004). Consequently they are ideal for testing landscape genetics hypotheses (Wang 2011) and are the most frequently used marker in landscape genetics studies (Storfer *et al.* 2010).

1.4 Loss and Fragmentation of Lowland Heathland

1.4.1 Lowland Heathland in Northwest Europe

Lowland heathland habitat is a plagioclimax community dominated by dwarf shrub species, particularly heathers such as *Calluna vulgaris* and *Erica* spp. which is found in northwest Europe. Heathland was created by forest clearances in areas with sandy soils 3000-4000 years BP (Chapman *et al.* 1989) and maintained by subsequent clearance of successional vegetation for fuel and by grazing (Webb 1986). It has suffered significant levels of decline across much of its range in the past 200 years (Moore 1962; Webb 1990; Piessens *et al.* 2005; Tsaliki & Diekmann 2010) and it is estimated that heathland now covers < 10% of its previous extent (Rose *et al.* 2000). Lowland heathland is considered of particular biodiversity value as it supports a high number of specialised plant and animal species, and consequently it has been afforded a high level of protection under European legislation (Joint Nature Conservation Committee 2007).

1.4.2 Heathland Loss and Fragmentation in Dorset

Loss and fragmentation of lowland heathland in Dorset in the United Kingdom has been particularly well documented (Figure 1.4 and Figure 1.5). In 1796, heathland habitats covered an estimated 40,000 ha of the Poole Basin area of Dorset (Haskins 1978). Using historical mapping, Moore (1962) estimated the extent of heathland in Dorset as 30,000 ha in 1811. By 1896 this had declined to 26,000 ha, falling to 18,000 ha in 1934 and 10,000 ha in 1960. In 1978 it was estimated that only 6,000 ha of heathland remained (Webb & Haskins 1980). Webb (1990) estimated heathland cover in Dorset as 7,977 ha in 1987, however this was calculated using a different methodology to previous studies and corresponded to a decline of 5% since 1978. A further decline of 7% continued until 1996 (Rose *et al.* 2000). Hooftman & Bullock (2012) compared remotely sensed habitat data from 2007 to habitat maps from the 1930s which were compiled from field surveys and recorded that the total area of heathland in Dorset had decreased from 13,722 ha in the 1930s to 6,004 ha in 2000, a decline of 56%. In addition to direct loss, the remaining heathland has become increasingly fragmented; between 1987 and 1996 the number of heathland fragments in Dorset increased from 142 to 151, whilst the total area continued to decrease (Rose *et al.* 2000). Hooftman & Bullock (2012) recorded an 88% decrease in the mean patch size of heathland fragments and measured a significant loss of connectivity between heathland patches since the 1930s.

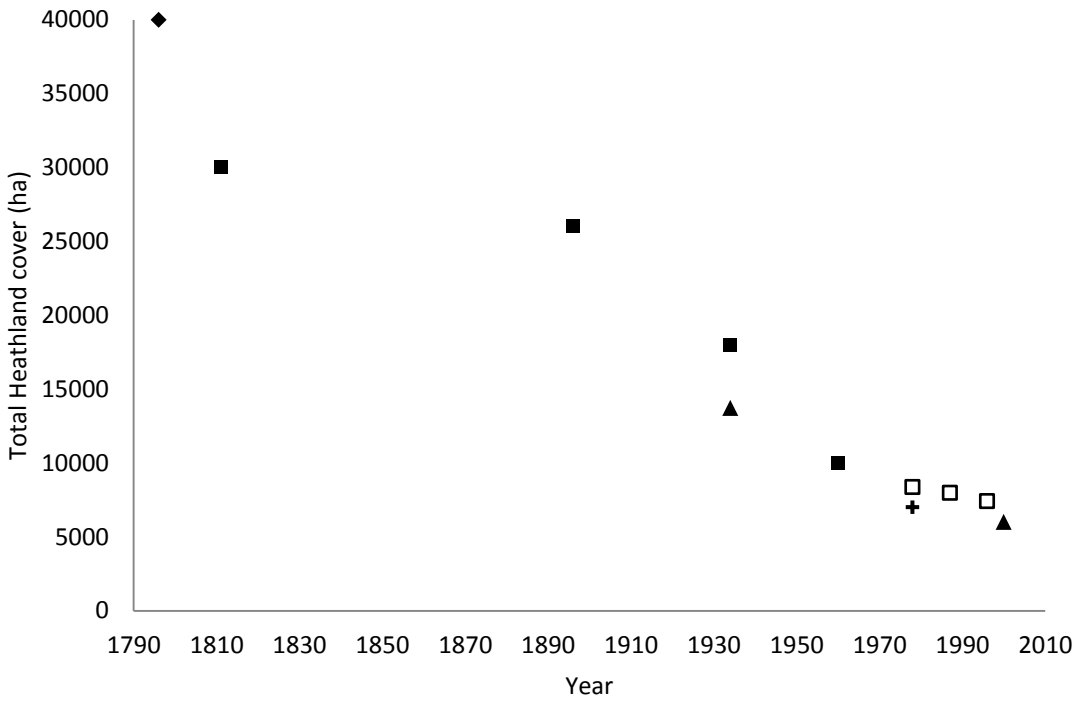


Figure 1.4. Estimated loss of heathland in Dorset from various studies. Diamonds = Haskins (1978), filled squares = Moore (1962), triangles = Hooftman & Bullock (2012), crosses = Webb & Haskins (1980), open squares = Webb (1990) and Rose *et al.* (2000)

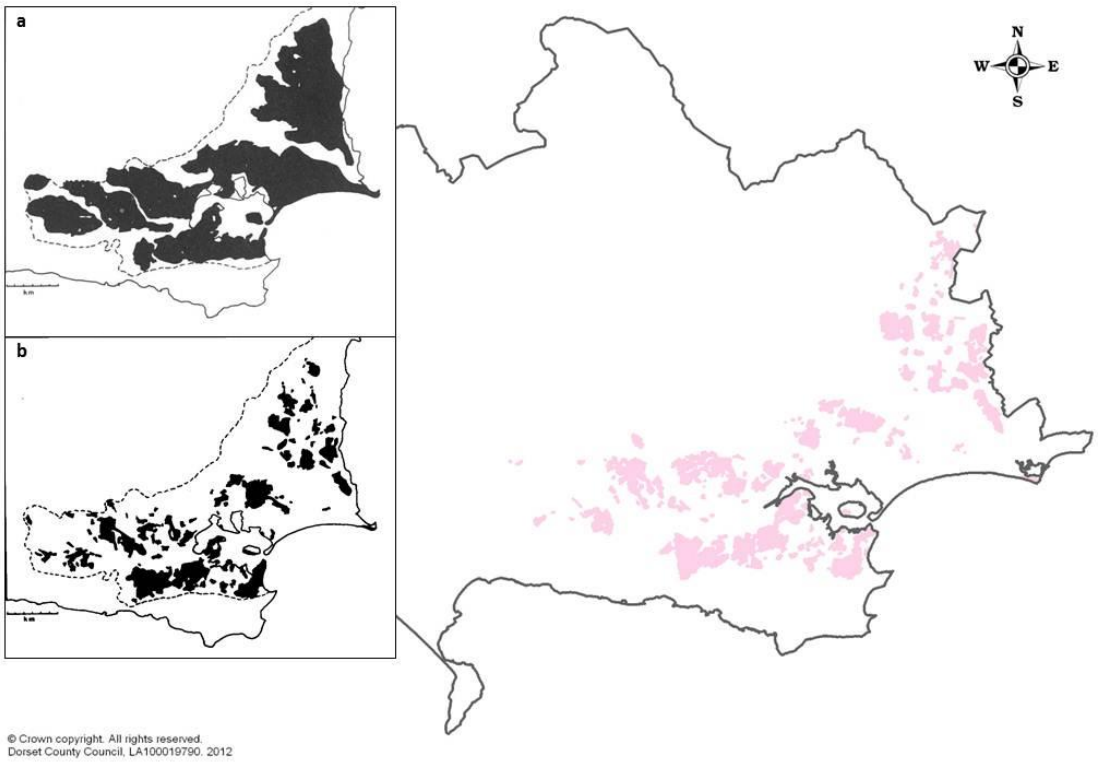


Figure 1.5. Current and historical extent of heathland in Dorset. In the main part of the figure the current extent of lowland heathland is shown as the shaded areas. Inset a) extent of heathland in the Poole basin in 1759; inset b) extent of heathland in 1962. The dashed line represents the boundary of the Tertiary deposits on which heathland develops. Insets reproduced from Webb & Haskins (1980).

The major cause of heathland loss has been identified as changes in land use such as urban expansion, conversion to agricultural use, commercial afforestation and mineral extraction (Moore 1962; Webb & Haskins 1980). However, significant losses have been attributed to natural succession as a result of declines in traditional management practices such as grazing and burning (Moore 1962; Webb 1990; Rose *et al.* 2000) which eventually result in changes to the soil chemistry and prevent future recolonisation by heathland species (Mitchell *et al.* 1997).

Where heathland loss and fragmentation has been caused by urbanisation, increased human use can have a further detrimental effect on the remaining habitat and fauna due to disturbance, increased incidence of fires and predation by domestic pets, effectively further reducing patch size for some species (van den Berg *et al.* 2001; Liley & Clarke 2003).

1.4.3 Effects of Fragmentation on Heathland Flora and Fauna

The effects of heathland fragmentation have been demonstrated in a number of plant, invertebrate and bird species. Plant diversity assessed by multiple indices decreases relative to patch size in heathland fragments in Britain (Webb & Vermaat 1990) and Germany (Dieckhoff *et al.* 2006), and the degree of patch isolation in Britain (Webb & Hopkins 1984) and Belgium (Piessens *et al.* 2005).

Similar effects have been recorded for invertebrates, with beetle diversity decreasing with patch size and the quantity of heathland within 2 km of the occupied patch (Webb & Hopkins 1984). This effect was not recorded in spiders in the same study, however Hopkins & Webb (1984) found that spiders with a poor dispersal ability were confined to larger patches of heathland and a similar pattern was found in ground beetles in the Netherlands (de Vries *et al.* 1996). The likelihood of occupation of heathland patches by the silver-studded blue *Plebejus argus* was linked to patch size in lowland heathland in Southern Britain (Thomas *et al.* 1998), where 100% of heathland patches larger than 50 ha were occupied compared to 50% of patches smaller than 33 ha. Webb *et al.* (1984) also found that the quality of the matrix affected invertebrate diversity on heathland with heathland patches surrounded by structurally diverse vegetation having a greater invertebrate diversity. Smaller and more isolated patches of heathland had a lower rate of occupation by breeding Dartford warblers *Sylvia undata*, as they were less likely to be found by dispersing juvenile birds seeking to establish new territories (van den Berg *et al.* 2001).

Heathland fragmentation has been demonstrated to have an effect at a molecular level in the flightless beetle *Poecilus lepidus* in Germany where allelic richness, number of alleles and

expected heterozygosity all had a significant relationship with patch size and it was therefore recommended that a minimum patch size of 50 ha was necessary to maintain genetic diversity (Drees *et al.* 2011). Conversely, a heathland bee species *Andrena fuscipes* did not seem to be affected by fragmentation with insignificant genetic structure between heathland patches (Exeler *et al.* 2010). This is likely to be a result of its greater dispersal ability when compared to flightless insects.

Although it has been considered a factor in the decline of the sand lizard *Lacerta agilis* and smooth snake *Coronella austriaca* in southern Britain (Corbett & Tamarind 1979; Beebee & Griffiths 2000), the effects of heathland fragmentation on reptiles are less well studied than other groups. However, Pernetta (2009) found that patch size was an important determinant of occupancy by *C. austriaca.* The same study looked at the population genetics of smooth snakes in fragmented heathland and detected some degree of structuring between populations in different fragmented heathland patches; it however did not unambiguously attribute this effect to fragmentation.

1.5 Sand Lizards

1.5.1 Introduction

The sand lizard *Lacerta agilis* in Britain is an ideal organism in which to investigate the effects of natural and anthropogenic processes on population genetics. *Lacerta agilis* (Figure 1.6 and Figure 1.7) is a widely distributed reptile with a range that stretches from northwest Europe across Asia into Mongolia (Gasc *et al.* 2004). The United Kingdom contains the westernmost *L. agilis* populations which are restricted to sites with specific habitats in the south and northwest, making it one of the country's rarest reptile species (Beebee & Griffiths 2000). It has declined significantly as a result of habitat loss (Corbett 1988b) and consequently is a target of significant conservation action (Corbett 1988a; Corbett & Moulton 1998; Moulton & Corbett 1999; Herpetological Conservation Trust 2009).

Figure 1.6. Male sand lizard *Lacerta agilis* in breeding colouration from Bergherbos, the Netherlands.

Figure 1.7. Female sand lizard *Lacerta agilis* from Wareham Forest, United Kingdom

1.5.2 Range, Distribution and Habitats

Throughout much of its range *L. agilis* inhabits a wide variety of habitat types including agricultural margins, grassland, steppe and hedgerows (Arnold & Ovenden 2002; Gasc *et al.* 2004). However at the north-western edge of the range it is restricted to specific habitats, typically on sandy substrates such as heathland, open woodland and coastal sand dunes (Strijbosch & Creemers 1988; Stumpel 1988; Beebee & Griffiths 2000; Berglind 2000; Ceirans 2008). Experiments into egg incubation suggest that the current northern limit of their range is determined by the climatic conditions required for successful incubation (Rykena 1987) and the amount of sunshine also appears to play a role in determining the species' range in Britain (Jackson 1978) and distribution at a local scale (Dent & Spellerberg 1987). Within Britain, *L. agilis* is particularly associated with long-established mature heathland habitats, dominated by dwarf shrub species, particularly heather *Calluna vulgaris* between 3 cm and 50 cm in height where there are frequent discontinuities in vegetation height resulting in many interfaces between areas of taller and shorter vegetation (House & Spellerberg 1983; Dent & Spellerberg 1987). Nicholson (1980) suggested that such structurally diverse habitats were preferred by *L. agilis* as they are richer in invertebrate prey, whereas House & Spellerberg (1983) attribute this to a requirement for a variety of basking sites for thermoregulation. Within such habitats, microhabitat features are particularly important, especially for basking, with south-facing banks utilised as well as individual features such as logs, stones and bushes (House & Spellerberg 1983). Suitable hibernation sites are also an important habitat feature (Spellerberg 1975) and a proportion of bare sand is required for egg-laying (Corbett & Tamarind 1979; Wouters *et al.* 2012). Habitat must also provide cover from terrestrial and aerial predators (van Bree *et al.* 2006).

1.5.3 Phylogeography

The main routes by which fauna and flora recolonised Europe from glacial refugia subsequent to the last ice-age are well illustrated in a number of species (Taberlet *et al.* 1998). Hewitt (1999, 2000) confirmed that the three southern peninsulas of Europe (Iberia, Italy and the Balkans) acted as glacial refugia and proposed three main post-glacial recolonisation routes illustrated by the meadow grasshopper *Chorthippus parallelus*, hedgehogs *Erinaceus* spp. and the brown bear *Ursus arctos* (Figure 1.8). Sand lizards colonised Europe from the Balkan-Carpathian glacial refugium (Kalyabina *et al.* 2001) following an earlier radiation from the Caucasian/Black Sea area (Joger *et al.* 2007). This reflects the 'grasshopper' paradigm (Hewitt 1999, 2000), where Northern Europe was rapidly colonised from a Balkan refugium during the most recent warm period, with dispersal from the Iberian and Italian refugia blocked by the

Pyrenees and Alps respectively, and expansion from Greece prevented by the presence of the species expanding from the east. A number of other species/species complexes show a similar pattern of post-glacial recolonisation to *C. parallelus* including the crested newts *Triturus* spp., (Wallis & Arntzen 1989) and common beech *Fagus sylvatica* (Demesure et al. 1996). The 'grasshopper' pattern of post-glacial recolonisation results in reduced levels of genetic diversity in the rapidly expanding northern species/populations when compared to those from southern refugium areas (Hewitt 1996), a pattern which is also seen in *L. agilis* and closely related species (Godinho *et al.* 2005). Sand lizards are able to exist in a much wider variety of habitats and altitudes throughout most of their range than related species and Godinho *et al.* (2005) suggested that the extensive present-day range of *L. agilis* could be explained by its superior dispersal ability when compared to closely related species such as *L. schreiberi* and *L. strigata* which are restricted to Iberia and the Caucasus respectively.

Figure 1.8. The three paradigms for the postglacial recolonisation of Europe (Hewitt 1999). *Lacerta agilis* mirrors the 'grasshopper' *Chorthippus parallelus* paradigm with dispersal from a Balkan refugium. Other closely related species were restricted to the Iberian and southern Balkan peninsulas. Reproduced from (Hewitt 1999).

It is not clear how *L. agilis* colonised the British Isles. The last glacial period came to an end approximately 18,000 years BP and by 12,500 years BP, average temperatures in Britain were warmer than the present day, allowing species which are now restricted to the Mediterranean to survive (Atkinson *et al.* 1987). This was followed by another period of cooling 11,000-10,000 years BP (the Younger Dryas) where mean daily average temperatures in Britain were between -5 °C and -2 °C, which ended in a short period of rapid warming (Dansgaard *et al.* 1989). Britain was connected to mainland Europe by a substantial land bridge across the North Sea and English Channel 10,300 years BP, which had disappeared by 8,700 years BP. However between 8,300 and 7,800 years BP a second bridge existed across the North Sea (Jelgersma 1979). Given the thermal requirements of *L. agilis* (Jackson 1978; Rykena 1987), they must have colonised

the British Isles subsequent to the Younger Dryas cooling period, and these land bridges offer potential routes for colonisation. The present day British fauna arrived via a variety of different routes, for example Zeisset & Beebee (2001) suggested the pool frog *Rana lessonae* colonised the Britain via a land bridge across the North Sea. Many species in Britain including the land snail *Cepaea nemoralis* (Davison 2000), natterjack toad *Bufo calamita* (Rowe *et al.* 2006) and water vole *Arvicola terrestris* (Piertney *et al.* 2005) show genetic evidence of an east-west split, suggesting they colonised from two separate glacial refugia.

1.5.4 Reproductive Ecology

The reproductive ecology of *L. agilis* is particularly well studied as it has been used as a model organism to test a number of hypotheses on reproductive strategy. This work has shed light onto the ecology of the species in the wild including the thermal requirements for successful reproduction. Sand lizards in northwest Europe emerge from hibernation in late March and April, females emerge later to avoid mating with functionally infertile males who have not had sufficient basking time for spermatogenesis (Olsson & Madsen 1996). Females are typically promiscuous and mate with several males in a season, leading to multiple paternity egg clutches (Gullberg *et al.* 1997a) which can result in higher quality offspring (Olsson & Madsen 2001a; Olsson *et al.* 2011b). Following copulation, males will guard their mates in order to prevent other males from mating with them (Olsson *et al.* 1996a). Male sand lizards will preferentially mate with large females (Olsson 1993a) and although females may mate with many males, the larger males are more successful at post-copulation mate-guarding and tend to sire a high proportion of the clutch (Gullberg *et al.* 1997a). Males with higher UV reflectivity (de Lanuza & Font 2007; Olsson *et al.* 2011a) and brighter green flank colouration (Anderholm *et al.* 2004), which correlate with fighting ability (Olsson 1994b) and other fitness related traits (Molnar *et al.* 2012), have greater reproductive success. Females exhibit a preference for males who are more distantly related, as assessed by Major Histocompatability Complex (MHC) genes (Olsson *et al.* 2003). The MHC is involved in immune response and male *L. agilis* with a particular MHC genotypes have lower levels of parasite infection, and higher mating and mate-guarding success (Olsson *et al.* 2005b). In *L. agilis*, females are the heterogametic sex and therefore more likely to be affected by recessive sex-linked genetic disorders. As a mechanism to avoid such conditions in their offspring, female sand lizards are more likely to produce daughters when they have mated with a high quality male (Olsson *et al.* 2005a; Olsson *et al.* 2005c).

External temperature during the spring mating season (April and May) plays an important role in the mating system of *L. agilis*. Warmer spring temperatures resulted in an increased number

of matings and a higher proportion of clutches with multiple paternity (Olsson *et al.* 2011c). Warm spring temperatures also result in earlier egg clutches which has a positive effect on hatchling condition (Olsson & Shine 1997b) and hatchlings from early clutches disperse further than those from later clutches in some lizard species (Warner & Shine 2008). As female sand lizards increase in size, the size of their offspring at hatching increases and large hatchlings have a higher survival rates in poor years than smaller ones (Olsson & Madsen 2001b).

1.5.5 Home Range and Dispersal

The maximum home range of *L. agilis* has been quantified at up to 648 m^2 for males and 398 m^2 for females (Nicholson & Spellerberg 1989). In displacement exercises, most sand lizards displaced by 100 m would return to their home range, whereas none returned home after a displacement of more than 150 m (Strijbosch *et al.* 1983). Male *L. agilis* generally disperse over wider distances than females; however both sexes show dispersal patterns related to reproductive success. From one breeding season to the next, males in poorer body condition tend to disperse further than other males and females with low reproductive success disperse further than successful breeders (Olsson *et al.* 1997). Following hatching, female *L. agilis* will disperse away from their offspring (Ryberg *et al.* 2004).

Triggers for dispersal between populations have not been investigated in *L. agilis*, however in the viviparous lizard *Lacerta* (*Zootoca*) *vivipara*, juvenile dispersal is triggered in response to increasing population density (Lecomte *et al.* 2004). Cote & Clobert (2010) suggested that the dispersal decisions of *L. vivipara* are influenced by their knowledge of the surrounding habitat, such as connectivity, ascertained from immigrants into their population. As with many reptiles (e.g. Johansson *et al.* 2008; Urquhart *et al.* 2009; Pernetta *et al.* 2011), *L. agilis* shows sex-biased dispersal patterns with male juveniles dispersing significantly further than females from their natal sites (Olsson *et al.* 1996b).

1.5.6 Population Genetics

A number of previous studies have investigated the population genetics of *L agilis*. Many of these have focused on Swedish populations where, as in Britain, *L. agilis* is at the edge of its range and is restricted to parts of the country where suitable habitat is present (Gullberg *et al.* 1998; Berglind 2000). Sand lizards colonised Sweden via a land bridge between Scandinavia and mainland Europe which was flooded by rising seawater approximately 9,000 years BP. Following this they spread throughout southern and central parts of the country until a drop in temperature in the late Holocene 5,000 years BP caused them to retreat to their current relictual distribution in particularly favourable habitats (Gullberg *et al.* 1998, 1999). As would

be expected from populations on the edge of a species' range (Lesica & Allendorf 1995), Swedish *L. agilis* from across the country were significantly less genetically diverse than a population from closer to the centre of the range in Hungary, in studies using microsatellites (Gullberg *et al.* 1997b; Gullberg *et al.* 1998; Madsen *et al.* 2000; Schwartz & Olsson 2008), minisatellites (Madsen *et al.* 2000) and DNA fingerprinting (Gullberg *et al.* 1999) (Table 1.1).

Gullberg *et al.* (1998) sampled *L. agilis* from ten sites in Sweden with varying degrees of isolation. Average heterozygosity in microsatellites varied between 0.21 and 0.69 (average 0.45), compared to 0.70 in a reference population from Hungary. Subdivision between the Swedish populations was also high with F_{ST} estimates between 0.192 and 0.299. The authors attributed the high levels of subdivision to a lack of gene flow between populations, and small population sizes and the low overall genetic diversity to a bottleneck of the founder population. Using minisatellite DNA fingerprinting, Gullberg *et al.* (1999) reported heterozygosity varying between 0.32 and 0.59 in Swedish *L. agilis* populations and 0.89 in a Hungarian one and observed higher levels of bandsharing (0.61) in the Swedish populations compared to the Hungarian one (0.19, typical of an outbred population). F_{ST} estimates within populations varied between 0.141 and 0.412 between the Swedish populations using microsatellites. In a genetic assessment of British *L. agilis* populations expected heterozygosity was between 0.500 and 0.691 and there were significant levels of subdivision with F_{ST} estimated between 0.133 and 0.241 (Beebee & Rowe 2001b) (Table 1.1).

Inbreeding has been attributed as the cause of a high incidence of malformed *L. agilis* offspring in a highly fragmented and isolated site in Sweden (Olsson *et al.* 1996b). Matings between siblings under laboratory conditions resulted in deformities such as short skulls and jaws, fused or missing toes and deformed or twisted limbs and tails. Similar deformities were recorded in 10% of wild hatchlings, which had a zero survival rate and the normal-looking siblings of deformed hatchlings also had a significantly lower survival rate compared to broods where all the neonates were normal.

Madsen *et al.* (2000) investigated the relationship between genetic diversity and population size in Swedish *L. agilis* and found different relationships depending on the type of loci used. Using loci under selection pressure (MHC Class I, which are involved in the immune response) genetic diversity was positively correlated with population size (i.e. larger and less isolated populations had higher levels of diversity). However, genetic diversity as assessed by minisatellite and microsatellite loci (neutral genetic markers not under selection) was surprisingly negatively correlated with population size and degree of isolation. The opposite

effect was observed in British *L. agilis*, where microsatellite heterozygosity was positively correlated with population size (Beebee & Rowe 2001b).

Table 1.1. Genetic diversity of *Lacerta agilis* from previous studies. H_o = observed heterozygosity, H_e = expected heterozygosity. Where multiple populations are considered the figures reported are an average across all populations**.**

Country	Marker type	No. of populations	Mean no. of alleles per locus	Average H_o	Average H_e	Reference
Sweden	microsatellites	10	3.3	0.468	0.451	Gullberg *et al.* (1998)
Sweden	DNA fingerprinting	6	-	-	0.450	Gullberg *et al.* (1999)
Sweden	microsatellites	5	-	-	0.461	Madsen *et al.* (2000)
Sweden	microsatellites	1	4.3	0.510	0.539	Schwartz & Olsson (2008)
Hungary	microsatellites	1	8.0	0.67	0.70	Gullberg *et al.* (1998)
Hungary	DNA fingerprinting	1	-	-	0.891	Gullberg *et al.* (1999)
Hungary	microsatellites	1	-	-	0.70	Madsen *et al.* (2000)
Hungary	microsatellites	1	11.2	0.677	0.828	Schwartz & Olsson (2008)
United Kingdom	microsatellites	3	4.17	0.514	0.623	Beebee & Rowe (2001b)

1.5.7 Sand Lizards in Britain

Sand lizards naturally occur in three distinct geographic areas of Britain (Figure 1.9): Merseyside in the northwest of England where they occur on coastal sand dune habitats; the Weald in Surrey in the southeast of England where they occur on lowland heathland habitats; and Dorset in the southwest of England where they also occur on lowland heathland (Beebee & Griffiths 2000). Sand lizards from the different geographic areas of Britain are genetically distinct (Beebee & Rowe 2001b) and appear to exhibit differences in colour and pattern (Simms 1970; Corbett 1988b), although this has not been demonstrated empirically.

Within Britain, its restricted distribution and specific habitat requirements have left *L. agilis* particularly vulnerable to the effects of habitat loss and fragmentation and the species has suffered significant declines (Corbett 1989). In addition to the significant loss of heathland in Dorset (section 1.4.2), similar levels of habitat loss have occurred within the other parts of the *L. agilis* range in Britain. Approximately 85% of heathland habitat was lost from the Weald in Surrey and the surrounding counties between the late 18th Century and the 1970s (Webb 1986). Between 1801 and the 1970s the coastal sand dunes of Merseyside decreased by 50%, with a further 12% modified (Figure 1.10). The remaining habitat existed in small patches divided by urban areas, roads, intensive agriculture and dense conifer plantations (Jackson 1979). British sand lizard population sizes have been estimated at 200-500, < 1,000 and 6,000-

8,000 adults in Merseyside, Surrey and Dorset respectively (Wheeler *et al.* 1993; Corbett 1994), although it is difficult to obtain accurate estimates for sand lizard populations sizes due to their cryptic behaviour (Fearnley 2009; Kery *et al.* 2009; Sewell *et al.* 2012) and these population estimates are based on a number of assumptions. Although habitat loss has been the primary driver of sand lizard decline in Britain, the role of climatic fluctuations has been debated (Jackson 1978; Langton 1988), predation by domestic cats *Felis catus* (Larsen & Henshaw 1998) and recent introductions of alien species such as wall lizards *Podarcis muralis* have been identified as potential concerns (Mole 2010).

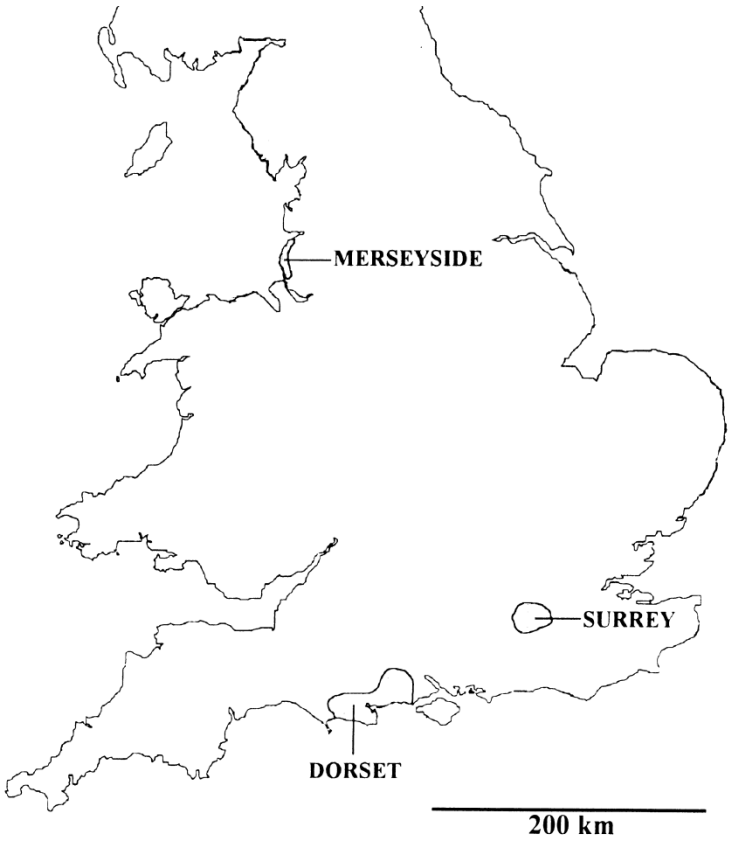


Figure 1.9. Distribution of *Lacerta agilis* in Britain, showing the three distinct geographic areas where it naturally occurs. Reproduced from Beebee & Rowe (2001b).

The declining status of *L. agilis* in Britain was recognised in the late 1960s (Corbett 1969) and by the early 1970s conservation action in the form of site management and population monitoring was being taken in response (Corbett & Tamarind 1979). Sand lizards received legal protection under the Wildlife & Countryside Act in 1981 and protection was extended to include their habitat under the Conservation (Natural Habitats &c) Regulations 1994 (the

United Kingdom's response to EU Directive in the Conservation of Habitats and Wild Fauna and Flora (92/43/EEC)). In 1994, a captive breeding programme was started with the aim of reintroducing *L. agilis* to many sites within its former range (Corbett 1994). Current conservation effort is governed by a Species Action Plan (Herpetological Conservation Trust 2009) under the auspices of the UK Biodiversity Framework (Joint Nature Conservation Committee 2012) and management activities include site protection and management, research and monitoring as well as captive breeding and reintroductions to restore its former range (Corbett & Moulton 1998; Moulton & Corbett 1999).

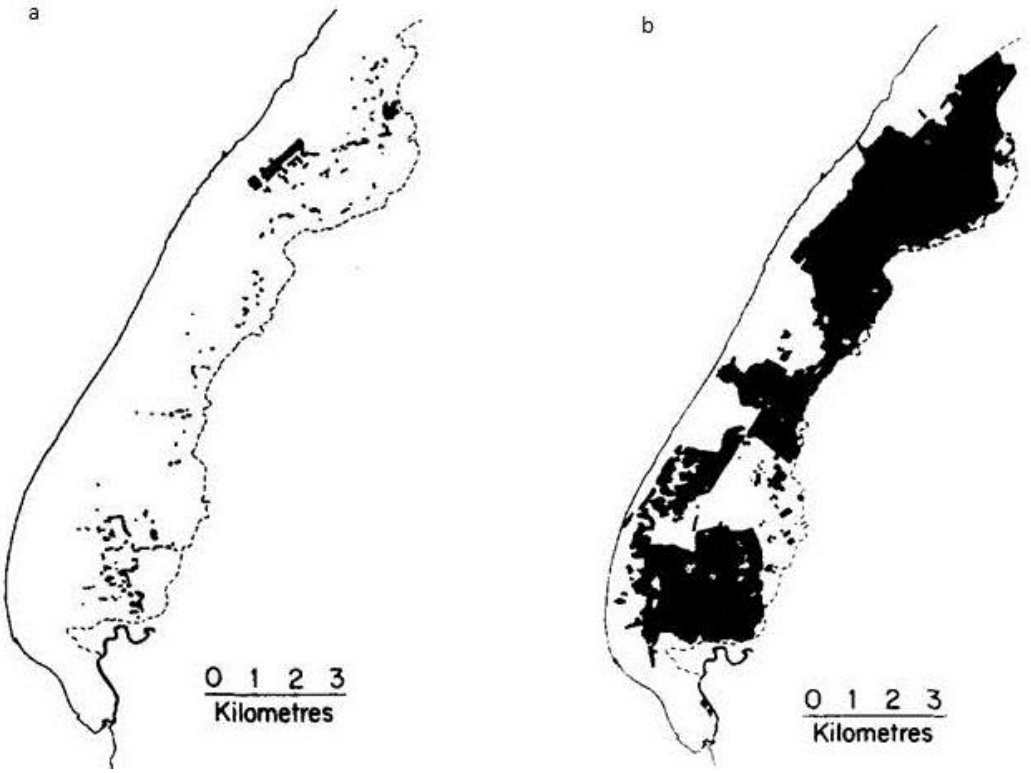


Figure 1.10. Building development and afforestation (shaded areas) on wind-blown sand between Southport and the River Alt. a) 1841, b) 1974. Reproduced from Jackson (1979).

1.6 Aims of this Thesis

The sand lizard has been the focus of significant conservation action within Britain which in many respects has been successful (Corbett & Moulton 1998). However, there are ongoing challenges in maintaining the conservation of the species in Britain, particularly relating to habitat fragmentation and degradation of heathland habitats due to recreational use, fire and

natural succession (Moulton & Corbett 1999), and it is unknown how sand lizards may react to global climate change.

Some preliminary investigation of the conservation genetics of *L. agilis* in Britain has been undertaken (Beebee & Rowe 2001b). This thesis aims to increase the understanding of the conservation genetics of sand lizards in Britain, including their biogeography, levels of genetic variability and landscape genetics, in order to inform ongoing conservation management of the species. The specific aims of this thesis are:

1. *To assess the genetic diversity of Lacerta agilis populations across the United Kingdom and compare this with populations from Europe*. Chapter 3 assesses the genetic diversity of British sand lizard populations using a variety of indices and compares this with a population sampled from the Netherlands as well as published values from other studies.
2. *To investigate the phylogeography of sand lizards in Britain and determine how this accounts for their disjunct range within the country*. Chapter 3 uses different methodologies to produce phylogenies of sand lizard populations across Britain and estimates historical divergence times between sand lizards in Britain and mainland Europe as well as between populations within Britain. The divergence times are then compared to data relating to historical climate and biogeography.
3. *To assess the genetic diversity of a mature translocated population and discuss the implications of this for current conservation management*. Chapter 3 quantifies genetic variation within sand lizards at Crooksbury Common in Surrey which were translocated here from Dorset in the late 1960s and early 1970s.
4. *To determine the origins of the population at Aberffraw*. Chapter 3 compares the recently discovered Aberffraw population from Anglesey with the nearest natural population in Merseyside and uses a variety of methods to assess genetic distance and differentiation as well as estimate divergence times between Aberffraw and the other populations.
5. *To determine the effects of heathland fragmentation on the genetics and population structure of sand lizards at various geographical scales*. Chapter 3 compares genetic variation and differentiation between populations within fragmented and unfragmented landscapes. Chapter 4 attempts to quantify the effects of the various landscape features, including, natural barriers such as rivers, anthropogenic barriers and habitat cover based on remotely sensed data, on the population genetics of sand lizards in Dorset at different geographical scales using a resistance surface. It also

investigates correlations between genetic population structure and historical landscape configuration.

6. *To investigate the apparent differences in colour and pattern between the different geographical areas of the Britain.* Chapter 5 quantifies variation in the pattern and colour of sand lizards from different geographic areas of Britain and compares this to genetic differences between the populations.

2 GENERAL MATERIALS AND METHODS

2.1 Introduction

General materials, methods and analytical processes relevant to all parts of this thesis are detailed in this chapter as are details of the study sites. Specific materials, methods and statistical analyses which are only applicable to parts of the study are contained within the relevant chapters.

2.2 Sampling Strategy

The study entailed investigating the genetics of *Lacerta agilis* at three differing geographic scales, therefore a sampling strategy which ensured the specific questions of the study could be addressed at each scale was employed. A nested sampling strategy (Haining 2003) was used which is appropriate for species with a naturally clustered distribution and allows analysis at different spatial scales (Storfer *et al.* 2007). This entails dividing the landscape into a series of sampling areas, which are then further subdivided (Figure 2.1).

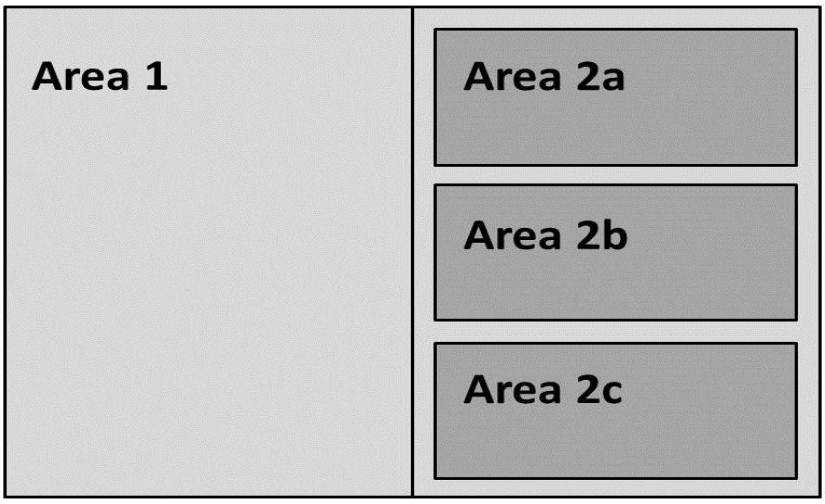


Figure 2.1. Diagrammatic representation of a nested sampling strategy. Areas 1 and 2 represent broad-scale sampling zones, Areas 2a, 2b and 2c represent smaller scale sampling zones.

At the national scale, the aims of the study were to allow a broad comparison between the three regions where *L. agilis* occurs (Dorset, Weald and Merseyside) (Figure 2.2) and investigate the phylogeography of sand lizards across Britain. Within each region, individual sample sites were selected on the basis of the size of the population and of ease of collecting

sufficient samples. Samples were also obtained from a site in the Netherlands (Bergherbos) to allow a comparison between British and mainland European populations

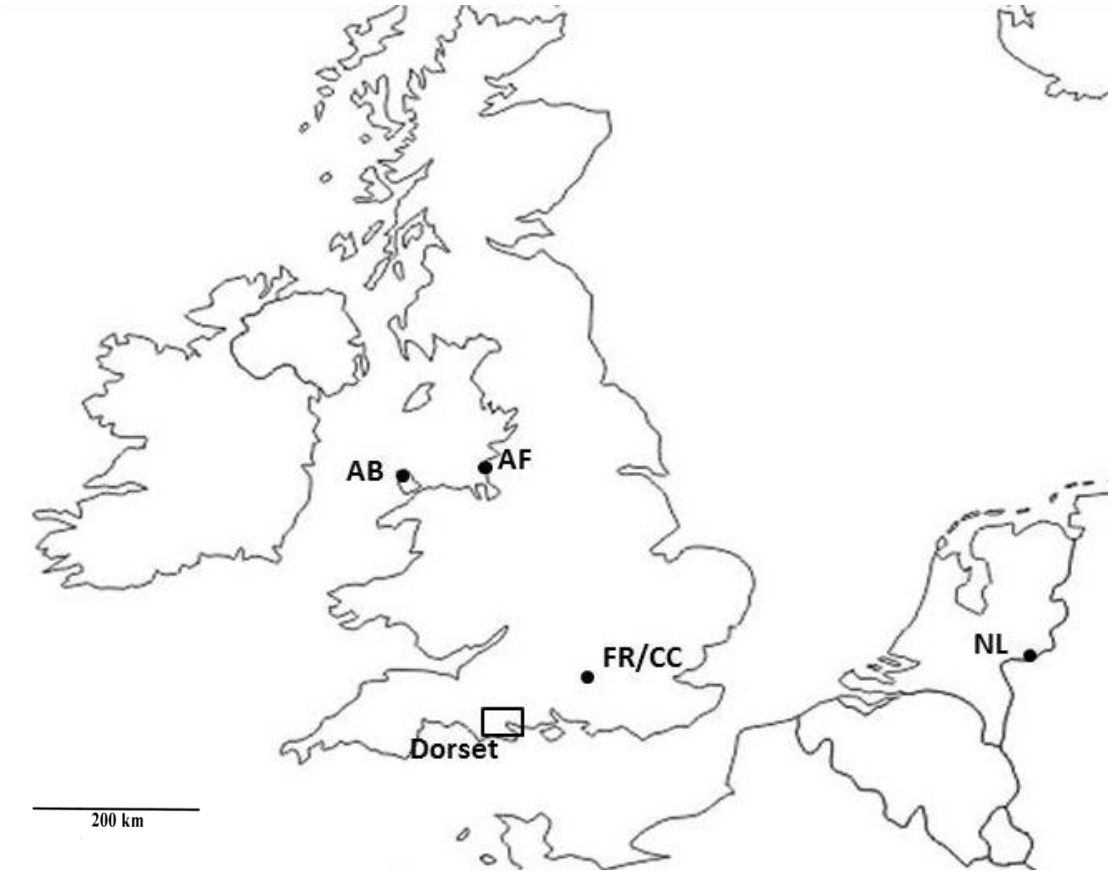


Figure 2.2. Location of sample sites at the national scale. AB = Aberffraw, AF = Ainsdale Frontal Dunes, FR = Frensham Common, CC = Crooksbury Common, NL = Bergherbos. Sampling locations within Dorset are shown in Figure 2.3

At the regional and local scales, the aim of the study was to investigate the effects of the landscape on *L. agilis* population structure. The range of *L. agilis* in Dorset is crossed by six rivers of differing sizes; these were used to define the boundaries of the sampling areas at a broad scale, creating six different areas. Two of these sampling areas were then subdivided to allow a comparison between fragmented (East Dorset between the River Stour and River Avon) and unfragmented (Wareham Forest) landscapes (Figure 2.3 and Figure 2.4). The largest populations within each sampling area were sampled. Many *L. agilis* populations within Britain have been affected by conservation action including translocations from sites threatened by fire or development. The sampling strategy avoided any sites which may be a result of anthropogenic processes and with the exception of Crooksbury Common and Aberffraw, all of the sampled populations are thought to be of natural origin.

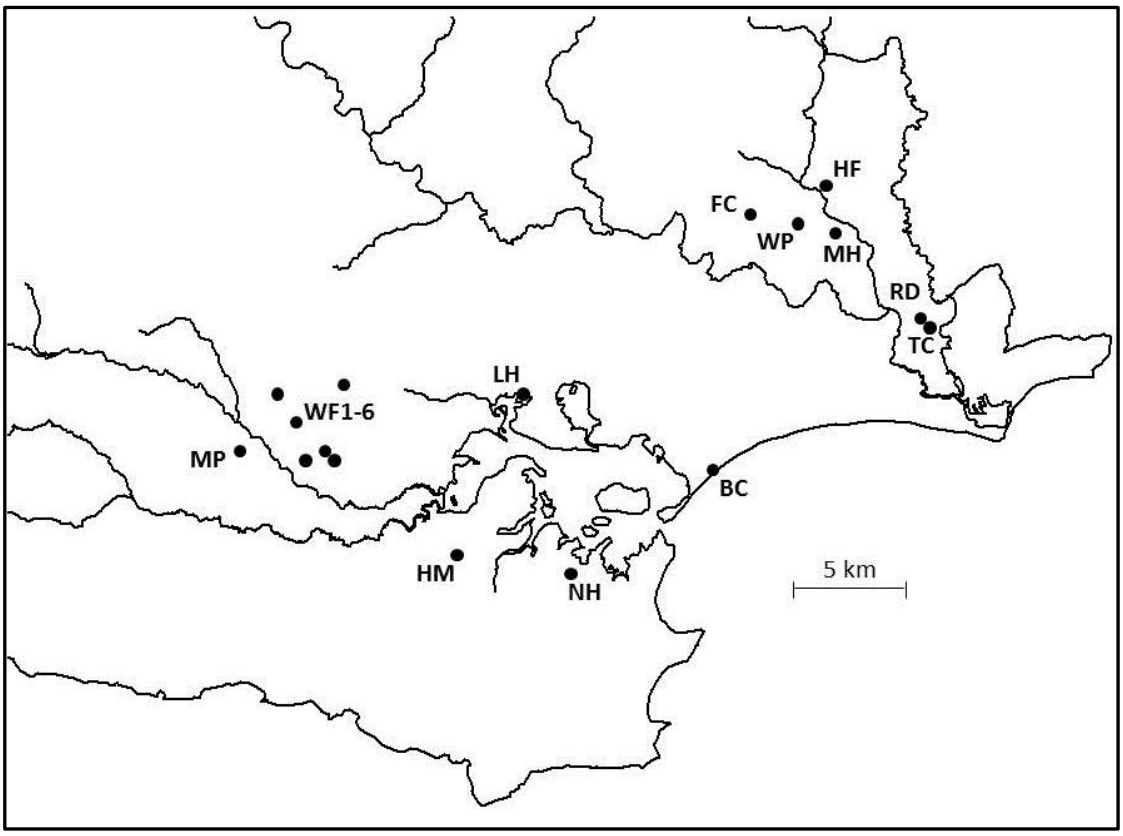


Figure 2.3. Locations of sampling sites within Dorset showing the main rivers. NH = Newton Heath, HM = Hartland Moor, MP = Master's Pit, WF = Wareham Forest, LH = Lytchett Heath, BC = Branksome Chine, FC = Ferndown Common, WP = West Parley, MH = Merritown Heath, HF = Hurn Forest, RD = Ramsdown, TC = Town Common.

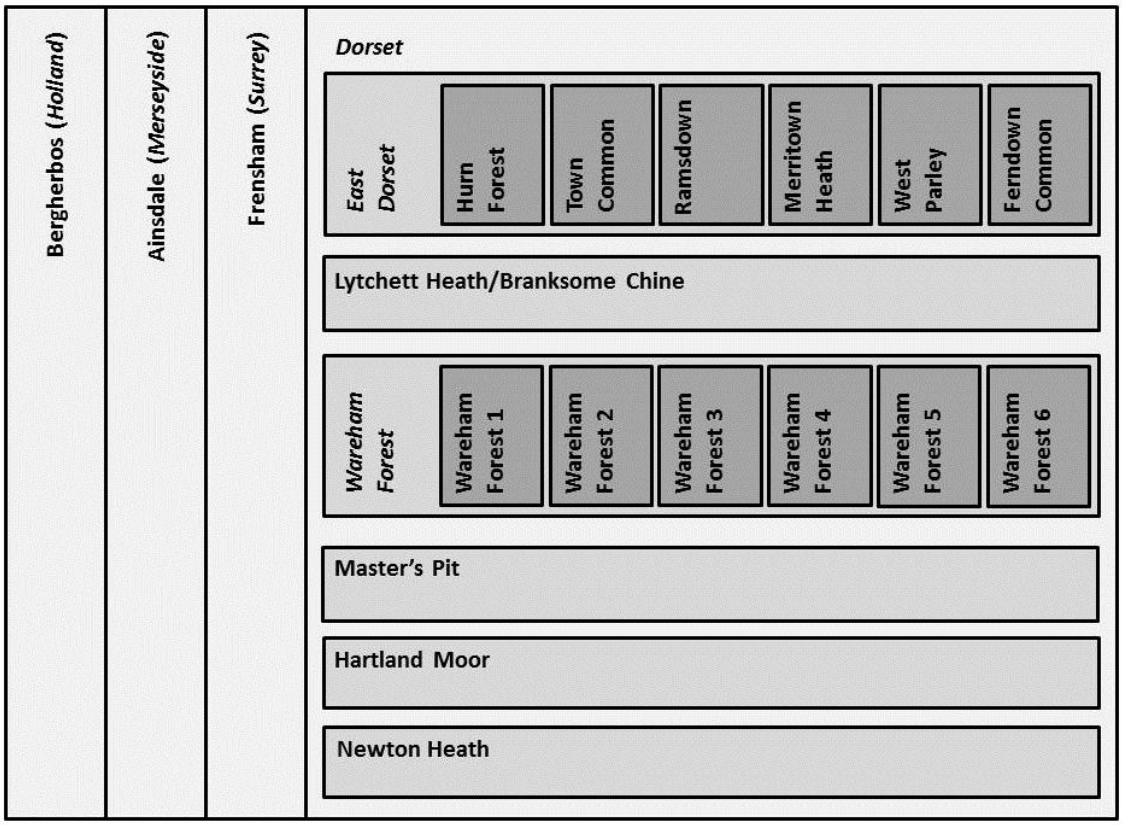


Figure 2.4. Schematic diagram of the nested sampling strategy showing all the sites sampled as part of this study.

2.3 Sample Sites

2.3.1 Town Common and Ramsdown

Town Common (173 ha) and Ramsdown (33 ha) are part of a formerly extensive area of dry and wet heath. Ramsdown was separated from the larger Town Common in the late 1970s by the construction of the four-lane A338 Bournemouth Spur Road. Further areas of heathland lie to the north, the River Avon valley to the east and agricultural land lie to the west. Ramsdown and part of Town Common are now managed for wildlife with a specific focus on reptiles.

2.3.2 Hurn Forest

Hurn Forest is a coniferous plantation on a former area of heathland, which was planted in the 1940s. Although much of the site is unsuitable for *L. agilis*, there are some areas of heathland within the forest matrix along ride edges and in former plantation areas which have been reclaimed for conservation purposes. Larger areas of heathland are still present to the east of the site.

2.3.3 Merritown Heath

Merritown Heath is a small heathland remnant to the north of Bournemouth (Hurn) Airport. It is separated from Hurn Forest by the small Moors River and from West Parley by approximately 2 km of agricultural land. Bournemouth Airport was built on primarily agricultural land to the south in 1941 and subsequently expanded. The site is currently managed for wildlife.

2.3.4 West Parley

West Parley Common is an isolated 145 ha remnant of lowland heathland which was formerly part of a much larger area of common land. Building between the 1930s and 1960s significantly reduced the extent of the site and the adjacent East Parley Common was converted to pasture in the 1970s. It is currently managed as a nature reserve, with an emphasis on reptiles.

2.3.5 Ferndown Common

Ferndown Common is an isolated fragment of heathland approximately 64 ha in area, surrounded by suburban development to the north and east and agricultural land to the south and west. It was initially separated from West Parley from by development in the 1950s, which increased until the 1980s. The site is currently managed for wildlife although is subject to a high level of public disturbance.

2.3.6 Lytchett Heath

Lytchett Heath is a small area of heathland on the northern shore of Poole Harbour. It is isolated by the town of Upton, most of which was built in the 1950s, and the A35 road built in the 1970s. Prior to this, the majority of the land surrounding the site was agricultural although other areas of heathland previously existed to the east.

2.3.7 Branksome Chine

The *L. agilis* population at Branksome Chine exists in a small area of sandy cliff on the seafront in Poole. It was connected to a formerly extensive area of heathland until the 1950s, although this was isolated from other heathland areas inland as early as the 1910s. Other similar small fragments of suitable habitat have persisted along the Poole and Bournemouth seafront, although these have become increasingly fragmented and this may be the last *L. agilis* population along the coastal strip (D. Bird, pers. comm.). Several introduced common wall lizard *Podarcis muralis* and at least one western green lizard *Lacerta bilineata* populations are now found along the seafront (Mole 2010) and *P. muralis* are encroaching into *L. agilis* areas.

2.3.8 Wareham Forest and Morden Bog

Wareham Forest is a large heathland/plantation mosaic which is ostensibly managed as a commercial conifer plantation, although also for recreational use and for conservation purposes. Morden Bog is a 149 ha National Nature Reserve containing dry and wet heathland habitats which lies to the northwest of Wareham Forest with which it forms a large continuous area of habitat. Most of Wareham Forest was heathland until afforestation began in the late 1930s, creating large areas of plantation interspersed with remnants of predominantly wetter heath, which were unsuitable for forestry. Despite the loss of heathland, *L. agilis* persisted in small remnant areas and along ride edges (Dent & Spellerberg 1987). Since the 1980s, activities such as selective clearance of small areas of trees and ride widening have been undertaken within Wareham Forest to improve the extent, quality and connectivity of the habitats for *L. agilis* and other reptile species (D. Bird, pers. comm.). Six sites were sampled within Wareham Forest and Morden Bog, generally comprising areas of dry heath and adjacent areas of grassland.

2.3.9 Master's Pit

Master's Pit is an active sand extraction site within an area of former heathland. The lizards sampled from the site were caught as part of an ongoing relocation project to remove them from areas affected by quarrying. Sand extraction began in the area in the 1950s and the area

affected by quarrying has been regularly extended since. The majority of the land surrounding the site remains as heathland.

2.3.10 Hartland Moor

Hartland Moor is a large (243 ha) area of wet and dry lowland heathland and bog which lies adjacent to other heathland sites making it part of the largest remaining heath in Dorset. It is National Nature Reserve.

2.3.11 Newton Heath

Newton Heath is part of a formerly extensive area of heathland which up until the 1940s covered most of the north of the Isle of Purbeck and would have been continuous with Hartland Moor. Planting of conifers began as early as the 1910s; however most of the area was not afforested until the 1940s. The plantation is interspersed with a number of small areas of heathland and heathland rides which support *L. agilis* populations.

2.3.12 Frensham Common

Frensham Common in Surrey is an area of approximately 400 ha of lowland heathland divided by a single carriageway road. Most of the original extent of the heathland remains, although some marginal areas were lost to afforestation in the 1940s. The surrounding area is a mixture of agricultural land and woodland with small villages. The site is currently managed to benefit wildlife, however some areas are subject to very high levels disturbance from recreational use.

2.3.13 Ainsdale Frontal Dunes

The Ainsdale and Birkdale sand dunes are part of the Sefton Coast sand dune system, which at 4,605 ha is the largest in England, although this is fragmented and much of the former extent has been lost to development and afforestation (Jackson 1979). The Ainsdale dunes contain vegetation at all successional stages, however sand lizards are primarily found in the embryo dunes which are characterised by marram grass *Ammophila arenaria* and a large proportion of open sand. Much of the development on the Sefton sand dune system has occurred further inland and therefore the Ainsdale frontal dunes remain relatively well connected to other frontal dunes areas along the coast.

2.3.14 Bergherbos

Bergherbos is a nature reserve located approximately 20 km southeast of Arnhem on the Dutch border with Germany and north of the River Rhine. It is an extensive area of woodland containing areas of agricultural land and heathland. Sand lizards are present within areas of heathland and forest rides.

2.3.15 Crooksbury Common

Crooksbury Common is an area of heathland located within a coniferous plantation in Surrey. The *L. agilis* population on the site derives from several sites within the centre of the Dorset range from which lizards were translocated in the early 1970s (J. Webster, pers. comm.) when their original sites were developed. Prior to the translocations, sand lizards were not present on the site, although smooth snakes *Coronella austriaca* were. It is now managed to improve the quality of the site for reptiles, particularly *L. agilis*.

2.3.16 Aberffraw

Aberffraw is an extensive but isolated sand dune system on the south west of the Isle of Anglesey, Wales. Sand lizards were first recorded at the site in 2010 (D. Cowley, pers. comm.) despite not being detected during previous surveys of the site. The lizards at the site are similar in appearance to those from nearest known natural populations in Merseyside; however the origin of the population is unknown.

2.4 Field Sampling

Sand lizards were captured in the field using a nylon noose and occasionally by hand. Between 30 and 37 lizards were sampled at each site (with the exception of Aberffraw which appears to have a very small population size), including at least ten animals of each sex, and all sampled lizards were in their third year or older. Once captured, the location of each animal was marked using a handheld GPS Unit (Garmin eTrex Legend HCx) and the reported accuracy of the GPS unit was noted; the location was not recorded until the reported accuracy was +/- 5 m or better and coordinates were subsequently checked for accuracy against large scale maps using ARCGIS v10 (ESRI, Redlands, California). A photograph of the dorsal pattern (and any other distinguishing marks) was taken to allow the individual identification of each lizard (Sacchi *et al.* 2010) and prevent resampling of the same animal. A DNA sample was obtained using a buccal swab (Cambio, Cambridge, UK); each lizard was encouraged to bite the swab and allowed to chew it for approximately one minute; the swab was then air-dried for ten minutes before being resealed in its tube and subsequently stored at -20 °C until required for DNA extraction. DNA was extracted using the BuccalAmp™ DNA Extraction Kit as per the manufacturer's instructions. This method for obtaining DNA samples was preferable to tissue or blood samples due to its reliability (Beebee 2008) and to take account of the welfare of the animals sampled (Parris *et al.* 2010).

2.5 Genotyping

2.5.1 Microsatellites

Microsatellite loci were amplified by the Polymerase Chain Reaction (PCR) using a series of primers developed specifically for *L. agilis* (Gullberg *et al.* 1997b; Schwartz & Olsson 2008) (Table 2.1). Samples were amplified using each of the primer pairs, with the exception of *La7*, which failed to amplify in previous studies of Swedish (Gullberg *et al.* 1997b) and British (Beebee & Rowe 2001b) *L. agilis*. PCR reaction mixes of 20 µl final volume contained 4 µl of the DNA extraction solution, 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 120 mM KCl, 2 mM $MgSO_4$, 0.2 µM of forward and reverse oligonucleotide primers, 100 µM dGTP, dCTP and dTTP, 10 µM unlabelled dATP, 3.7 KBq [α-^{33}P]dATP and 0.5 units of NEN *Taq* DNA polymerase (New England Biolabs). Where possible, loci were multiplexed using appropriate pairs (Table 2.1). The PCR reactions were carried out using either a Techne TC412 or Techne Genius thermal cycler, using one of three programs depending on the annealing temperature of the primer (Table 2.2). The PCR products were then electrophoresed through a 6% w.v. polyacrylamide gel alongside an M13 sequence marker. Gels were then visualised using autoradiography and the size of the alleles scored against the M13 marker. Any samples which failed to amplify at any locus were repeated once under the original PCR conditions and a second time using a PCR cycle with a lower annealing temperature if required. Following genotyping, 100 samples were randomly selected and blindly re-genotyped at one locus or multiplex pair each in order to allow an assessment of the genotyping error rate (Bonin *et al.* 2004).

2.5.2 Mitochondrial DNA

A subset of samples (12 each from Frensham Common, Ainsdale and Bergherbos, 12 randomly selected from sites in the west of Dorset, 12 randomly selected from sites in the east of Dorset and all seven from Aberffraw) were amplified in the presence of universal cytochrome *b* primers (Kocher *et al.* 1989). PCR reaction mixes of 20 µl final volume contained 4 µl of the DNA extraction solution, with reagent concentrations as per the microsatellite PCR (excluding the radiolabelled dATP). The PCR conditions were 94 °C x 4 min (94 °C x 1 min, 55 °C x 1 min, 72 °C x 1 min) x 35 cycles, 72 °C x 1 min. Following amplification, PCR products from one sample from each geographical area were electrophoresed through a 1.5% agarose gel to check for successful amplification and size differences in the PCR products between the different areas. The remaining samples were purified using Qiagen™ kits as per the manufacturer's protocol, purified products were sequenced by Macrogen Sequencing Services and the sequences were then scrutinised for errors and aligned using BIOEDIT (Hall 1999).

Table 2.1. Details of PCR primers used in this study. Details of the PCR programs referred to are given in a separate table (Table 2.2).

Locus	Primer sequence	Microsatellite repeat motif	PCR program	Multiplex pair	Reference
La1	AGGTTTCCTGGCTTGGAG ATTTGCACAAAACAGCAGC	$(GT)_{16}$	1	1	Gullberg *et al.* (1997b)
La2	GCTTAAATTGGAACCAGATTG AAGCAGCCAGAACACAGAG	$(GT)_{16}$	1	1	Gullberg *et al.* (1997b)
La3	ACTAGGAGCGAGAAGAATCAG GACATATGGCAGAAGAGCAG	$(GA)_{28}$	1	2	Gullberg *et al.* (1997b)
La4	CATGAGCAAAGCAATGAGC TGGAATGTGTCATTGAACTCTG	$(GT)_{19}$	1	3	Gullberg *et al.* (1997b)
La5	TAGATGCACTCAGAATGACTTC AACACTATTCTAAGGCTGTTC	$(GA)_{20}$	2	-	Gullberg *et al.* (1997b)
La6	GACTGGCGCATTCTATAAAAC GCCTTAAAGGGCCATCAG	$(GT)_{17}$	1	3	Gullberg *et al.* (1997b)
La7	CCTTTGTGGTCTCTTCCAAC CCTCATAGGGTTGTCGTGAG	$(GT)_{16}$	-	-	(Gullberg *et al.* 1997b)
La8	AACCACTAGCAGAAATCTCATTC GACCTTGGAATTTTCACCTG	$(GT)_{14}$	2	-	Gullberg *et al.* (1997b)
La9	AGATGCTTTTATATATGCAACTTC GTGCCTTCATTTGTTTACTTC	$(GT)_{12}$	3	4	Gullberg *et al.* (1997b)
La10	CCCTGATAAAGCCCCAC CACTAGCTGAAATAAGAATGAGG	$(GT)_{15}$	2	-	Gullberg *et al.* (1997b)
La01	AACGGAGGTAGAATGTCATAGC CTTGAAGGGAAAGAGCTACTGC	$(GT)_2AT(GT)_{15}$	2	-	Schwartz & Olsson (2008)
La02	TGCCTGCAAGACTATAATCCAAG GGAATGGCATGAGATATGGTG	$(GT)_{23}$	2	-	Schwartz & Olsson (2008)
La3E	AAAGTTGGTCTGCACTGACG CAATTCAAAATGCACACAACG	$(GT)_{13}AT(GT)_{10}$	2	-	Schwartz & Olsson (2008)
La04	CTAGGCATGGAGAATGGATGTG AGCCACTTCCCTAAGTGTGTCC	$(CA)_{20}$	3	5	Schwartz & Olsson (2008)
La10	TAATAAAGCAGGCGCAAACC TGCAGCTAATCTTCATTTAGGATG	$(CA)_5(GA)_4GGGA$ $(GACA)_9(CA)_9(GA)_{17}$	2	-	Schwartz & Olsson (2008)
La12	CAGAGTTCATGGAAAGTGAAGG GGAGACTCTGCTGGTCATTC	$(CA)_{18}$	1	6	Schwartz & Olsson (2008)
La27	AAATGCAAGCGAGCAACAAT ATCTGGCGGAGGGATGAG	$(GT)_{11}(AT)_{26}$	1	6	Schwartz & Olsson (2008)
La37	TTTGCTTGGAGCTTCTGTCC GATGCAGGACGGAGAGTAGC	$(GT)_{19}$	1	-	Schwartz & Olsson (2008)
La40	GGGAACCGTTGTACTAAGTTTGG ATGCATTCAGATGTCTCCCAAG	$(CA)_{19}$	3	5	Schwartz & Olsson (2008)
La45	CAGAGTTCATGGAAAGTGAAGG AAGGAGACTCTGCTGGTCATTC	$(CA)_{18}$	2	-	Schwartz & Olsson (2008)
La47	CCCACTAGAGAAATGAGCTTCTG CAAACAAGGAGGGTAAGGAATG	$(GT)_{18}$	1	-	Schwartz & Olsson (2008)
La50	AGGTAGCCCAGGTGTCATACAG TGGGTCTTACATGAGCTGAATC	$(GT)_{21}$	1	2	Schwartz & Olsson (2008)
La55	TCCCTCATTACAGGCATAGGAG TCTGAACAAAACATGGGACTTG	$(CA)_{19}$	2	-	Schwartz & Olsson (2008)
La58	CAGTTCTGGGGATTTTCTCCTAC CATTGTAATTGGAGCACAAAGC	$(CA)_{18}$	2	-	Schwartz & Olsson (2008)
La64	AGATGCTGAACTACCAGCTTGC GCTATCCTGGCTGACCATTAAG	$(CA)_{16}$	3	4	Schwartz & Olsson (2008)

Table 2.2. PCR conditions for each microsatellite primer used within the study.

PCR Program	Conditions
1	1): 94 °C x 4 min (x1); 2): 94 °C x 1 min, 58 °C x 1 min, 72 °C x 1 min (x35); 3): 72 °C x 1 min; 4): 4 °C
2	1): 94 °C x 4 min (x1); 2): 94 °C x 1 min, 54 °C x 1 min, 72 °C x 1 min (x35); 3): 72 °C x 1 min; 4): 4 °C
3	1): 94 °C x 4 min (x1); 2): 94 °C x 1 min, 50 °C x 1 min, 72 °C x 1 min (x35); 3): 72 °C x 1 min; 4): 4 °C

2.6 Analysis of Microsatellite Data

2.6.1 Screening for Scoring Errors and Loci under Selection

Allele sizes for each individual at each locus were initially recorded in a standard GENEPOP v4 (Raymond & Rousset 1995) file format and converted into file formats suitable for other programs using CREATE v1.35 (Coombs *et al.* 2008) software. The dataset was then screened for scoring errors caused by stuttering and the likely presence of null alleles using the Brookfield 1 method (Brookfield 1996) implemented in MICRO-CHECKER (Van Oosterhout *et al.* 2004) as it was likely that some alleles failed to amplify as a result of DNA degradation. Compliance with Hardy-Weinberg Equilibrium (HWE) expectations was tested using GENEPOP v4.0 (Raymond & Rousset 1995), as was linkage disequilibrium using a Markov chain method. Population genetics studies frequently adjust critical *P*-values using the Bonferroni correction for multiple comparisons. This approach has been criticised as the power to correctly reject false null hypotheses is reduced. Therefore *P*-values were adjusted using a False Discovery Rate (FDR) procedure (Narum 2006), a less conservative correction for multiple comparisons. This is calculated by dividing the desired critical level (e.g. 0.05) by the sum of one divided by the number of tests for each test (e.g. for five tests, $\alpha = 0.05/(1/1 + 1/2 + 1/3+ 1/4 + 1/5) = 0.022$). Prior to undertaking full analysis of the dataset, each microsatellite locus was screened for its suitability for use in the study using samples from a lizard population close to the centre of the range in Dorset (Wareham Forest 1).

During the initial screening *La37* failed to amplify and *La47* produced three alleles for some individuals; these loci were consequently excluded from further use. Loci *La5, La8, La10* (Gullberg *et al.* 1997b), *La01, La45 and La55* (Schwartz & Olsson 2008) showed significant evidence of null alleles and did not conform to Hardy-Weinberg expectations after applying the FDR test, therefore these loci were also excluded from use within the study. Although not excluded after this initial screening, *La58* failed to amplify consistently and subsequently showed evidence of null alleles and Hardy-Weinberg non-conformity in several other populations and was consequently excluded from the data analysis. This left a suite of 15 microsatellite loci: *La1, La2, La3, La4, La6, La9* (Gullberg *et al.* 1997b), *La02, La3E, La04, La10,*

La12, La27, La40, La50 and *La64* (Schwartz & Olsson 2008). These results reflected those of Gullberg *et al.* (1997b) who reported the potential presence of null alleles in *La5, La8* and *La10* in Swedish *L. agilis*. Schwartz & Olsson (2008) found that all the loci they described were in HWE in Swedish sand lizard populations, but not in populations from Hungary. However, *La6* successfully amplified in this study whereas in a previous study of British *L. agilis* it produced no PCR products (Beebee & Rowe 2001b).

2.6.2 Assessment of Genetic Variation

Using the 15 remaining loci, standard measures of genetic diversity were calculated for each sample site. Observed and expected heterozygosity (H_o and H_e) were estimated using ARLEQUIN v3.5.1.2 (Excoffier & Lischer 2010) and Allelic richness (A_R) for each sample site was calculated using FSTAT v2.9.3 (Goudet 1995), as was the inbreeding coefficient (F_{IS}) for each population. Significant differences in measures of genetic diversity were tested for between all populations and between all Dorset populations using a Kruskal-Wallis one way analysis of variance implemented in MYSTAT v12 (Systat Software, Chicago, USA). The effective population size (N_e) for each population was calculated using a linkage disequilibrium method (Hill 1981) implemented in NeESTIMATOR v1.4 (Peel *et al.* 2004). Recent genetic bottlenecks were tested for using the program BOTTLENECK v1.2.02 (Cornuet & Luikart 1996); 1000 iterations were run of a two-phase mutation model comprising 90% stepwise mutation model and significance assessed using mode-shift analysis of allele frequencies and by the Wilcoxon test.

2.6.3 Assessment of Population Structure

Genetic differentiation and structuring between populations was assessed using a number of different metrics. Pairwise F_{ST} values (Weir & Cockerham 1984), a measure of population structure, between each pair of sampled populations were estimated. There has been recent criticism of the use of F_{ST} and its analogs (particularly with microsatellites) due to their reliance on within-population diversity which means they can approach zero even when two populations are completely differentiated, especially when using highly polymorphic markers (Jost 2008; Ryman & Leimar 2009; Meirmans & Hedrick 2011). Therefore G'_{ST} (Hedrick 2005), a standardised measure of structuring which accounts for the high heterozygosity of microsatellites, and D (Jost 2008), a differentiation measures which uses the effective number of alleles rather than expected heterozygosity in calculating differentiation, were also calculated. Other authors have defended F_{ST} and criticised G'_{ST} and D as insensitive when mutation is high relative to migration (Whitlock 2011). Calculating F_{ST} values is also useful, not least because it has been widely used in population genetics studies and therefore its calculation allows comparisons to be made with previous work. Most of the discussion relating

to measures of genetic differentiation is based on modelling and theoretical datasets, however using empirical data Raeymaekers *et al.* (2012) found that different measures of genetic differentiation were useful at different scales with *D* more useful at determining broader scale colonisation history, and G_{ST} having greater sensitivity when investigating more recent demography. F_{ST} values between each pair of sampled populations were estimated using FSTAT v2.9.3 (Goudet 1995). Pairwise G'_{ST} and *D* values between each population pair were calculated using SMOGD (Crawford 2010). The similarity of F_{ST}, G'_{ST} and *D* was investigated using a series of Spearman's rank correlations.

2.6.4 Defining Populations using Bayesian Methods

Population structure was assessed using two different Bayesian assignment methodologies implemented by the programs STRUCTURE v2.3.3 (Pritchard *et al.* 2000; Falush *et al.* 2003) and BAPS v5.3 (Corander *et al.* 2003; Corander *et al.* 2008), both of which had have high success in identifying true numbers of subpopulations when levels of genetic differentiation are low (Latch *et al.* 2006). STRUCTURE uses Markov chain Monte Carlo (MCMC) algorithms to infer the probability of predefined numbers of subpopulations (*K*). BAPS uses a greedy stochastic optimisation algorithm to estimate the most likely value of *K*. Separate analyses were carried out at each geographic scale with the Crooksbury Common and Aberffraw sites included in the national scale analysis which used Town Common as a representative Dorset site. The regional (Dorset) scale analysis included Town Common, West Parley, Lytchett Heath, Wareham Forest 5, Master's Pit, Hartland Moor and Newton Heath.

In STRUCURE three iterations of each value of *K* (from *K* = 1 to *K* = 10) were performed with an MCMC chain length of $1x10^6$ and a burn-in period of $1x10^5$ used for each run. Low standard deviations for log likelihood values for each probability of *K* indicated that further replicated runs were unnecessary. Where possible the true value of *K* was determined using the Δ*K* method (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER (Earl & Vonholdt 2012) and by visual inspection of the graphical output of the program. The Δ*K* method identifies the highest level of structure within a dataset (Waples & Gaggiotti 2006) and therefore will often give a result of *K* = 2 when the graphical output of STRUCTURE indicates the presence of more than two populations, suggesting a hierarchical population structure. When this occurred during the analyses, STRUCTURE was rerun using each of the subgroups identified within the first analysis as per Coulon *et al.* (2008) and Murphy *et al.* (2010b) until no further subdivision occurred. Initially the programme was run without using any prior information about the location of each sample, if this failed to produce a clear value of *K*, the analysis was repeated with USELOCPRIOR selected (USELOCPRIOR=1), which allows *a priori* specification of groups of

samples. All simulations were run using the default correlated alleles model (which assumes allele frequencies maybe similar in populations with a shared ancestry or migration). Admixture was excluded from analysis at the national scale due to the large geographic distances and unsuitable habitat separating the three different areas. Separate analyses allowing and excluding admixture were carried out for the regional scale and admixture was included at the local scale. BAPS was run in both individual mode which uses genotype data alone to determine the most likely value for *K* and group clustering mode which uses genotype data and sample group information to determine the most likely value for *K*. The maximum value of *K* was set to 20 in both modes.

2.6.5 Detection of Migrants

After the most likely value of *K* had been determined, an additional STRUCTURE run with a burn-in of $1x10^6$ and a run length of $1x10^6$ was undertaken with *K* fixed at the most likely value determined by the previous STRUCTURE analysis to detect migrants at each geographic scale (Latch *et al.* 2011). The value of *q* (the proportion of each individual's genome assigned to each population) was used to assign an each individual to a population at a 90% and 50% threshold.

2.6.6 AMOVA

An analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was undertaken using ARLEQUIN v3.5 (Excoffier & Lischer 2010). Separate analyses were carried out at a national scale (including Town Common from Dorset) and at a regional scale using all the sample sites from within Dorset. AMOVA requires the population structure to be defined in advance and therefore the populations defined by the STRUCTURE and BAPS analysis were used. At the national scale, two groups of populations were defined, one containing all the British populations and one containing Bergherbos only. For the Dorset scale analysis groups were defined on the basis of the nested sampling strategy (section 2.2, Table 2.3).

Table 2.3. Groups of populations defined for the Dorset scale AMOVA.

Group	Populations
1	Hurn Forest, Town Common, Ramsdown
2	Merritown Heath, West Parley, Ferndown Common
3	Lytchett Heath, Branksome Chine
4	Wareham Forest (all sample sites as a single population)
5	Masters Pit
6	Hartland Moor, Newton Heath (as a single population)

3 CONSERVATION GENETICS AND PHYLOGEOGRAPHY OF BRITISH SAND LIZARDS

3.1 Introduction

Populations at the edge of a species' range often show lower genetic diversity and are more vulnerable to inbreeding and genetic drift than those from nearer the centre (Allendorf & Luikart 2007; Bohme *et al.* 2007), and consequently are at greater risk of becoming extinct (Lande 1988; Frankham 1995). The vulnerability of such populations can be increased by anthropogenic habitat loss and fragmentation which causes further reductions in population size, prevents migration between populations and can increase the likelihood of stochastic genetic events (Mills & Allendorf 1996; Couvet 2002). Edge populations may also have particular conservation value as they often have to adapt to less favourable conditions and are therefore subject to stronger selection pressures than populations nearer the centre of a species' range, consequently they may be important for the survival of the species in the event of environmental change (Lesica & Allendorf 1995).

The sand lizard *Lacerta agilis* reaches the western edge of its distribution in Britain where it occurs in three widely separated geographically and genetically distinct regions: Merseyside, Surrey and Dorset (Beebee & Griffiths 2000; Beebee & Rowe 2001b). Within Britain, *L. agilis* is restricted to sandy habitats such as lowland heathland and coastal sand dunes (Beebee & Griffiths 2000) and therefore its distribution reflects the availability of these habitats. However, some areas of apparently suitable habitat, particularly in the east of the country are not occupied. Climatic factors also influence the distribution of British sand lizards as all extant populations occur to the southwest of the 6.5 hour May isohel where the mean daily bright sunshine typically exceeds this figure (Jackson 1978). Although the role of fluctuating sunshine hours in the decline of *L. agilis* in north-west England has been disputed (Langton 1988), temperature plays an important role in reproduction (Olsson *et al.* 2011b; Olsson *et al.* 2011c). Warm spring temperatures also allow lizards to emerge from hibernation earlier and consequently produce earlier egg clutches which tend to be larger, have higher hatching success and higher hatchling survival (Olsson & Shine 1997b) and Rykena (1987) suggested the temperature required for egg incubation limits the range of the species.

Sand lizard habitat in Britain has suffered significant loss and fragmentation in the past 200 years (Jackson 1979; Webb 1986; Rose *et al.* 2000) and populations have declined as a result

(Jackson 1979; Corbett 1988b) making the species a conservation priority within the UK (Corbett & Moulton 1998; Moulton & Corbett 1999).

In Sweden, *L. agilis* is also at the edge of its range and as in Britain it exists in relatively isolated populations where particularly favourable habitat persists (Berglind 2000). Swedish sand lizards showed reduced genetic diversity when compared to a Hungarian population from near the centre of the species' range (Gullberg *et al.* 1998, 1999; Madsen *et al.* 2000). In comparison, *L. agilis* populations from Britain were found to typically have greater genetic variability than in Sweden, and in the same study, variability was significantly correlated with estimated population size (Beebee & Rowe 2001b). Of the three geographic areas, Merseyside was found to have the lowest variability and showed evidence of a genetic bottleneck.

This chapter investigated the population and conservation genetics of *L. agilis* populations within Britain. It builds upon the preliminary investigation of Beebee & Rowe (2001b) which provided some initial estimates of genetic variability of British sand lizards and the differentiation between the three geographic areas occupied by the species. The genetic variability of a number of populations from across Britain was assessed and compared to a population from mainland Europe. Population structure was investigated in sites across the core part of the sand lizard's British range in Dorset, comparing structure within fragmented and unfragmented landscapes. The phylogeography of British sand lizards was also investigated in order to establish possible explanations for their current disjunct range. In addition, a mature translocated population was investigated as well as a newly discovered population of unknown origins.

3.2 Materials and Methods

3.2.1 Introduction

General materials and methods relating to field sampling, genotyping and initial analysis of the genetic data are given in Chapter 2. The materials and methods given below relate to analytical methods specific to this chapter.

3.2.2 Construction of Phylogenetic Trees

General materials and methods for field sampling, genotyping and the initial analysis of the microsatellite data are detailed within Chapter 2. Following these initial processes phylogenetic relationships between sample sites were inferred using the PHYLIP v3.69 (Felsenstein 2009) software package. Data were bootstrapped 1000 times in the SEQBOOT

program and trees were constructed using Maximum Likelihood, Neighbour-joining and UPGMA. Cavalli-Sforza chord distances (Cavalli-Sforza & Edwards 1967) were used for all trees as this measure is particularly effective at identifying the correct topology of trees for closely related populations using microsatellites (Takezaki & Nei 1996). In order for a tree to indicate the direction of evolution it requires rooting, typically using an outgroup which is most distantly related to all the other populations. Sand lizards colonised Europe from the Balkans in a rapid westward expansion (Joger *et al.* 2007), reaching Northern Europe 10,000-12,000 years BP (Gullberg *et al.* 1998; Kalyabina *et al.* 2001). They are not found in northern France and southern Belgium despite the presence of suitable habitat (Gasc *et al.* 2004). As recently as 10,000 years BP, climatic conditions in Britain were unsuitable for sand lizards (Atkinson *et al.* 1987; Dent & Spellerberg 1987; Rykena 1987; Dansgaard *et al.* 1989), so colonisation must have occurred since this time. Jelgersma (1979) identified land bridges across the North Sea and English Channel approximately 10,000 BP and across the North Sea approximately 8,000 years BP. The timing and location of the land bridges by which sand lizards could reach Britain indicate colonisation across the North Sea and therefore, in the absence of samples from a more distantly related population, trees were rooted using the Bergherbos population from the Netherlands as the outgroup. This assumes a single colonisation event, which given the narrow period in which a land bridge existed and climatic conditions were suitable, is the most likely scenario. Once constructed, trees were visualised using TREEVIEW software (Page 1996).

3.2.3 Estimation of Divergence Times and Historical Population Sizes

Historical divergence times were investigated at a national scale (using Town Common from Dorset, Frensham Common, Ainsdale, Bergherbos and Aberffraw) using the program IMa2 (Hey 2010a, b). IMa2 uses a MCMC algorithm to simulate genealogies from genetic data from which maximum likelihood estimates of population splitting times, effective population sizes and migration rates are derived. When analysing microsatellite data IMa2 uses a stepwise mutation model (Kimura & Ohta 1978) which precludes the use of complex loci which may violate this model. Three loci (*La6, La3E, La10*) had complicated repeat units and were therefore excluded from the analysis, two additional loci (*La9, La27*) had allele sizes which differed by one base pair implying a mutation in the flanking region, which may also violate the stepwise mutation model. Consequently, only loci with simple repeat units (*La1, La2, La3, La4, La02, La04, La12, La40, La50* and *La64*) were used in the analysis. None of the loci used in the IMa2 analysis showed any evidence of linkage disequilibrium (Table 3.1) in the populations analysed. A phylogenetic tree based on the Neighbour-joining and UPGMA trees produced by PHYLIP (which

agreed on the arrangement of these populations relative to each other) was specified when running the model.

IMa2 also requires a mutation rate (*u*) for each locus to be specified. Estimates of microsatellite mutation rates for squamate reptiles include 0.0002 – 0.004 per generation in Mauritian skinks *Gongylomorphus* spp. (Nichols & Freeman 2004) and 0.01 in the Australian lizard *Egernia stokesii* (Gardner *et al.* 2000). There are no published estimates of microsatellite mutation rates for *Lacerta agilis*; however estimates are available for other members of the Lacertidae. A rate of 0.009 per locus per generation was estimated for an unstable tetranucleotide microsatellite in the Lacertid lizard *Darevskia unisexualis* (Tokarskaya *et al.* 2004). This is within published microsatellite mutation rate estimates (Weber & Wong 1993; Ellegren 2000), however this species exhibits parthenogenic reproduction, which may affect mutation rates (Badaeva *et al.* 2008). In a study of gene flow between island populations of the Skyros wall lizard *Podarcis gaigeae*, Runemark *et al.* (2012) used a figure of 0.0001 mutations per generation, an intermediate value between estimates of divergence time between *P. gaigeae gaigeae* and the subspecies *P. gaigeae weigandi* (Poulakakis *et al.* 2005) and between *P. gaigeae* and the closely related *P. milensis*. Male *L. agilis* typically breed in their second year, whilst females first breed in their third year (Simms 1970). Strijbosch & Creemers (1988) reported than the majority of net reproduction in a Dutch *L. agilis* population was supplied by females in their 4^{th}, 5^{th} and 6^{th} years and recorded a maximum age of 8 for males and 12 for females. Therefore assuming a 1:1 sex ratio (Strijbosch & Creemers 1988), average generation time (*g*) was estimated at 5 years. Consequently the mutation rate of $u = 0.0001/5 = 0.00002$ per locus per year was used for all loci when running IMa2.

Due to the large geographic distances and unsuitable habitat between each of the populations and the results of a STRUCTURE analysis to detect migrants, a model with no migration between populations was run. The upper bound for the prior distribution of the time of divergence (*t*) was set at $t = 1$. Therefore, with the assumed mutation rate of 0.0001 and generation time of 5 years, time of divergence $T = t \times g/u = 50{,}000$ years. Given that during the peak of the Pleniglacial 22,000-18,000 years BP, summer temperatures in Britain were below 10°C (Atkinson *et al.* 1987) and therefore unsuitable for *L. agilis* (Rykena 1987), Britain must have been colonised since that time.

The upper bounds of prior distribution of the population size parameter when running IMa2 was set using the geometric mean of estimates of $4Nu$ (θ), where *u* is the mutation rate and *N* is the population size. $4Nu$ for each of the loci in each population was estimated using a MCMC

method implemented in the program MISAT v1.0 (Nielsen 1997; Nielsen & Palsboll 1999) and the geometric mean 4*Nu was* calculated for each population. The largest value of the geometric means was then multiplied by five to give an upper bound for the prior distribution of population size of 66.07 (Hey 2010b).

IMa2 can have extremely long run times when analysing microsatellite data and therefore 20 individuals (40 gene copies) were randomly selected from each population used in the analysis (with the exception of Aberffraw where only seven individuals were sampled). When analysing large datasets IMa2 utilises multiple metropolis-coupled Markov chains (Geyer 1991) where swapping between the chains improves the overall level of mixing. Initial runs of the program were undertaken to assess the appropriateness of the priors and whether the heating terms were leading to adequate swap rates between 150 metropolis-coupled chains. After the heating terms were optimised, an 'M-mode' run with an indefinite burn-in period was started and monitored by visual inspection of trend plots of splitting time. Stationarity was reached after approximately 300,000 steps when no discernible trends were observable (Hey 2010b), at which point the burn-in period was terminated and sampling runs commenced. Two independent runs were conducted, each simulating in excess of 20,000 genealogies which were used in the final analysis. Maximum likelihood estimates of population parameters were averaged across the two runs to give a final estimate.

3.3 Results

3.3.1 Genotyping Errors and Microsatellite Screening

A total of 691 sand lizards were sampled across the 22 sample sites. Of these, 664 (96.09%) samples successfully amplified at sufficient loci to be included within the subsequent analysis. The blind re-genotyping identified four mis-scored alleles out of 224 giving a genotyping error rate of 1.78%. Bonin *et al.* (2004) recommend that an error rate of 2% or less is unlikely to significantly bias the results of population genetics studies and the estimation of genetic diversity, especially for large datasets and therefore the 1.78% error rate was considered acceptable. However, the estimation of some parameters, such as effective population size (N_e), can be particularly affected by genotyping errors (Paetkau 2003) and therefore this was considered when evaluating population size estimates.

Of the loci retained for analysis, MICRO-CHECKER indicated the possible presence of null alleles at a number of loci and some were also found to deviate from HWE after an FDR correction

(adjusted nominal 5% level for *P* = 0.00784, for 1% level, *P* = 0.00157) in some populations (Table 3.1). Some pairs of loci also showed evidence of linkage disequilibrium in some populations (Table 3.1) after an FDR correction (adjusted nominal 5% level for *P* = 0.00601, for 1% level, *P* = 0.00120). However, no patterns in the loci and sampling sites affected were discernible and the affected loci were not consistent across sampling sites. Therefore all the remaining loci were retained for use within the study. It is likely that the identification of some possible null alleles was due to DNA degradation after defrosting during transport.

Table 3.1. Loci showing potential evidence of null alleles, deviation from Hardy-Weinberg Equilibrium and linkage disequilibrium. Loci indicated show significant evidence of deviation from HWE or linkage disequilibrium at the 5% level, * = significant at a 1% level. For HWE, adjusted nominal 5% level for *P* = 0.00784, for 1% level, *P* = 0.00157, for linkage disequilibrium adjusted nominal 5% level for *P* = 0.00601, for 1% level, *P* = 0.00120.

Sample site	Evidence of null alleles	Deviation from HWE	Evidence of Linkage Disequilibrium
Hurn Forest	-	-	-
West Parley	*La12*	*La02, La12*	*La1/La6*
Lytchett Heath	*La40*	*La40**	*La3/La02, La3E/La10*
Wareham Forest 1	-	*La27*	-
Masters Pit	*La9, La3E, La12, La50*	*La9*, La3E, La12**	-
Hartland Moor	*La27*	*La1*	-
Newton Heath	-	*La9*	-
Town Common	-	-	-
Ramsdown	*La40*	-	*La3/La27*
Merritown Heath	*La6, La9, La10, La12*	*La6*, La9, La10*	*La6/La64**
Ferndown Common	*La2, La3, La4, La3E*	*La6**	-
Branksome Chine	-	-	*La02/La04, La12/La64*
Wareham Forest 2	*La1, La6, La9, La10, La27*	*La1, La6*, La10, La27*	*La2/3E*
Wareham Forest 3	*La2, La02, La12*	*La2, La27**	-
Wareham Forest 4	*La6, La3E, La10, La12*	*La6*, La12**	-
Wareham Forest 5	*La9, La02, La10, La27*	*La2, La3E*, La10*, La12*	*La9/La40*
Wareham Forest 6	*La6 La9, La10, La12*	*La6**	*La12/La64*
Frensham Common	*La6, La9, La10*	*La6*, La9*, La02, La3E*	-
Ainsdale Frontal Dunes	*La6, La02, La3E, La10, La27, La50*	*La6*, La02*,La10, La27*	*La3E/La27*
Bergherbos	*La4, La6, La3E, La27*	*La4*, La6*, La3E*	-
Crooksbury Common	*La02, La10*	*La10**	-
Aberffraw	*La02*	*La02**	-

3.3.2 Mitochondrial DNA

Of the 65 samples sent for sequencing, 51 were successful and three different genotypes of 307 base pairs were identified. However, two genotypes were found in only one individual

each, with all the remaining samples sharing the same genotype. The most commonly found genotype is shown in Table 3.2, the others consisted of single base substitutions: G to A at position 104 was found in a lizard from Newton Heath in Dorset, and: T to A was detected at position 235 in a lizard from Frensham Common in Surrey.

Given the low levels of variation, no further analysis of the mitochondrial DNA was undertaken. It is noteworthy that Godinho *et al.* (2005) also found very low levels of variation across five nuclear and mitochondrial markers (including cytochrome *b*) in *L. agilis* from Holland, Austria and Germany which was attributed to the rapid colonisation of Northern Europe from a glacial refuge in the Balkans and is typical of rapidly colonising species (Nichols & Hewitt 1994).

Table 3.2. Cytochrome *b* sequence found in 49 of 51 successfully amplified samples. Other sequences were identical except of a G to A substitution at position 104 in one genotype and a T to A substitution at position 235 in one genotype.

1	11	21	31	41	51
CTTTG GATCA	CTACT AGGCC	TATGC CTCAT	TATTC AAACC	ATTAC AGGTC	TCTTC TTAGC
61	71	81	91	101	111
CATAC ATTAT	ACTGC AGACA	TCTCC TCTGC	ATTTT CATCT	GTAGC CCATA	TTCAC CGAGA
121	131	141	151	161	171
TGTAC AACAT	GGATG ATTAA	TTCGT AATCT	ACACG CTAAC	GGCGC ATCCA	TATTC TTTAT
181	191	201	211	221	231
CTGCA TTTAC	CTCCA CATTG	GACGT GGATT	ATACT ATGGC	TCCTA CATCT	ATACT GAAAC
241	251	261	271	281	291
CTGAA ACATT	GGAAT CCTCC	TCCTT CTAAT	AGTGA TAGCC	ACAGC TTTCA	TAGGC TATGT
301					
ATTAC CG					

3.3.3 Genetic Diversity

Mean allelic richness (which, in this study is more appropriate than the mean number of alleles per locus as it compensates for sample size), observed and expected heterozygosities, F_{IS} and effective population size were calculated for each sample site (Table 3.3). Significant correlations were observed between all pairwise combinations of A_R, H_o and H_e (Table 3.4).

Genetic diversity was high across Dorset (average A_R = 5.322, H_o = 0.6949, H_e = 0.7448) and did not vary significantly between the populations (Kruskal-Wallis one-way test: A_R: H = 16.728, P = 0.403; H_o: H = 17.292, P = 0.367; H_e: H = 9.805, P = 0.877). These levels of diversity were comparable to those observed in Bergherbos (A_R = 5.444, H_o = 0.6580, H_e = 0.7473). All

measures of genetic diversity were lower in Frensham Common (A_R = 3.780, H_o = 0.5514, H_e = 0.6245) and lower still in Ainsdale (A_R = 3.505, H_o = 0.4555, H_e = 0.5470). Crooksbury Common (A_R = 4.518, H_o = 0.5931, H_e = 0.6981) exhibited lower levels of diversity than populations from Dorset from where it originated, but higher then Frensham Common in Surrey, the nearest natural population to where it was translocated to. Aberffraw exhibited the lowest levels of diversity by all measures (A_R = 3.400, H_o = 0.4381, H_e = 0.5011).

Table 3.3. Standard indices of genetic diversity for each sampling location. n = number of samples, *N* = average number of alleles per locus, A_R = allelic richness, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = inbreeding coefficient, N_e = effective population size. Notes: 1. Newton Heath is considered to be the same population as Hartland Moor for some analyses. 2. All Wareham Forest sites are considered to be the same population for some analyses.

Sample site	**n**	***N***	A_R	H_o	H_e	F_{IS}	N_e **(95% confidence limits)**
Hurn Forest	28	7.867	5.508	0.7315	0.7695	0.049	112.3 (76.2-203.8)
West Parley	32	8.733	5.583	0.7396	0.7701	0.040	105.2 (78.1-157.1)
Lytchett Heath	33	5.867	4.478	0.6780	0.6958	0.026	112.3 (72.9-226.1)
Wareham Forest 1	32	8.467	5.632	0.7458	0.7541	0.119	788.7 (569.0-1147.3)
Master's Pit	33	8.933	5.786	0.6989	0.7854	0.112	220.5 (133.1-591.4)
Hartland Moor	33	8.067	5.228	0.7393	0.7265	0.029	154.9 (124.1-203.3)
Newton Heath[1]	32	7.600	5.135	0.7102	0.7318	-	-
Town Common	33	8.800	5.635	0.7465	0.7674	0.028	280.4 (151.6-1487.4)
Ramsdown	31	7.733	5.184	0.7259	0.7509	0.034	194.4 (112.7-622.7)
Merritown Heath	31	8.467	5.645	0.6816	0.7615	0.107	145.4 (96.2-283.1)
Ferndown Common	33	7.800	5.351	0.6681	0.7588	0.121	117.2 (81.4-201.0)
Branksome Chine	30	5.133	4.131	0.7177	0.6899	-0.041	46.7 (35.8-65.7)
Wareham Forest 2[2]	30	7.867	5.292	0.6178	0.7322	-	-
Wareham Forest 3[2]	32	8.200	5.366	0.6217	0.7077	-	-
Wareham Forest 4[2]	29	8.333	5.594	0.6596	0.7526	-	-
Wareham Forest 5[2]	31	7.933	5.374	0.6717	0.7493	-	-
Wareham Forest 6[2]	32	8.400	5.559	0.6590	0.7576	-	-
Frensham Common	34	5.000	3.780	0.5514	0.6245	0.118	135.1 (77.9-417.9)
Ainsdale Frontal Dunes	29	4.929	3.505	0.4555	0.5470	0.170	38.6 (29.1-55.3)
Bergherbos	34	8.333	5.444	0.6580	0.7473	0.121	156.3 (104.2-298.8)
Crooksbury Common	25	6.267	4.518	0.5931	0.6981	0.153	59.0 (42.5-92.5)
Aberffraw	7	4.000	3.400	0.4381	0.5011	0.135	14.4 (8.7-34.4)
Average (Dorset)	*31.47*	*7.894*	*5.322*	*0.6950*	*0.7450*	*0.067*	-
Average (total)	*30.18*	*7.367*	*5.051*	*0.6595*	*0.7172*	*0.088*	-

Table 3.4. Spearman's rank correlations between various genetic diversity measures. A_R = allelic richness, H_e = expected heterozygosity, H_O = observed heterozygosity.

Correlation	Spearman's ρ	*P*
A_R x H_e	0.8927	<0.0001
A_R x H_o	0.5155	0.0150
H_e x H_o	0.6093	0.0031

Significant variation between sample sites was found for all measures of genetic diversity regardless of whether the non-natural sites (Crooksbury Common and Aberffraw) were included (Kruskal-Wallis one-way test: A_R: H = 56.833, P < 0.001; H_o: H = 52.075, P < 0.001; H_e: H = 42.135, P = 0.004; F_{IS}: H = 46.768, P = 0.001) or excluded (Kruskal-Wallis one-way test: A_R: H = 43.75, P = 0.001; H_o: H = 38.549, P = 0.005; H_e: H = 33.191, P = 0.023; F_{IS}: H = 44.503, P = 0.001) from the analysis (Figure 3.1). No significant differences in allelic richness, observed and expected heterozygosities were found between sites within Dorset (Kruskal-Wallis one-way test: A_R: H = 16.728, P = 0.403; H_o: H = 17.292, P = 0.367; H_e: H = 9.805, P = 0.877), however there was significant variation in F_{IS} (H = 42.739, P < 0.001). Sites from the fragmented East Dorset landscape did not differ significantly in A_R from the unfragmented Wareham Forest landscape (Student's *t*-test: t = 0.157, P = 0.879), however H_o and H_e were significantly different (H_o: t = 2.291, P = 0.048; H_e: t = 2.481, P = 0.045).

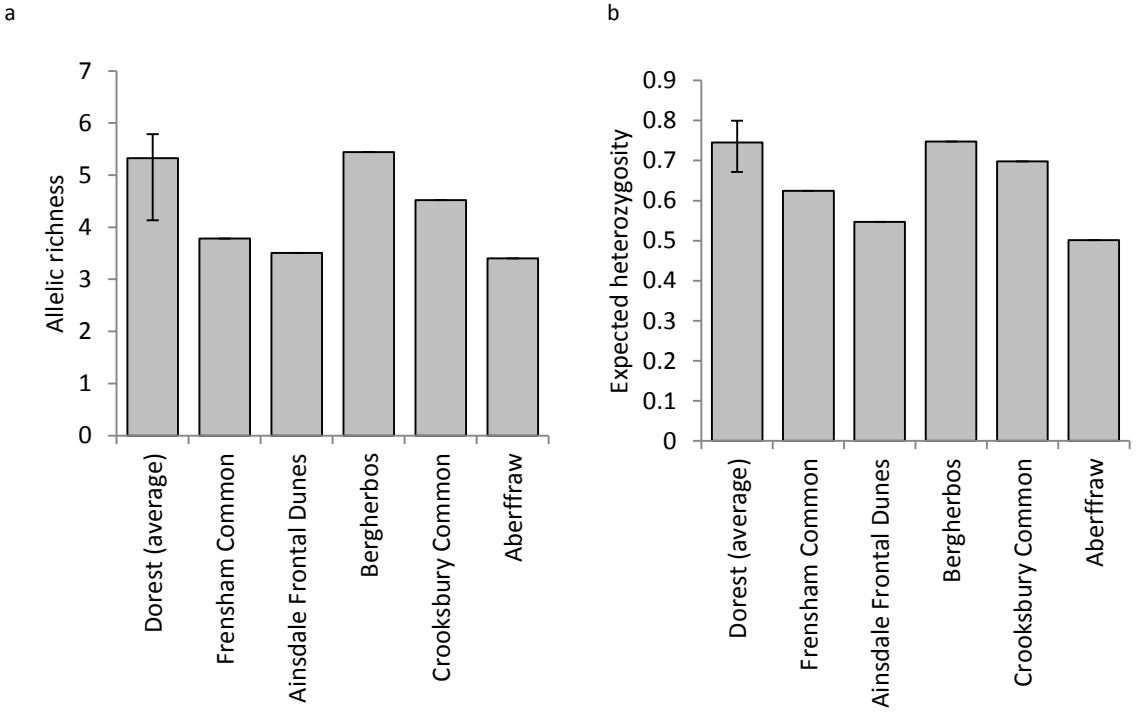


Figure 3.1. Allelic richness (a) and expected heterozygosity (b) across sampled populations. Error bars encompass 100% of data values for Dorset.

Inbreeding was low across populations with an average F_{IS} across Dorset of 0.067, and F_{IS} in Frensham Common was within the range of that of the Dorset populations. The highest level of inbreeding was observed in Ainsdale (0.170). There was significant variation in F_{IS} estimates across all sites (Kruskal-Wallis one-way test: H = 34.366, P = 0.005) and across all Dorset sites (H = 23.579, P = 0.015). There was no significant difference in F_{IS} between sites from the fragmented East Dorset landscape and the unfragmented Wareham Forest landscape (Student's t-test: t = -1.736, P = 0.115).

Only one sample site (Branksome Chine) showed signs of a recent population bottleneck with a significant one-tailed Wilcoxon test for heterozygote excess (P = 0.00418). However, in common with all the other sites, this site showed a normal L-shaped allele frequency distribution. The F_{IS} estimate for Branksome Chine was -0.041. Negative F_{IS} estimates occur when there is heterozygote excess, typical of a bottlenecked population. Allelic richness was also lower in this population compared to other Dorset populations whilst heterozygosity was comparable. This pattern would be expected in a bottlenecked population as A_R is more sensitive to the loss of rare alleles which occurs in a bottleneck (Nei *et al.* 1975).

3.3.4 Genetic Differentiation and Structuring

With the exception of two comparisons within Wareham Forest, all pairwise F_{ST} estimates were significant (Table 3.6). At the national scale high levels of differentiation were observed between the three geographic areas of Britain and the Netherlands although the three different estimators used disagreed in which populations where most highly differentiated from each other, with G'_{ST} and D most often in agreement (Table 3.7). Two of the estimators agreed that the least differentiated populations were Dorset (average) and Frensham Common (G'_{ST} = 0.476, D = 0.324), whereas F_{ST} predicted that Dorset and Bergherbos were least differentiated (0.132 compared to 0.158 between Dorset and Frensham Common). Frensham Common and Bergherbos were the most highly differentiated populations according to G'_{ST} (0.636) and D (0.510) estimates, however according to F_{ST}, Frensham Common and Ainsdale were the most highly differentiated (0.244 compared to 0.210 between Frensham Common and Bergherbos). All measures agreed that Ainsdale was more differentiated from Dorset (F_{ST} = 0.204, G'_{ST} = 0.561, D = 0.395) than from Bergherbos (F_{ST} = 0.211, G'_{ST} = 0.547, D = 0.381).

Low to moderate levels of differentiation were observed among populations across Dorset (F_{ST} = 0.034 – 0.130, G'_{ST} = 0.181 – 0.446, D = 0.081 – 0.323), with the small and isolated population of Branksome Chine the most highly differentiated (average F_{ST} = 0.109, G'_{ST} = 0.376, D = 0.240). At the local scale, differentiation between populations was typically lower, although

significantly higher in the fragmented East Dorset Area (average F_{ST} = 0.051*,* G'_{ST} = 0.216, D = 0.126) than the unfragmented Wareham Forest Area (average F_{ST} = 0.016*,* G'_{ST} = 0.081, D = 0.029) (Mann-Whitney test: F_{ST}: U = 219, P < 0.0010*;* G'_{ST}: U = 218, P < 0.001; D: U = 217, P < 0.001). Each of the genetic differentiation metrics were highly correlated with F_{ST} and D showing the closest relationship (Table 3.5).

Table 3.5. Spearman's rank correlations between F_{ST}, G'_{ST} and D.

Correlation	**Spearman's ρ**	***P***
F_{ST} x G'_{ST}	0.8510	<0.0001
F_{ST} x D	0.9161	<0.0001
G'_{ST} x D	0.8831	<0.0001

Table 3.6. Pairwise matrix of intersite F_{ST} estimates for all sites in the study. HF = Hurn Forest, WP = West Parley, LH = Lytchett Heath, WF = Wareham Forest, MP = Master's Pit, HM = Hartland Moor, NH = Newton Heath, TC = Town Common, RD = Ramsdown, MH – Merritown Heath, FeC = Ferndown Common, BC = Branksome Chine, FrC = Frensham Common, AFD = Ainsdale Frontal Dunes, BNL = Bergherbos, CC = Crooksbury Common, AB = Aberffraw.

	HF	WP	LH	WF1	MP	HM	NH	TC	RD	MH	FeC	BC	WF2	WF3	WF4	WF5	WF6	FrC	AFD	BNL	CC	AB
HF	-	0.062	0.118	0.073	0.069	0.111	0.101	0.035	0.037	0.039	0.080	0.092	0.057	0.069	0.063	0.052	0.059	0.146	0.183	0.121	0.078	0.156
WP		-	0.116	0.084	0.053	0.105	0.108	0.047	0.063	0.030	0.052	0.114	0.079	0.080	0.077	0.072	0.070	0.155	0.190	0.119	0.091	0.192
LH			-	0.099	0.109	0.129	0.126	0.115	0.115	0.119	0.129	0.099	0.092	0.120	0.103	0.087	0.091	0.173	0.223	0.155	0.158	0.228
WF1				-	0.048	0.093	0.105	0.093	0.095	0.077	0.086	0.099	0.033	0.025	0.016	0.034	0.017	0.136	0.200	0.132	0.096	0.187
MP					-	0.077	0.078	0.068	0.071	0.061	0.083	0.111	0.067	0.076	0.059	0.057	0.058	0.138	0.201	0.115	0.085	0.200
HM						-	0.046	0.097	0.110	0.099	0.120	0.124	0.107	0.104	0.099	0.091	0.097	0.196	0.239	0.171	0.136	0.225
NH							-	0.106	0.102	0.096	0.117	0.130	0.109	0.112	0.108	0.094	0.107	0.204	0.240	0.162	0.148	0.224
TC								-	0.018	0.043	0.073	0.099	0.067	0.083	0.077	0.058	0.069	0.144	0.208	0.119	0.073	0.189
RD									-	0.048	0.093	0.105	0.074	0.081	0.080	0.061	0.070	0.160	0.208	0.123	0.082	0.188
MH										-	0.043	0.098	0.065	0.069	0.071	0.063	0.066	0.149	0.184	0.109	0.068	0.183
FeC											-	0.115	0.096	0.104	0.091	0.097	0.086	0.154	0.238	0.127	0.105	0.224
BC												-	0.077	0.100	0.090	0.086	0.086	0.216	0.224	0.134	0.129	0.226
WF2													-	0.015	0.020	0.009	0.009	0.148	0.186	0.124	0.084	0.179
WF3														-	0.007	0.016	0.009	0.157	0.186	0.152	0.097	0.183
WF4															-	0.014	0.003	0.135	0.196	0.127	0.093	0.177
WF5																-	0.006	0.140	0.177	0.133	0.087	0.178
WF6																	-	0.138	0.179	0.120	0.084	0.169
FrC																		-	0.244	0.210	0.198	0.252
AFD																			-	0.211	0.223	0.135
BNL																				-	0.097	0.183
CC																					-	0.198
AB																						-

Table 3.7. Pairwise matrix of intersite *D* (top half) and G'_{ST} (bottom half) estimates for all sites in the study. HF = Hurn Forest, WP = West Parley, LH = Lytchett Heath, WF = Wareham Forest, MP = Master's Pit, HM = Hartland Moor, NH = Newton Heath, TC = Town Common, RD = Ramsdown, MH – Merritown Heath, FeC = Ferndown Common, BC = Branksome Chine, FrC = Frensham Common, AFD = Ainsdale Frontal Dunes, BNL = Bergherbos, CC = Crooksbury Common, AB = Aberffraw.

	HF	WP	LH	WF1	MP	HM	NH	TC	RD	MH	FeC	BC	WF2	WF3	WF4	WF5	WF6	FrC	AFD	BNL	CC	AB
HF	-	0.156	0.335	0.203	0.208	0.272	0.242	0.080	0.085	0.120	0.192	0.191	0.140	0.169	0.164	0.151	0.185	0.281	0.363	0.373	0.205	0.329
WP	0.277	-	0.324	0.264	0.136	0.276	0.277	0.110	0.160	0.052	0.128	0.272	0.189	0.185	0.217	0.163	0.198	0.371	0.384	0.356	0.212	0.370
LH	0.440	0.446	-	0.225	0.276	0.296	0.289	0.320	0.315	0.336	0.352	0.166	0.189	0.257	0.242	0.189	0.204	0.348	0.406	0.413	0.346	0.413
WF1	0.321	0.381	0.354	-	0.101	0.207	0.266	0.311	0.280	0.234	0.251	0.244	0.056	0.049	0.041	0.072	0.033	0.247	0.404	0.348	0.225	0.334
MP	0.330	0.259	0.399	0.199	-	0.208	0.231	0.230	0.210	0.179	0.249	0.323	0.137	0.149	0.127	0.142	0.153	0.310	0.483	0.361	0.207	0.481
HM	0.419	0.407	0.431	0.351	0.324	-	0.081	0.223	0.282	0.257	0.297	0.260	0.217	0.225	0.224	0.190	0.251	0.383	0.470	0.509	0.251	0.431
NH	0.387	0.432	0.430	0.399	0.341	0.181	-	0.258	0.263	0.235	0.279	0.282	0.229	0.252	0.239	0.210	0.278	0.419	0.467	0.435	0.330	0.446
TC	0.151	0.219	0.406	0.397	0.318	0.360	0.396	-	0.032	0.101	0.191	0.198	0.182	0.230	0.238	0.144	0.228	0.299	0.429	0.367	0.172	0.371
RD	0.167	0.278	0.403	0.386	0.313	0.432	0.391	0.090	-	0.130	0.256	0.247	0.178	0.185	0.202	0.149	0.187	0.335	0.415	0.349	0.181	0.374
MH	0.187	0.147	0.435	0.360	0.276	0.387	0.377	0.175	0.203	-	0.094	0.225	0.131	0.127	0.194	0.130	0.180	0.351	0.374	0.307	0.157	0.361
FeC	0.314	0.226	0.458	0.373	0.370	0.432	0.426	0.291	0.370	0.182	-	0.303	0.239	0.245	0.236	0.250	0.247	0.340	0.580	0.401	0.269	0.530
BC	0.341	0.428	0.316	0.365	0.420	0.404	0.438	0.339	0.376	0.381	0.432	-	0.152	0.208	0.208	0.179	0.207	0.452	0.378	0.342	0.275	0.377
WF2	0.252	0.326	0.318	0.138	0.256	0.368	0.372	0.289	0.292	0.268	0.376	0.261	-	0.015	0.041	0.008	0.018	0.283	0.303	0.283	0.120	0.266
WF3	0.271	0.317	0.380	0.114	0.275	0.366	0.396	0.312	0.280	0.267	0.373	0.322	0.065	-	0.008	0.028	0.015	0.260	0.280	0.326	0.136	0.259
WF4	0.280	0.333	0.368	0.095	0.252	0.386	0.405	0.333	0.311	0.320	0.371	0.313	0.110	0.049	-	0.026	0.007	0.265	0.389	0.316	0.195	0.304
WF5	0.238	0.298	0.297	0.149	0.243	0.338	0.349	0.240	0.234	0.256	0.374	0.284	0.048	0.068	0.087	-	0.011	0.291	0.277	0.366	0.138	0.283
WF6	0.289	0.310	0.317	0.095	0.259	0.385	0.414	0.312	0.287	0.298	0.361	0.313	0.060	0.050	0.045	0.042	-	0.265	0.313	0.307	0.178	0.267
FrC	0.448	0.487	0.492	0.424	0.456	0.549	0.582	0.442	0.477	0.464	0.473	0.606	0.458	0.441	0.423	0.441	0.429	-	0.388	0.510	0.347	0.397
AFD	0.536	0.546	0.582	0.567	0.620	0.625	0.635	0.587	0.586	0.542	0.690	0.542	0.486	0.472	0.553	0.473	0.488	0.581	-	0.381	0.372	0.054
BNL	0.507	0.482	0.533	0.490	0.475	0.617	0.565	0.464	0.473	0.438	0.517	0.471	0.424	0.473	0.461	0.479	0.432	0.636	0.547	-	0.209	0.243
CC	0.300	0.347	0.484	0.359	0.339	0.427	0.480	0.278	0.297	0.276	0.396	0.414	0.265	0.272	0.333	0.277	0.297	0.520	0.558	0.351	-	0.274
AB	0.489	0.539	0.585	0.515	0.626	0.601	0.617	0.547	0.560	0.539	0.652	0.553	0.471	0.456	0.494	0.479	0.448	0.589	0.233	0.460	0.493	-

3.3.5 Bayesian Assignment of Population Structure

STRUCTURE and BAPS showed high levels of agreement in most of the analyses carried out (Table 3.8). Using the Δ*K* method (Evanno *et al.* 2005) STRUCTURE identified the most likely value of *K* as five at the national scale. The graphical output (Figure 3.2) showed that Morden Bog, Frensham Common, Bergherbos and Crooksbury Common were well separated, however the program was unable to separate samples from Ainsdale and Aberffraw regardless of whether USELOCPRIOR was selected or not. BAPS identified six populations in the individual clustering analysis but did not separate Ainsdale and Aberffraw in the group analysis (Figure 3.2).

At the regional (Dorset) scale, the no admixture model in STRUCTURE identified the most likely value for *K* as six using the Δ*K* method, failing to separate Hartland Moor and Newton Heath (Figure 3.3). Using an admixture model, the Evanno procedure identified the most likely value for *K* as two. The Δ*K* method identifies the highest level of population structure (Waples & Gaggiotti 2006) and as the graphical output of STRUCTURE showed six well defined populations for all values of $K > 5$, in agreement with the no admixture model, it is likely that the East Dorset populations exhibit a hierarchical structure. Rerunning the analysis with USELOCPRIOR = 1 resulted in the same arrangement of populations. As the graphical output enabled the differentiation of the populations and the clusters coincided with those of the no admixture model, no further simulations were run. In group clustering mode BAPS identified six populations, in agreement with STRUCTURE, failing to separate Hartland Moor and Newton Heath (Figure 3.3). In individual clustering mode eight populations were identified, however most individuals were grouped into six main clusters corresponding with the clusters found in the group clustering analysis and the remaining groups comprising single individuals.

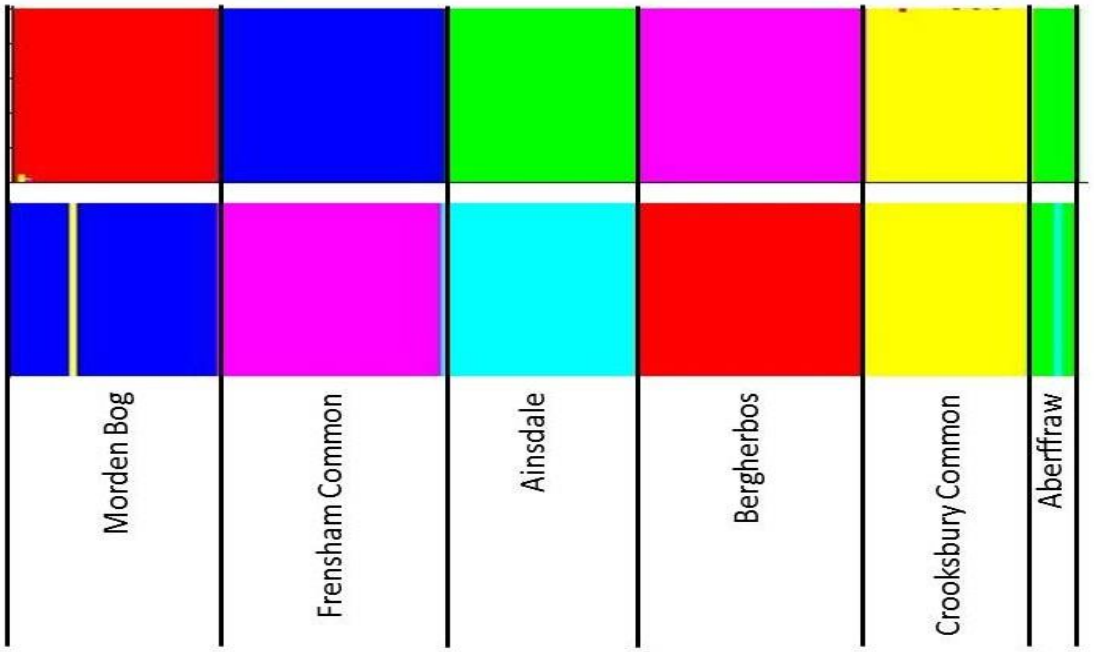
Using the Δ*K* method, STRUCTURE analysis of the data from the fragmented East Dorset area gave the most likely value for *K* as two for both admixture and no admixture models and when USELOCPRIOR = 1. However, the graphical output from the program indicated the potential presence of more than two populations (Figure 3.4) indicating a hierarchical structure (Waples & Gaggiotti 2006). Therefore the STRUCTURE analysis was repeated on each of the subgroups identified within the previous analysis. Using this method five populations were identified with STRUCTURE unable to separate Town Common and Ramsdown. BAPS identified the most likely value of *K* as three in group clustering mode (Figure 3.4) and five in individual clustering mode. However the arrangement of individuals in the individual clustering mode output corresponded with the arrangement on the group clustering mode with a few individuals assigned to additional populations.

At the local scale STRUCTURE was unable to distinguish any clear population structure within the unfragmented landscape of Wareham Forest (Δ*K* method, USELOCPRIOR = 0, *K* = 2; USELOCPRIOR = 1, *K* = 3, however the clusters did not correspond to the geographic locations of the samples). BAPS failed to identify any separate populations in group mode and gave a most likely value of *K* as eight in the individual mode with most individuals in a large single cluster and the remaining clusters comprising single individuals (Figure 3.5).

Table 3.8. Most likely arrangement of populations given by STRUCTURE and BAPS.

Scale of Analysis	Sample Site	Population Assignment	
		STRUCTURE	BAPS
National	Morden Bog (Dorset)	1	1
	Frensham Common	2	2
	Ainsdale Frontal Dunes	3	3
	Bergherbos	4	4
	Crooksbury Common	5	5
	Aberffraw	3	6
Regional (Dorset)	Town Common	1	1
	West Parley	2	2
	Lytchett Heath	3	3
	Morden Bog	4	4
	Master's Pit	5	5
	Hartland Moor	6	6
	Newton Heath	6	6
Local (Wareham Forest)	Wareham Forest 1 (Cold Harbour)	1	1
	Wareham Forest 2	1	1
	Wareham Forest 3	1	1
	Wareham Forest 4 (Phil Shillitoes)	1	1
	Wareham Forest 5 (Morden Bog)	1	1
	Wareham Forest 6	1	1
Local (East Dorset)	Hurn Forest	1	1
	Town Common	2	2
	Ramsdown	2	2
	Merritown Heath	3	3
	West Parley	4	3
	Ferndown Common	5	3

a



b

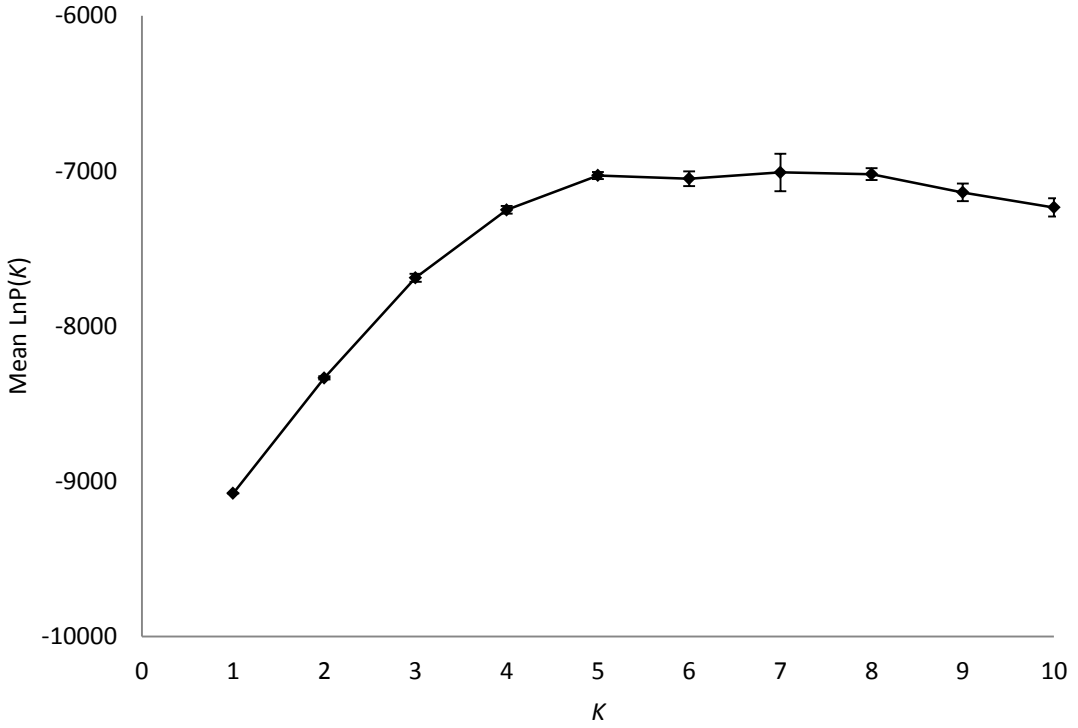
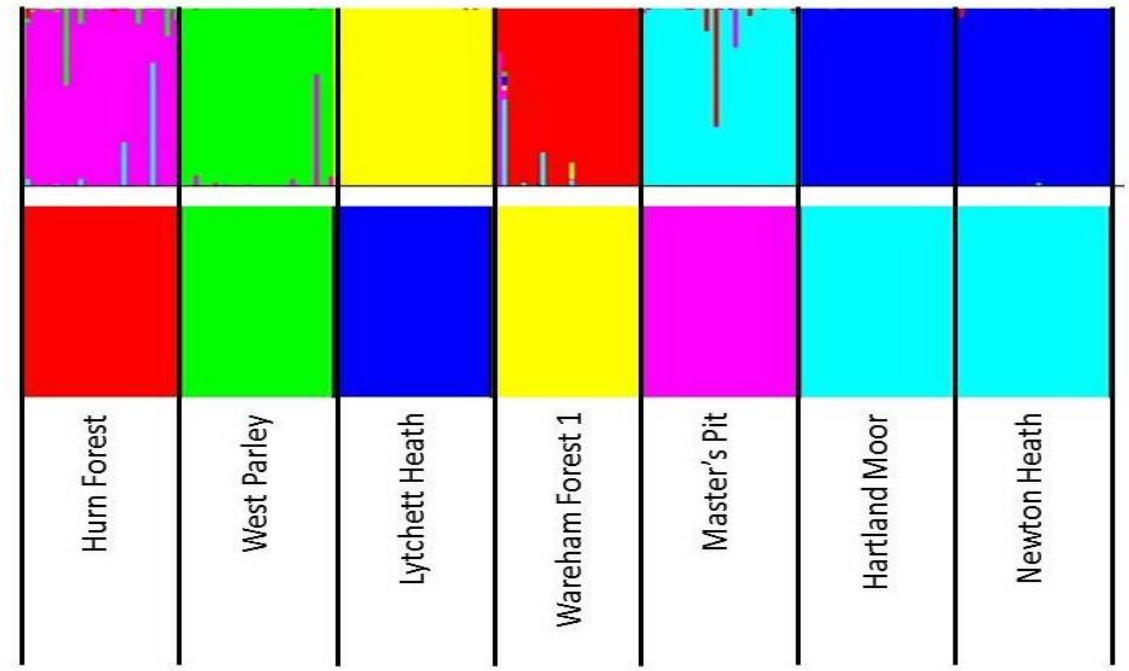


Figure 3.2. Results of the STRUCTURE (*K* = 5) and BAPS (*K* = 6) analysis at the national scale. a) Both STRUCTURE (top) and BAPS (below) outputs showed well defined populations (individual BAPS analysis shown). STRUCTURE indicated the true value of *K* as 5, failing to separate Ainsdale from Aberffraw, whereas BAPS showed these as separate populations (*K* = 6). b) Graph of the log likelihood of each value of *K* including standard deviation bars.

a



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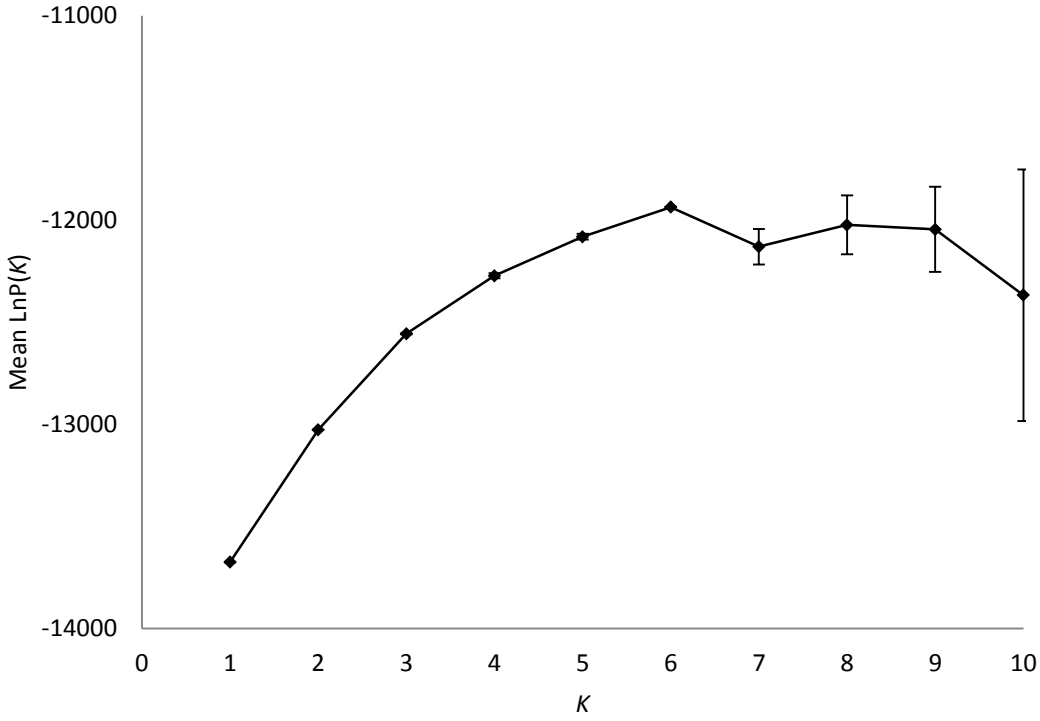

Figure 3.3. Results of the STRUCTURE (*K* = 6) and BAPS (*K* = 6) analysis at the regional scale. a) Both STRUCTURE (top) and BAPS (below) outputs showed well defined populations, with *K* = 5. b) Graph of the log likelihood of each value of *K* including standard deviation bars.

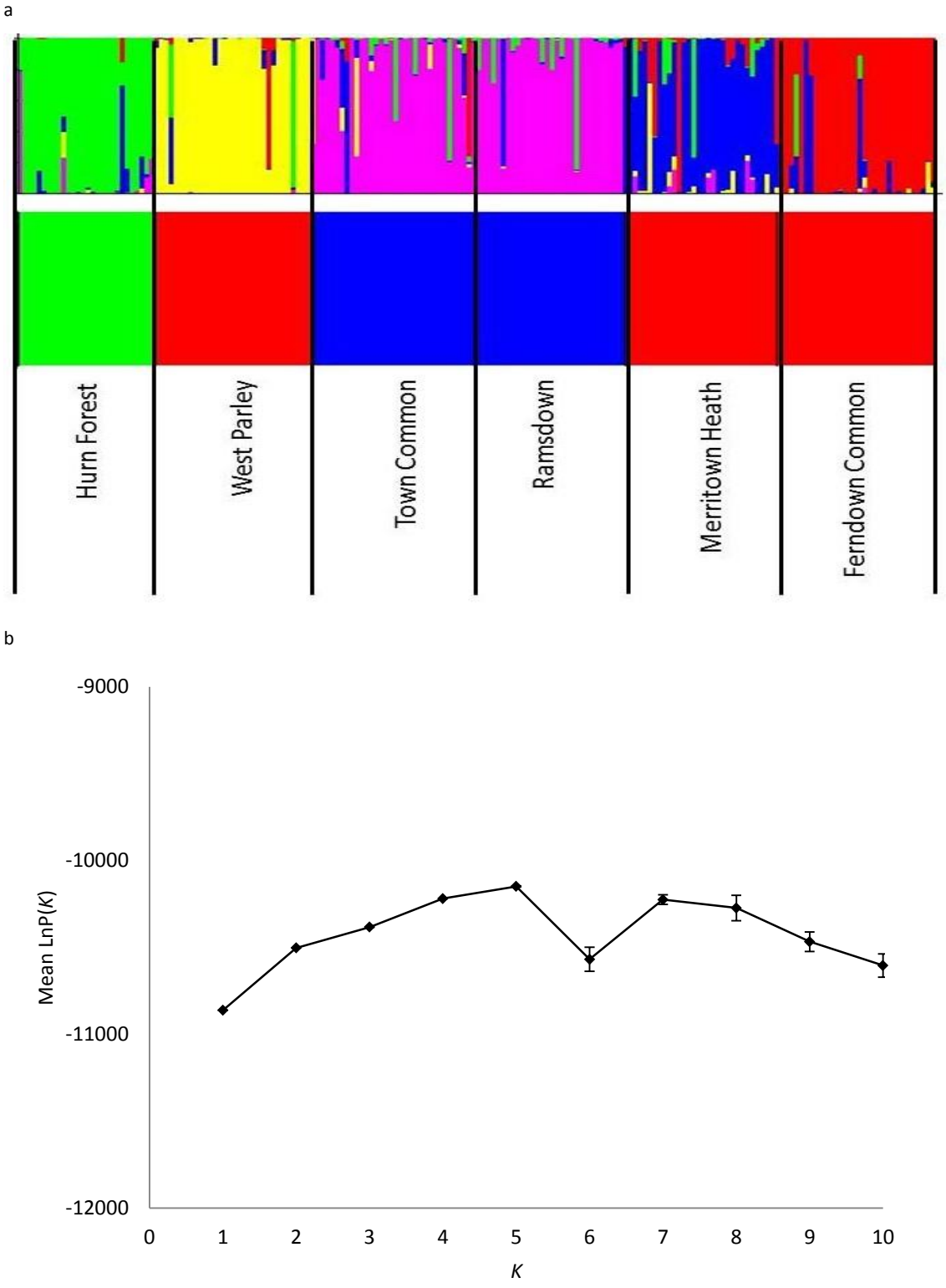

Figure 3.4. Results of the STRUCTURE (*K* = 6 after a hierarchical analysis) and BAPS (*K* = 3) analysis at the local scale in East Dorset (fragmented landscape). a) Both STRUCTURE (top) and BAPS (below) did not separate Town Common and Ramsdown; BAPS did not separate Merritown Heath, West Parley and Ferndown Common, which STRUCTURE identified as separate populations in a hierarchical analysis. b) Graph of the log likelihood of each value of *K* including standard deviation bars from the initial STRUCTURE run.

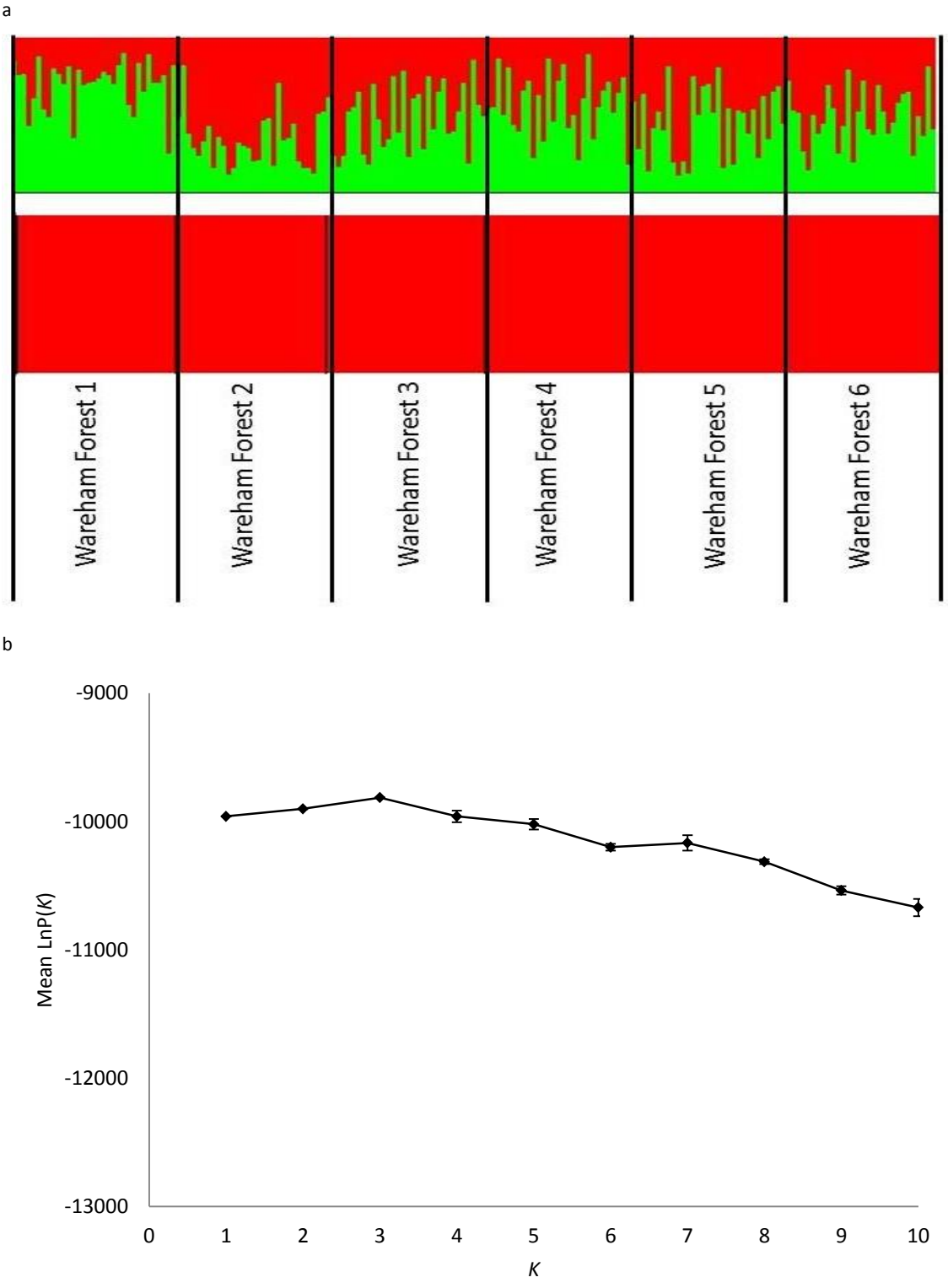

Figure 3.5. Results of the STRUCTURE (*K* = 2) and BAPS (*K* = 1) analysis at the local scale in Wareham Forest (unfragmented landscape). Both STRUCTURE and BAPS were unable to identify any subpopulations. b) Graph of the log likelihood of each value of *K* including standard deviation bars.

3.3.6 Detection of Migrants

No migrants were identified between populations at the national scale with 100% of individuals assigned to their sample site. At the regional scale (Table 3.9), no migrants were identified at the 90% threshold, although there were a number of individuals that could not be unambiguously assigned to any population; at the 50% threshold, two potential migrants were identified (Figure 3.6). At the local scale within East Dorset (Table 3.10), two potential migrants were identified at the 90% threshold, with several individuals unassigned. A total of 23 potential migrants were identified at the 50% threshold. Merritown Heath and Ferndown Common exchanged the most migrants with two moving in each direction. Lizards assigned to Hurn Forest were detected at all sample sites, only one potential migrant originating from Town Common/Ramsdown was detected (Figure 3.6). No subpopulations were identified within Wareham Forest and therefore no attempt was made to detect migrants in this area.

Table 3.9. Assignment of individuals to populations at the regional (Dorset) scale using STRUCTURE. Individuals are assigned at a 50% (and 90%) threshold. TC = Town Common, WP = West Parley, LH = Lytchett Heath, WF5 = Wareham Forest 5, MP = Master's Pit, HM = Hartland Moor, NH = Newton Heath. Hartland Moor and Newton Heath are considered to be one population.

Sample site	Assigned population							Total
	TC	WP	LH	WF5	MP	HM/NH	Not assigned	
Town Common	32 (26)				1		(7)	33
West Parley	1	31 (31)					(1)	32
Lytchett Heath			33 (33)					33
Wareham Forest 1				30 (27)			1 (4)	31
Master's Pit				1	32 (31)		(2)	33
Hartland Moor						33 (33)		33
Newton Heath						32 (32)		32

Table 3.10. Assignment of individuals to populations at the local (East Dorset) scale using STRUCTURE. Individuals are assigned at a 50% (and 90%) threshold. HF = Hurn Forest, TC = Town Common, RD = Ramsdown, MH = Merritown Heath, WP = West Parley, FC = Ferndown Common. Town Common and Ramsdown are considered to be one population.

Sample site	Assigned population						Total
	HF	TC/RD	MH	WP	FC	Not assigned	
Hurn Forest	25 (20)	1		1		1 (8)	28
Town Common	2	26 (21)	1	2	1	1 (12)	33
Ramsdown	1	29 (24)	1			(7)	31
Merritown	1		26 (16)	1	2	1 (15)	31
West Parley	1 (1)			29 (26)	1	1 (5)	32
Ferndown Common	1		2 (1)		29 (24)	(8)	33

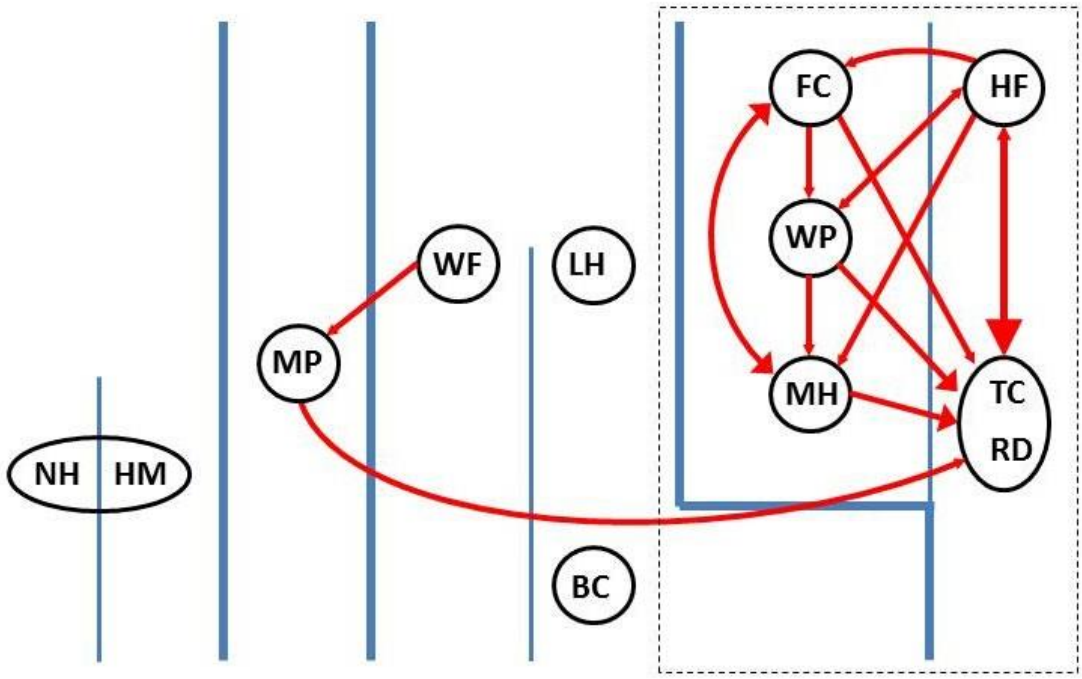

Figure 3.6. Diagram of movement of migrants identified by STRUCTURE, all migrants identified at the 50% threshold are shown. NH = Newton Heath, HM = Hartland Moor, MP = Master's Pit, WF = Wareham Forest 5, LH = Lytchett Heath, BC = Branksome Chine, FC = Ferndown Common, WP = West Parley, MH = Merritown Heath, HF = Hurn Forest, TC = Town Common, RD = Ramsdown, small arrowheads = one migrant, medium arrowheads = two migrants, large arrowheads = three migrants, blue lines represent rivers. The figure is based on separate local (East Dorset, sites within the dashed line) level and the regional (Dorset) level analyses in which East Dorset was represented by Town Common. Therefore not all possible combinations of populations were investigated.

3.3.7 AMOVA

The AMOVA of sites at the national scale showed insignificant variation between the British sites and Bergherbos in the Netherlands (Table 3.11). Most of the variation (81.91%) was within populations, with 18.54% among the British populations. At the regional scale in Dorset (Table 3.12) the highest level of variation was again observed within populations (91.82%), whilst the lowest level of variation (3.41%) was observed among populations within the groups.

Table 3.11. AMOVA of sample sites at a national scale.

Source of variation	d.f.	Sum of squares	% of variation	*P*
Among groups	1	73.439	-0.45	0.7507
Among populations within groups	2	145.331	18.54	< 0.0001
Within populations	256	1203.776	81.91	< 0.0001
Total	259	1422.546		

Table 3.12. AMOVA of all Dorset sample sites.

Source of variation	d.f.	Sum of squares	% of variation	*P*
Among groups	5	363.805	3.41	< 0.0001
Among populations within groups	5	116.973	4.78	< 0.0001
Within populations	1059	5815.925	91.82	< 0.0001
Total	1069	6296.703		

3.3.8 Phylogeography

Each of the three tree production methods showed a similar arrangement of the populations, with one significant difference in the Maximum Likelihood tree, which nested Frensham Common within the Dorset populations. Within Dorset the three methods differed in the location of Master's Pit, which was grouped with the Wareham Forest populations in the UPGMA tree (Figure 3.7) and Hartland Moor and Newton Heath in the Neighbour-joining (Figure 3.8) and Maximum Likelihood trees (Figure 3.9). Both arrangements are feasible when the landscape and site locations are considered. The trees also differed in the arrangement of some of the sites within Wareham Forest and in the Neighbour-joining tree many of the nodes within Wareham Forest had low support. Given the low genetic distances between them this is unsurprising. Bootstrap values were highest for the UPGMA tree with most nodes supported by more than 500 replicates. Bootstrap support was lowest for the Maximum Likelihood tree. Both the Neighbour-joining and UPGMA trees grouped Ainsdale and Aberffraw together with 100% support.

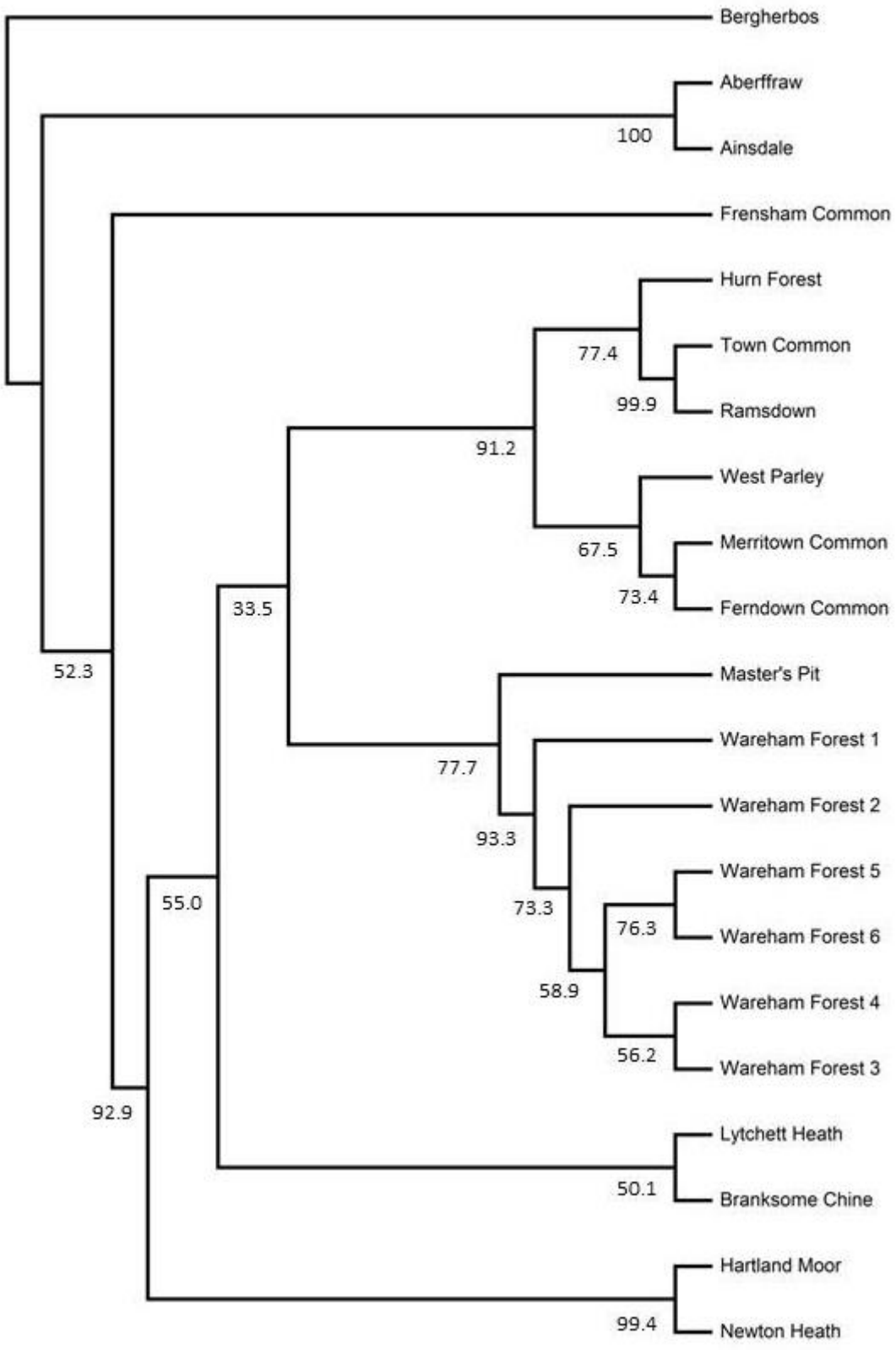

Figure 3.7. UPGMA tree of all sample sites based on Cavalli-Sforza genetic distances. Figures below the nodes refer to the percentage bootstrap support for each node.

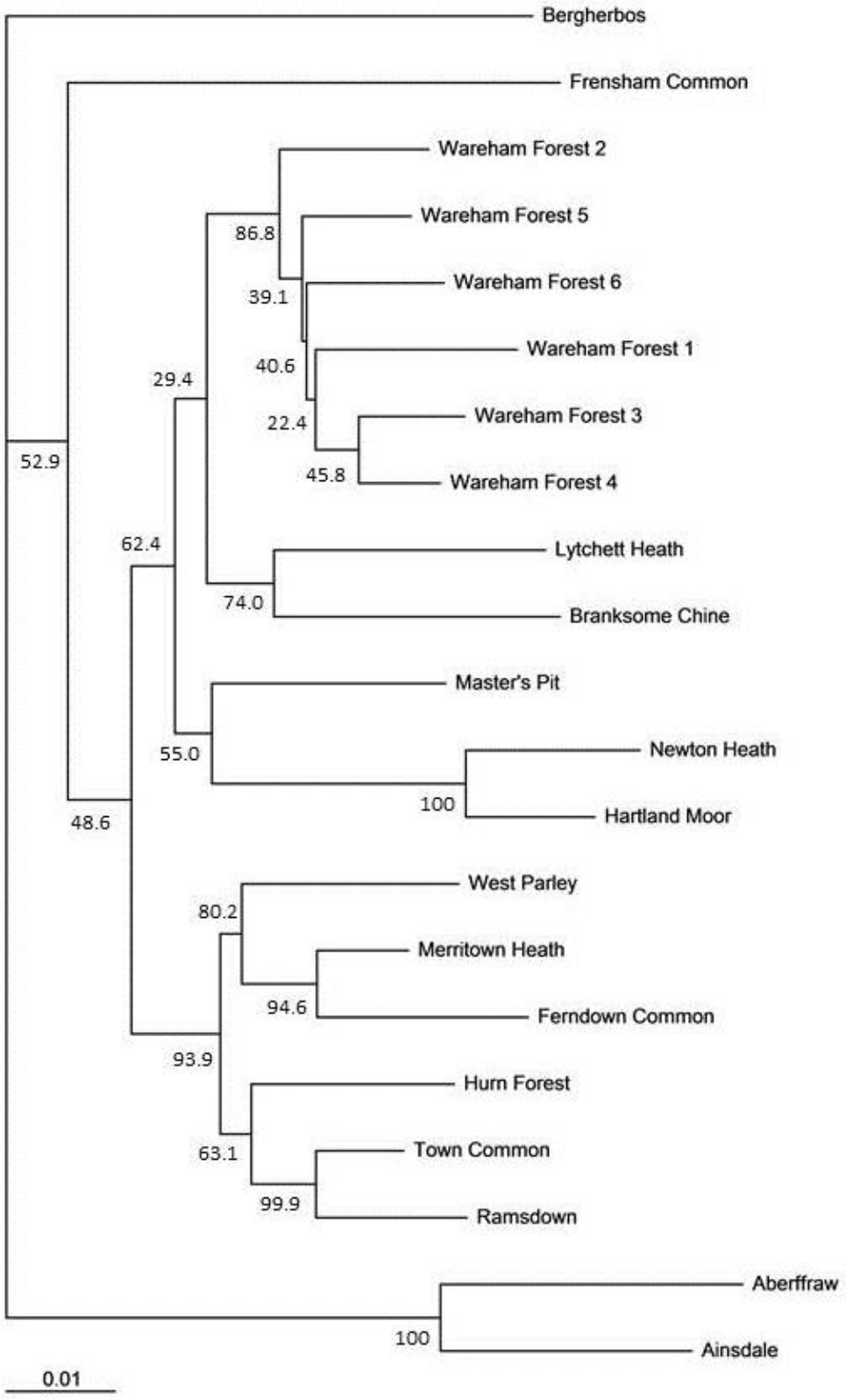

Figure 3.8. Neighbour-joining phylogram of all sample sites based on Cavalli-Sforza genetic distances. Figures below the nodes refer to the percentage support for each node.

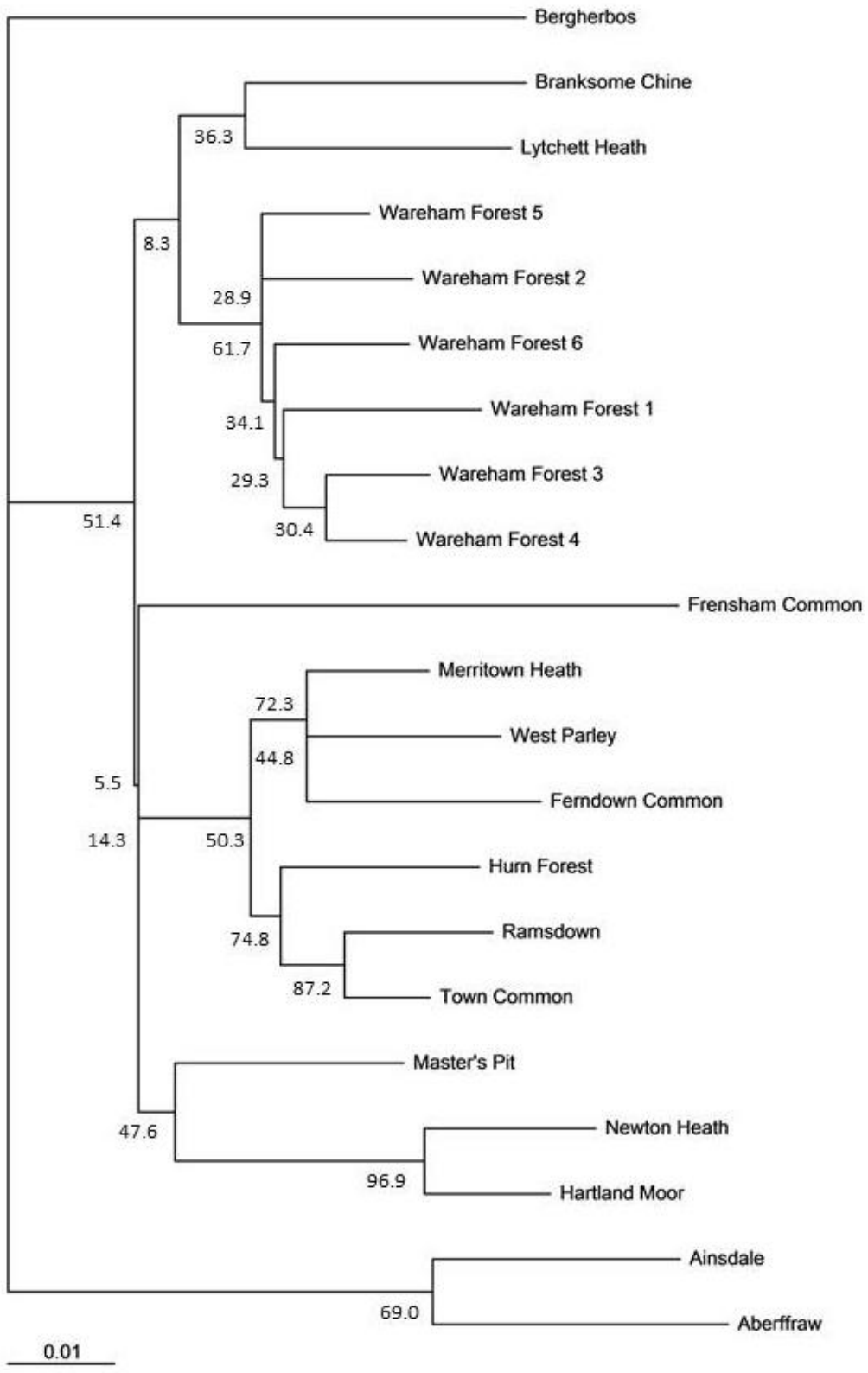

Figure 3.9. Maximum Likelihood phylogram of all sample sites based on Cavalli-Sforza genetic distances. Figures below the nodes refer to the percentage support for each node. The arrangement of Frensham Common differs to the UPGMA and Neighbour-joining trees.

3.3.9 Historical Divergence and Population Sizes

Estimates of historical divergence times and present and historical population sizes produced by IMa2 are shown in Table 3.13 and Table 3.14 respectively. The phylogenetic tree specified when running IMa2 is given in Figure 3.10 and shows the parameters estimated by the program. Estimates of *t* are converted to the divergence time in years (*T*) by multiplying *t* by the mutation rate per generation (*u*) over the generation time in years (*g*). Using the mutation rate of 0.0001 of *Podarcis gaigeae* (Runemark et al. 2012) and the generation time of 5 years discussed in section 3.2.3, $T = t \times 50{,}000$. The population size (*N*) was estimated from the population size parameter estimate (*q*) by dividing with 4*u* ($N = q/4u$). Therefore $N = q/0.0004$. Estimates of *q* for Ainsdale and Aberffraw were highly correlated implying they are in effect a single population, and consequently the estimates were considered unreliable. The upper 95% confidence estimate for *q*7 exceeded the upper bound of the prior estimate of maximum population size.

Table 3.13. Maximum likelihood estimates of historical divergence times. BP = before present. Divergence times are shown in Figure 3.10.

Parameter	**Estimate of *t* (95% confidence limits)**	**Divergence time (95% confidence limits) (years BP)**
*t*1 (Ainsdale x Aberffraw)	0.01325 (0.00075 – 0.03)	662.5 (37.5 – 1500)
*t*2 (Frensham Common x Town Common)	0.023945 (0.005 – 0.0475)	1197.25 (250 – 2375)
*t*3 (*q*6 x *q*7)	0.06877 (0.026 – 0.1195)	3438.5 (1300 – 5975)
*t*4 (*q*8 x Bergherbos)	0.1607 (0.0575 – 0.2865)	8035 (2875 – 14325)

Table 3.14. Maximum likelihood estimates of population sizes (*N*). *q*1 – *q*5 are present day populations, *q*6 – *q*9 are historical populations (Figure 3.10). * Estimates of *q* for Ainsdale and Aberffraw were highly correlated indicating that these are in effect one population; therefore estimates of *q* are not reliable. § The upper 95% confidence limit for *q*7 exceeded the prior for the upper bound of population size.

Parameter	**Estimate of *q* (95% confidence limits)**	**Population size (95% confidence limits)**
*q*1 (Aberffraw)*	-	-
*q*2 (Ainsdale)*	-	-
*q*3 (Frensham Common)	0.155 (0.05 – 0.371)	387.5 (125 – 927.5)
*q*4 (Town Common)	0.566 (0.2025 – 1.7925)	1390 (506.25 – 4481.25)
*q*5 (Bergherbos)	1.409 (0.729 – 2.599)	3522.5 (1822.5 – 6497.5)
*q*6	0.238 (0.0735 – 0.604)	595 (183.75 – 1510)
*q*7	42.7955 (1.167 – >66.07)§	106988.8 (2917.5 – >165175)§
*q*8	1.385 (0.0935 – 4.0635)	3462.5 (233.75 – 10158.75)
*q*9	36.8285 (23.9085 – 58.973)	92071.25 (59771.25 – 147432.5)

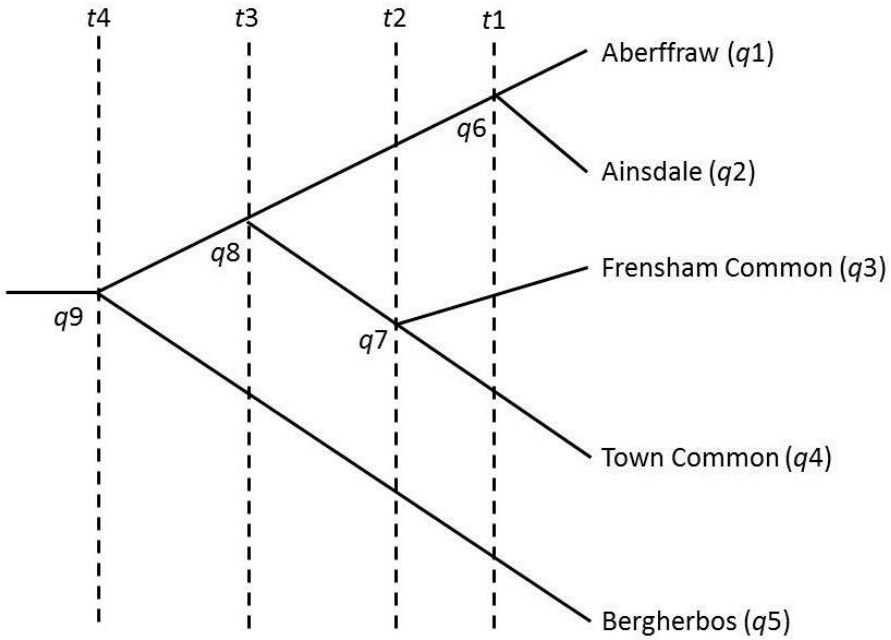

Figure 3.10. Phylogenetic tree specified when running IMa2. The tree was derived from the Neighbour-joining and UPGMA trees produced in PHYLIP. *t1* – *t2* are historical divergence times, *q*1 – *q*5 are present day populations, *q*6 – *q*9 are historic ancestral populations.

3.4 Discussion

3.4.1 Genetic Diversity and Differentiation

Expected heterozygosity in sand lizards from Dorset (average 0.7448) compared favourably with the continental population of Bergherbos (0.7473) and a large population in Hungary (0.67) (Gullberg *et al.* 1998; Schwartz & Olsson 2008); it was considerably higher than in a number of populations in Sweden which averaged 0.451 across ten sites (Gullberg *et al.* 1998). Within Dorset, even isolated populations with a small effective population size and which may have experienced a possible bottleneck such as Branksome Chine (N_e = 46), exhibited relatively high levels of genetic diversity (H_e = 0.6899). However, the populations from Surrey and Merseyside, which have had historically lower population sizes, were significantly less diverse. Surprisingly high levels of genetic diversity have been observed in other very small populations of Lacertid lizards including the closely related *L. viridis* (Bohme *et al.* 2007) and the Iberian rock lizard *Iberolacerta monticola* (Remon *et al.* 2012) and it has been demonstrated that many reptiles, including *L. agilis,* are able to avoid mating with close kin (Olsson *et al.* 2003), which may enable small populations to maintain high levels of genetic diversity. The differences in the degree of diversity between British and Swedish *L. agilis* could be a result of

the colonisation history of the areas and implies the British founder population may have been larger than that which colonised Sweden.

The genetic diversity of sand lizards makes an interesting comparison with that other restricted amphibian and reptile species in Great Britain. Throughout large parts of its range in Britain *L. agilis* is sympatric with the smooth snake *Coronella austriaca* (Beebee & Griffiths 2000). Smooth snakes in Wareham Forest exhibited lower levels of genetic diversity than sand lizards from the same site (A_r = 2.559, H_o = 0.428, H_e = 0.532) (Pernetta *et al.* 2011). The natterjack toad *Bufo calamita* also has a similar disjunct distribution in Britain and like *L. agilis*, has undergone recent declines (Beebee & Griffin 1977). Various studies have given very low estimates of genetic variability in British *B. calamita*; expected heterozygosity ranged from 0 – 0.021 (Hitchings & Beebee 1996) as assessed by allozymes, and between 0.291 and 0.391 using microsatellites (Beebee & Rowe 2000).

Levels of inbreeding as assessed by F_{IS} estimates were low in most of the populations in the study, the highest value of 0.170, being recorded in Ainsdale. Excluding one population where a negative value was recorded, F_{IS} estimates for Dorset ranged between 0.026 and 0.121 (average 0.067). By contrast, a range of 0.056 – 0.354 (average 0.192) was recorded in *C. austriaca* from sites across Dorset

Only one population (Branksome Chine) exhibited any evidence of having undergone a genetic bottleneck. This population is the most isolated of all the populations sampled within Dorset and has been separated from most other populations for over 100 years due to the urban expansion of Poole. This is the last remaining sand lizard population on the Poole seafront (D. Bird, pers. comm.), although other small populations persisted until recently. No evidence of a bottleneck was found in the Frensham Common or Ainsdale populations which is in contrast to the results of Beebee & Rowe (2001b). The samples for that study were collected from wild-caught animals which were part of the captive breeding programme at the time and their results were based on a small sample size.

The relatively high genetic diversity and low inbreeding of *L. agilis* in apparently small and fragmented populations may be in part attributable to reproductive strategies which minimise inbreeding. This has been observed in the skink *Gnypetoscincus queenslandiae* (Stow & Sunnucks 2004) as well as *L. agilis* in Sweden where females exhibit a mating preference for males with dissimilar MHC genes (Olsson *et al.* 1999; Olsson *et al.* 2003).

3.4.2 Genetics of a Translocated Population

The Crooksbury Common population exhibited lower levels of genetic diversity than all the sampled populations from Dorset (where its founder animals were translocated from), including the small isolated population from Branksome Chine, and had a smaller effective population size than all Dorset populations with the exception of Branksome Chine. However, it exhibited significantly more variation and had a larger effective population size than the nearest natural population at Frensham Common and the degree of inbreeding was not significantly different from populations in Dorset.

Genetic bottlenecks occur due to a sudden decline in breeding adults within a population. No evidence of such a bottleneck was found in the sand lizards from Crooksbury Common indicating that the capture part of the translocation was successful with a significant proportion of the adults translocated from the donor populations.

These results imply that the translocation has resulted in a loss of genetic diversity compared to the source population(s). However, this loss seems unlikely to have a significant effect on the long-term persistence of the population. The absence of evidence of a bottleneck and the comparable levels of genetic diversity to the Dorset populations suggests that a sufficient number of animals were translocated to avoid a founder effect and levels of genetic diversity are sufficient to limit the effects of genetic stochasticity.

Both STRUCTURE and BAPS identified Crooksbury Common as a separate population from the nearest native population and from Dorset populations near to its geographic origin. No migrants were identified within the Crooksbury samples and no lizards from other sample sites were identified as being from Crooksbury. This implies that the population has remained isolated from the natural populations within the wider vicinity.

3.4.3 Population Structure

As would be expected considering the large geographic distances and unsuitable habitat between them, sand lizards from the three geographic areas of Britain are well differentiated from each other and no migrants could be detected at the national scale. Across Dorset, most populations were well differentiated and at the regional scale few migrants were identified. Neither STRUCTURE nor BAPS could separate Hartland Moor and Newton Heath, although the genetic distance between them was significant as assessed by the estimate of F_{ST}. This is perhaps unsurprising as these sites were part of the same large heathland area until the 1940s and unlike many of the sample sites; they have been separated by afforestation rather than development, which may allow higher levels of migration between the populations.

In the comparison between the fragmented East Dorset and unfragmented Wareham Forest areas, differences in population structure were marked. Although there was significant genetic structuring between most of the sample sites within Wareham Forest, all the sample sites were part of the same population.

In East Dorset, STRUCTURE was able to identify each sample site as a true population with the exception of Town Common and Ramsdown, whereas BAPS did not separate Merritown Heath, West Parley and Ferndown Common. Several potential migrants were identified between populations. Merritown Heath, West Parley and Ferndown Common were previously part of the same large extent of heathland which was fragmented between the 1930s and 1980s; Hurn Forest, is separated from these sites by a similar geographic distance, but also by the relatively small Moors River. This implies that *L. agilis* population structure within the East Dorset more closely reflects natural historic barriers than recent habitat fragmentation. The effect of current and historical landscape on the population genetics of *L. agilis* in Dorset is considered further in Chapter 4.

Although it was not possible to separate Town Common from Ramsdown using the methods implemented by STRUCTURE or BAPS, the construction of a four-lane road between them in the late 1970s means that there is an extremely low likelihood of individuals being able to pass between them and the genetic similarity between them is likely to be a result of genetic 'time-lag'. Landguth *et al.* (2010) used a variety of migration models to quantify the time it would take before a barrier to dispersal could be detected using genetic methods and found that it may take up to 200 generations to detect the effect of a barrier using population-based approaches based on F_{ST} estimates. Given a generation time for *L. agilis* of 4-5 years, approximately 7-9 generations would have passed since the construction of the road and therefore genetic effects would not yet be detectable, as was the case in fragmented populations of the Florida sand skink *Plestiodon reynoldsi* after a similar number of generations (Richmond *et al.* 2009; McCoy *et al.* 2010). It may be possible to detect the effect of such barriers using an individual-based approach (Murphy *et al.* 2010a; 2010b; Latch *et al.* 2011), for example Murphy *et al.* (2008) were able to detect barriers to gene flow after just five generations.

The phylogenetic trees offered two slightly different arrangements of the populations within Dorset. The UPGMA tree grouped Master's Pit with the Wareham Forest populations (77.7% bootstrap agreement), whereas the Neighbour-joining tree grouped it with the Purbeck populations of Hartland Moor and Newton Heath (55.0% bootstrap agreement). The UPGMA

tree also indicated an earlier divergence of the Lytchett Heath and Branksome Chine populations compared to the Neighbour-joining tree, which grouped these with the Wareham Forest populations (albeit with low bootstrap support). Both trees showed a high level of congruence with the geographic locations of the sampled populations and the rivers which separate them, particularly the Neighbour-joining tree.

3.4.4 Colonisation History of Sand Lizards in Britain

The majority of the genetic variation of British *L. agilis* was found within populations rather than between them and variation between British sand lizards and the population from Bergherbos was insignificant when compared to variation within the populations. This pattern of high within population variation compared to between population variation implies a relatively recent divergence time between the populations. The lack of variation in the cytochrome *b* gene also supported a relatively recent separation from mainland European populations.

The maximum likelihood estimate for the time of divergence between British and continental European *L. agilis* populations (8,035 years BP) approximately coincides with the presence of a land bridge across the North Sea 8,300 – 7,800 years BP (Jelgersma 1979) and this offers the most likely explanation for how *L. agilis* colonised the Britain. During the mid-Holocene 8,500 – 5,500 years BP, a 'Thermal Maximum' occurred when during which sea surface temperatures were 1.5 °C warmer than today (Calvo *et al.* 2002; Matthews & Dresser 2008). Following the initial colonisation of Britain, it is likely that *L agilis* spread rapidly throughout the country reaching the northern limits of their current range in Merseyside during this warmer period. Warmer temperatures are likely to have allowed the species to occupy a wider variety of habitats than they currently do in Britain, thus facilitating their spread through the country. *Lacerta agilis* are able to utilise many different habitats and occur up to 2,200 m in altitude over much of their range (Gasc *et al.* 2004). Cooling of the climate subsequently caused sand lizards to retreat to areas of particularly favourable habitat, similar to that in which they are found today, resulting in the divergence of the Merseyside populations from the southern populations between 5,975 and 1,300 years BP, during a period of change from a warmer, drier climate to a cooler, wetter one (Barber 1982; Dark 2006). This pattern of range expansion during the mid-Holocene followed by a contraction to a relictual range is mirrored by Swedish *L. agilis* (Gullberg *et al.* 1998) and explains the similarity in the species' distribution between the two countries.

IMa2 estimated that the Surrey and Dorset populations diverged 1,197.25 years BP (95% confidence range of 250 – 2,375 years BP). This range encompasses the 'Little Ice Age' when average temperatures across north-west Europe were 0.9 °C cooler than today and levels of precipitation increased (Grove 1988; Wigley & Kelly 1990; Seppa & Birks 2002; Nesje & Dahl 2003; Moberg *et al.* 2005; Dong *et al.* 2012). This has been implicated in extinctions and range contractions in a number of invertebrates (Girling 1984; Thomas 1993), potentially in combination with the effects of habitat destruction (Buckland & Wagner 2001). It also appears to have caused changes in the population dynamics of small rodent communities (Henden *et al.* 2009). The Little Ice Age followed a period of warmer temperatures in Europe (Moberg *et al.* 2005; Dong *et al.* 2012). IMa2 estimated an historical effective population for *L. agilis* in southern Britain of approximately 107,000 at this time and may have occupied large areas in between Surrey and Dorset, including much of the New Forest from where it persisted until the 1970s (Corbett 1988b). The present combined size of the Dorset and Surrey populations was estimated at < 10,000 (Corbett 1994).

The limit of the range of many species of reptile is determined by the environmental conditions required for successful incubation of their eggs (Fitch & Fitch 1967; Ultsch 2006) and many reptiles have evolved viviparity as an adaptation to cold climates (Tinkle & Gibbons 1977; Shine 1983). Of the six reptile species native to Britain, only *L. agilis* and the grass snake *Natrix natrix* are oviparous. *Natrix natrix* typically lays its eggs in compost or manure heaps where the heat generated by decomposition aids the incubation of the clutch (Lowenborg *et al.* 2012) which enables the species to extend its range further north than other oviparous reptile species (Lowenborg *et al.* 2010). Climate is very important to the successful reproduction of *L. agilis*. The link between offspring viability and spring temperatures was demonstrated by Olsson *et al.* (2011b), who showed that higher temperatures in April and May increased the incidence of females mating with multiple males, resulting in increased sperm competition and healthier offspring (Olsson *et al.* 2011c). Cloudier weather has also been shown to result in smaller offspring in Swedish sand lizards (Olsson & Shine 1997a), which have a lower survival rate than larger ones (Olsson & Madsen 2001b). Even in a continental climate with comparatively more sunshine hours than northwest Europe, up to 90% of adult and 75% of juvenile active time is spent basking (Nemes 2003).

The climatic conditions required for successful egg incubation may be particularly important. The northern limit of the range of *L. agilis* appears to be limited by the minimum requirements for egg incubation (Rykena 1987) and cold, wet summers with low sunshine have coincided

with the failure of almost all sand lizard egg clutches in some years within an isolated Swedish population (Berglind 2000).

The hydrology and thermal properties of sand offer a possible explanation for the distribution of *L. agilis* within Britain. Female sand lizards exhibit careful selection of nest sites, often excavating several nest burrows which are abandoned before depositing a clutch in a burrow with suitable conditions. Nest sites are typically in unshaded areas of bare sand, within the nests sand humidity typically ranges between 15-18% and average temperature between 16.5 °C and 20 °C (Elbing 1993; Beebee & Griffiths 2000). Sand lizards in Britain are typically found in habitats with sandy soils characterised by low organic matter and large particle size (Chapman 1979; Webb 1986; Beebee & Griffiths 2000). Such soils have lower water retention than soils with higher organic matter content and smaller particles (Hollis *et al.* 1977). Sandy soils also typically have a greater thermal diffusivity (thermal conductivity relative to density and specific heat capacity) than clay soils (Abu-Hamdeh 2003) and therefore a clutch of eggs buried in a sandy soil would heat up more quickly and attain a higher temperature on a warm day than in a clay soil. Conversely they would also cool more quickly on a cold day or at night. The water balance of reptile eggs during incubation can have a significant impact on egg and hatchling viability (Packard 1991; Phillips & Packard 1994; Marco *et al.* 2005) and the rate of water exchange between reptile eggs and the nest medium is significantly influenced by temperature and the thermal conductivity of the medium (Ackerman 1994). Tracy (1980) demonstrated a significant relationship between egg mortality and the saturation of the medium in which they are incubated, in particular, eggs reared under wet conditions had a high incidence of mortality and a greater rate of infection by fungi, itself a cause of mortality.

The climate of north-west Europe, in particular Britain, is heavily influenced by the North Atlantic Oscillation (NAO) weather system which typically results in higher precipitation than central and eastern Europe (Wibig 1999). It is hypothesised that sandy soils provide suitable hydrological conditions for the incubation of sand lizard clutches with the precipitation levels of Britain, however given their high thermal diffusivity such soils can only reach, and more importantly maintain, suitable temperatures for incubation in areas with a high sunshine index. This offers an explanation of the current range of *L. agilis* in Britain, accounting for the apparent relationship between distribution and spring sunshine (Jackson 1978). In central European parts of the range with less precipitation, soil humidity is likely to fluctuate less and therefore *L. agilis* are not restricted to certain soil types for egg incubation, allowing the species to occupy a wider variety of habitats across much of its range (Gasc *et al.* 2004). This could also account for the pattern of range expansion during warmer, drier periods and

contraction into sandy areas under cooler, wetter conditions in Britain and Sweden since the colonisation of these countries.

The recent biogeography of *L. agilis* is reflected in other reptile species with a dependence on the climate for successful reproduction. For example the European pond terrapin *Emys orbicularis*, which is dependent on warm summer temperatures for egg incubation (Schneeweiß 2004), expanded from a Balkan glacial refuge as per the 'grasshopper' paradigm (Hewitt 1999) reaching both Britain and Scandinavia before climatic cooling caused its extinction in northern parts of its range (Joger *et al.* 2007; Sommer *et al.* 2009). Adult *E. orbicularis* can survive in Britain, however there are no records of successful breeding (Langton *et al.* 2011).

3.4.5 Provenance of the Aberffraw population

Based on the analyses undertaken, it seems highly unlikely that the Aberffraw population is of a natural origin. Anglesey became tidally separated from mainland Britain 8,800-8,400 years BP and the tidal causeway completely submerged between 5,800 and 4,600 years BP (Roberts *et al.* 2011). Given the estimates of divergence time between the Aberffraw and Ainsdale populations (37.5 – 1,500 years BP), for it to be a natural population, Aberffraw must have been colonised at several thousand years subsequent to this. It is not inconceivable that *L. agilis* reached Anglesey by rafting, for example several species of Lacertidae are thought to have crossed the Strait of Gibraltar between Iberia and North Africa in both directions (Harris *et al.* 2004; Carranza *et al.* 2006; Paulo *et al.* 2008). However, levels of differentiation between Aberffraw and Ainsdale were low (F_{ST} = 0.1354, G'_{ST} = 0.2328, D = 0.0540) when compared with other pairwise comparisons between sample sites, for example similar levels of differentiation were observed between sample sites within the unfragmented landscape of Wareham Forest (as estimated by G'_{ST} and D), which both STRUCTURE and BAPS assigned to the same population. Given the low genetic diversity and small effective population size of both populations, and the relatively large geographic distance between Aberffraw and Ainsdale, if these populations had been separated for any length of time it would be expected that high levels of genetic drift would result in high levels of differentiation given the geographic distance and unsuitable habitat (including sea water) between the two sites. Low levels of differentiation combined with the failure of STRUCTURE to separate Aberffraw from Ainsdale and the grouping of Aberffraw and Ainsdale with 100% support in both the Neighbour-joining and UPGMA trees implies high similarity between the populations. This supports the hypothesis of a recent unnatural origin for the Aberffraw population, most likely an introduction of animals originating from Merseyside. This is further supported by the results of the IMa2 analysis which

implies that Aberffraw and Ainsdale are in effect the same population. However, the Aberffraw lizards were found to possess some alleles which were not found in the Ainsdale population (but were found on Dorset populations). This may be due to them originating from a Merseyside population other than Ainsdale where these alleles are present, or from captive stock where one or more of their ancestors were lizards from outside of Merseyside.

The population at Aberffraw is within the theoretical historical range of *L. agilis* in Britain, as indicated by habitat type and climate (Jackson 1978; Corbett 1994) and other anthropogenic populations, such as a population introduced to the Outer Hebridean island of Coll, have persisted despite being much further north than the natural range (Beebee & Griffiths 2000). However, despite suitable climate and habitat conditions, small population size and low genetic variation of the population mean that it is at high risk of genetic stochasticity, which may have an influence on its long-term persistence.

3.4.6 Conclusion

Although many edge populations exhibit low levels of genetic diversity, this appears not to be the case in British sand lizards over much of their range. Despite considerable recent habitat loss and fragmentation, populations in Dorset compare favourably in terms of genetic diversity to populations from mainland Europe, and are more diverse than other edge populations in Sweden, as well as being higher than in other species with similar distribution patterns in Britain. The smaller populations of Surrey and Merseyside are notably less diverse than the Dorset populations, a pattern which is closer to expectations of small, isolated edge populations.

Habitat fragmentation appears to be having some effect on sand lizard populations by increasing genetic differentiation between them, although this may not be significant when compared with natural historic barriers (this topic is considered in detail in Chapter 4).

The current disjunct range of *L. agilis* in Britain is a result of its colonisation history and subsequent climatic changes which have caused a restriction in range, possibly as a result of the conditions required for successful egg incubation. When considered in conjunction with landscape genetics data, the pattern of range expansion during warm periods and contraction during cooler ones may enable predictions to be made about how sand lizards will react in the face of climate change and this is discussed in Chapter 6.

4 LANDSCAPE GENETICS OF SAND LIZARDS IN DORSET

4.1 Introduction

Understanding the processes that influence patterns of genetic diversity is a crucial aim in conservation biology and vital to the effective conservation of species, particularly in the face of anthropogenic threats such as global climate change (Thomas *et al.* 2004; Stork 2010), and habitat loss and fragmentation (Fahrig 2001; Brooks *et al.* 2002; Fahrig 2002, 2003). Patterns of genetic diversity within species are influenced by colonisation history (Hewitt 1999; Beebee & Rowe 2000; Hewitt 2000; Bernatchez 2001), landscape configuration (Hutchison & Templeton 1999; Storfer *et al.* 2007) and environmental gradients (Jorgensen *et al.* 2005; Schwartz *et al.* 2009).

Anthropogenic habitat loss and fragmentation have been cited as the most significant drivers of biodiversity loss (Fahrig 1997; Hanski & Ovaskainen 2000; Fahrig 2003; Stuart *et al.* 2004). In addition to the direct impacts of the physical removal of habitat, habitat loss and fragmentation render the remaining small populations more vulnerable to destructive stochastic events (Shaffer 1981; Caughley 1994). Small fragmented populations are more likely to be affected at a molecular level due to disruption to genetic process such as gene flow, resulting loss of diversity, the accumulation of harmful genetic mutations, inbreeding depression and reduced fitness (Saccheri *et al.* 1998; Higgins & Lynch 2001; Keller & Waller 2002; Reed & Frankham 2003).

Assessing the effect of habitat fragmentation on genetic diversity poses a challenge for conservation biologists. Although it is relatively easy to quantify levels of genetic diversity (Frankham 1995, 1996), the degree of inbreeding within populations and genetic differentiation between populations (Wright 1931; Nei 1972), it is more difficult to quantify the underlying causes of the observed patterns. Landscape genetics (Manel *et al.* 2003; Storfer *et al.* 2007; Holderegger & Wagner 2008) uses a combination of genetic and spatial data to investigate the effect of landscape configuration on processes of gene flow and genetic drift, and provides an approach to explaining patterns of genetic diversity. Landscape genetics approaches can be used to assess levels of gene flow across different habitat types (Wang *et al.* 2009; Murphy *et al.* 2010b), identify natural and anthropogenic barriers (Arens *et al.* 2007; Epps *et al.* 2007; Millions & Swanson 2007; Moore *et al.* 2011) and corridors for gene flow (Vignieri 2005; Clark *et al.* 2008; Perez-Espona *et al.* 2008; Zhu *et al.* 2010), investigate the effect of environmental conditions (Schwartz *et al.* 2009; Murphy *et al.* 2010b) and disentangle

the relative effect of historical versus contemporary gene flow (Moore *et al.* 2008; Zellmer & Knowles 2009; Apodaca *et al.* 2012).

Different aspects of a landscape can influence genetic diversity at different spatial scales (Galpern *et al.* 2012; Ortego *et al.* 2012). For example, habitat cover was the most important factor affecting gene flow at a local scale in the western toad *Bufo boreas,* whilst environmental gradients were more important at larger scales (Murphy *et al.* 2010b). In the European tree frog *Hyla arborea*, large rivers were significant barriers to gene flow at a large scale whilst roads and forests were more important locally (Angelone *et al.* 2011). Failure to address issues of scale in landscape genetic studies may result in incorrect conclusions (Cushman & Landguth 2010a; Wasserman *et al.* 2010).

Many landscape genetics studies utilise a resistance surface (Adriaensen *et al.* 2003; Zeller *et al.* 2012) to quantify the 'effective' or 'ecological' distance between populations or individuals. A resistance surface typically uses a Geographical Information System (GIS) approach to construct a representation of the landscape divided into pixels, each of which is assigned a resistance value which relates to the relative difficulty for an organism (and therefore genetic information) to pass through it. The resistance surface can then be used to calculate a distance metric which gives a more meaningful estimate of the effective distance between populations than Euclidean distance. The two most commonly used distance metrics are Least Cost Path (LCP) and Isolation by Resistance (IBR). An LCP is defined as the path between two populations which has the lowest cumulative cost (i.e. resistance) along its entire length (Adriaensen *et al.* 2003). Whereas LCP defines a single pathway between populations, IBR assesses gene flow between populations across an entire landscape. IBR is based on electrical circuit theory where the resistance of landscape features to gene flow is analogous to electrical resistance (McRae 2006; McRae *et al.* 2008). Pairwise geographic distance metrics between populations can be compared to genetic differentiation or distance metrics in order to investigate hypotheses about the influence of various landscape variables on gene flow.

The number of published landscape genetics studies has rapidly increased over the past ten years (Storfer *et al.* 2010) as conservation biologists have sought to gain greater insight into genetic processes at a landscape level. A great many of these studies have focused on mammals, which account for 86% of published works using a resistance surface between 2000 and 2011. Amphibians and birds have also been popular subjects (18% and 17% respectively), whilst reptiles account for only 8% (Zeller *et al.* 2012). Reptiles, and lizards in particular, are potentially interesting subjects for landscape genetics studies as they typically exhibit low

vagility and small body size which may render them more vulnerable to the effects of habitat fragmentation (Gibbons *et al.* 2000; Jenkins *et al.* 2007).

The sand lizard *Lacerta agilis* is a widely distributed reptile which reaches the edge of its range in Great Britain where its specific habitat requirements have left it restricted to a few small areas of the country (Beebee & Griffiths 2000). The largest *L. agilis* populations in Britain are found in lowland heathland habitat in Dorset, which has suffered extensive loss and fragmentation in the past 200 years and currently covers less than 15% of its original extent (Moore 1962; Rose *et al.* 2000; Hooftman & Bullock 2012). Habitat loss and fragmentation have been identified as the primary cause of the significant decline of *L. agilis* (Corbett 1969, 1988b), and the species is now a conservation priority in the United Kingdom (Corbett & Tamarind 1979; Corbett 1988a; Moulton & Corbett 1999; Herpetological Conservation Trust 2009).

The genetic effects of habitat fragmentation have been investigated in Swedish *L. agilis* where small fragmented populations show low diversity and a high level structuring (Gullberg *et al.* 1998, 1999; Madsen *et al.* 2000) resulting in inbreeding and a loss in fitness of offspring (Olsson *et al.* 1996b). Similarly a closely related species, the green lizard *Lacerta viridis* in Germany, shows high levels of genetic differentiation between isolated populations within a relatively limited geographical range (Bohme *et al.* 2007). This was attributed to genetic drift arising due to habitat fragmentation and isolation in small populations. Chapter 3 of this thesis investigated the population genetics of *L. agilis* across Great Britain. It showed that whilst there is a significant degree of genetic structuring between populations, even over short geographic distances, levels of genetic diversity within populations remain relatively high when compared to populations from Sweden and are similar to larger populations in Europe.

This chapter investigated the effect of landscape configuration on patterns of genetic diversity in *L. agilis* across Dorset at different geographic scales, and specifically whether habitat type and potential barriers to gene flow between populations were a better predictor of patterns of genetic diversity than a null (Isolation by Distance) hypothesis. A resistance surface was created encompassing the entire Dorset range of the species and used to assess the relative effects of habitat cover and potential natural and artificial barriers on gene flow. A number of different parameterisation models were compared to enable the selection of the resistance surface which explained the greatest proportion of the pattern of genetic diversity across the study area and within smaller areas of fragmented and unfragmented landscape. This was then used to identify significant barriers to gene flow and the relative importance of landscape

features to facilitate dispersal. The parameterisation of the resistance surface also allowed inferences about the relative importance of historical and recent gene flow to be made. The relative ability of different genetic distance and differentiation markers to detect recent landscape change was also investigated.

4.2 Materials and Methods

4.2.1 Introduction

General materials and methods relating to the field sampling, sampling strategy, and the collection and initial analysis of DNA samples is provided in Chapter 2. The materials and methods presented here are specific to the analysis conducted in this chapter, particularly the use of a resistance surface to investigate the landscape genetics of *L. agilis* in Dorset.

4.2.2 Genetic Distance Metrics

The majority of landscape genetics studies have used traditional genetic differentiation and distance metrics from population genetics such as F_{ST} (and its analogs) to assess the effect of the landscape on genetic processes (Storfer *et al.* 2010). However, measures of structure such as F_{ST} and distance measures such as *D* and their analogs assume equilibrium when assessing gene flow and therefore, in anthropogenically fragmented landscapes, such assumptions may not hold true. Consequently, Storfer *et al.* (2010) recommended the use of D_{PS} (Bowcock *et al.* 1994) which is based on the proportion of shared alleles between populations and avoids assumptions of equilibrium. D_{PS} was found to be more effective at detecting recent landscape changes than F_{ST} (Murphy *et al.* 2010b). Therefore, in addition to F_{ST} (Weir & Cockerham 1984), G'_{ST} (Hedrick 2005) and *D* (Jost 2008) used elsewhere in this thesis, pairwise D_{PS} was calculated for each pair of populations using MICROSATELLITE ANALYSER (Dieringer & Schlotterer 2003). Populations were defined by their sampling location.

4.2.3 Parameterisation of the Resistance Surface

Resistance surfaces have been parameterised using a number of variables relevant to the ecology of the study species such as habitat type (Wang *et al.* 2009; Angelone *et al.* 2011), topography (Spear *et al.* 2005; Murphy *et al.* 2010b) or environmental conditions (Jorgensen *et al.* 2005; Schwartz *et al.* 2009). However, the geographic area considered within this study is relatively small compared to many other studies and all the sample sites were contained within a 20 km by 30 km area with little variation in elevation and climate. The habitat requirements of *L. agilis* in north-west Europe have been studied in depth and it is well

documented that the species is highly associated with lowland heathland and sand dune habitats, whilst rarely being found in other habitat types (Spellerberg 1975; Corbett & Tamarind 1979; House & Spellerberg 1983; Dent & Spellerberg 1987; Nicholson & Spellerberg 1989; Spellerberg 1989; Moulton & Corbett 1999; Berglind 2000; Wouters *et al.* 2012). Habitat type is also known to be an important factor in the dispersal behaviour of *L. vivipara* (Zajitschek *et al.* 2012). Therefore it was considered that the primary determinant of sand lizard distribution and dispersal in the study area was likely to be habitat type.

The resistance surface used in this study was based on the Land Cover Map 2007 (LCM2007) (Morton *et al.* 2011), which maps land cover across the United Kingdom at a 25 m resolution based on satellite imagery combined with digital cartography (Figure 4.1). LCM2007 categorises land cover into 23 different habitat types using spectral remote sensing and has a reported accuracy of 83% (Morton *et al.* 2011). As the resolution of LCM2007 is 25 m, any features < 0.5 ha in area or linear features < 20 m in width, including most rivers and all roads within the study area, are typically not recorded on the map. As linear features such as roads and rivers may be significant barriers to gene flow, Ordnance Survey Meridian™2 data (Ordnance Survey, Southampton, UK) containing major and minor roads and rivers were incorporated into the resistance surface (Figure 4.1). Both LCM2007 and Meridian™2 data were supplied in a vector format which was converted to a raster layer with a 25 m x 25 m pixel size using ARCGIS v10 (ESRI, Redlands, California).

The Amphibian and Reptile Conservation Trust (ARC) maintains a database of reptile and amphibian records which, as of September 2011, contained 15,906 records of *L. agilis* in Dorset recorded post 1990. The records were from a number of sources including presence-absence surveys, population monitoring and casual observations. Records with duplicate co-ordinates were removed from the dataset and the remaining 14,553 records were plotted in ARCGIS (Figure 4.1). In order to define the study area, a minimum convex polygon was created which encompassed all the ARC database records (with the exception of two outliers) to which a 1 km buffer was added. Where the study area met the sea, the boundary was defined by excluding sub-tidal marine habitats. The number of *L. agilis* records within each LCM2007 habitat type was recorded and the proportion of pixels of each habitat type within the study area containing a record was calculated (Figure 4.2).

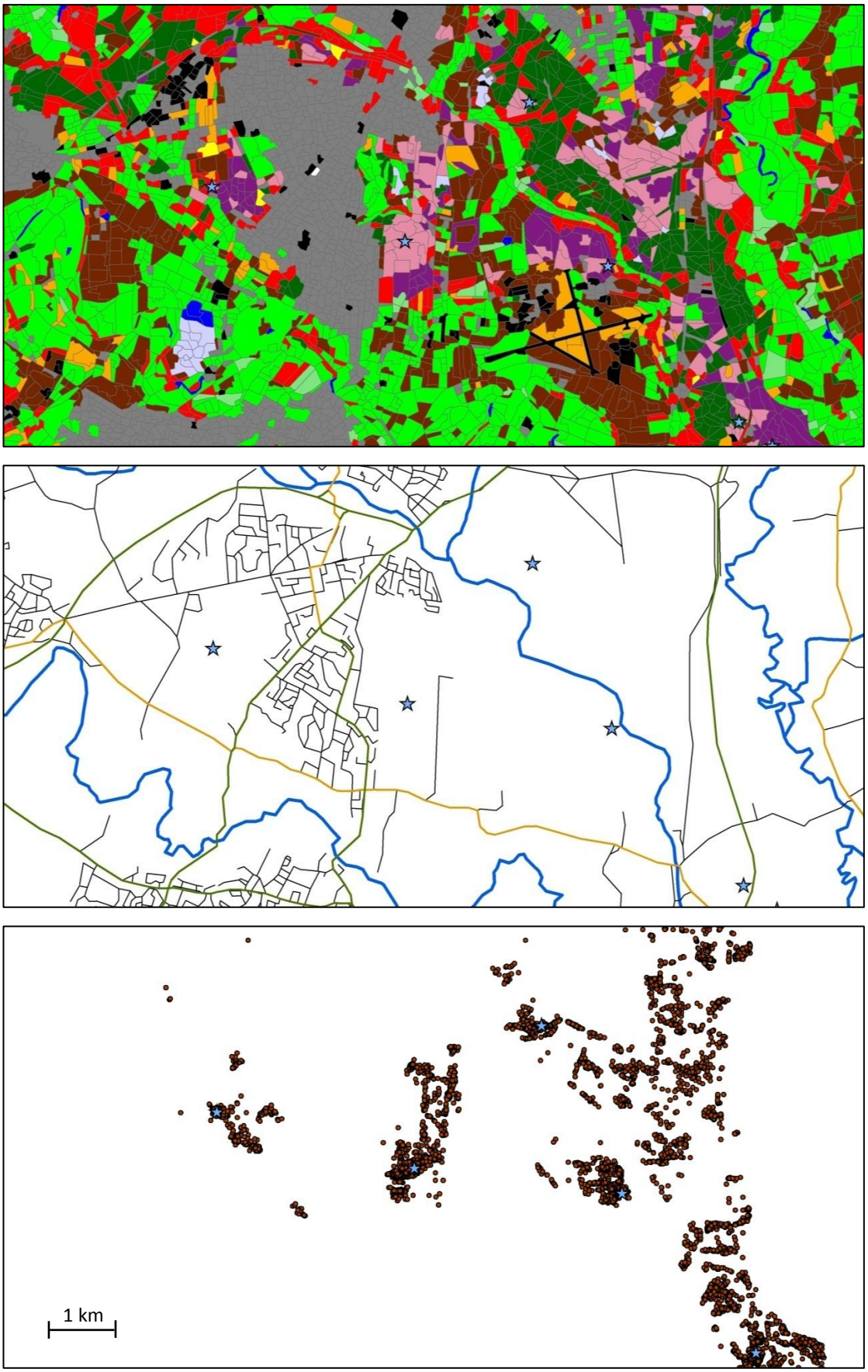

Figure 4.1. Data components of the resistance surface. Detail of the study area from East Dorset, top: LCM2007 habitat data; middle: OS Meridian™2 data; bottom: ARC database *L. agilis* records. Blue stars are sample sites.

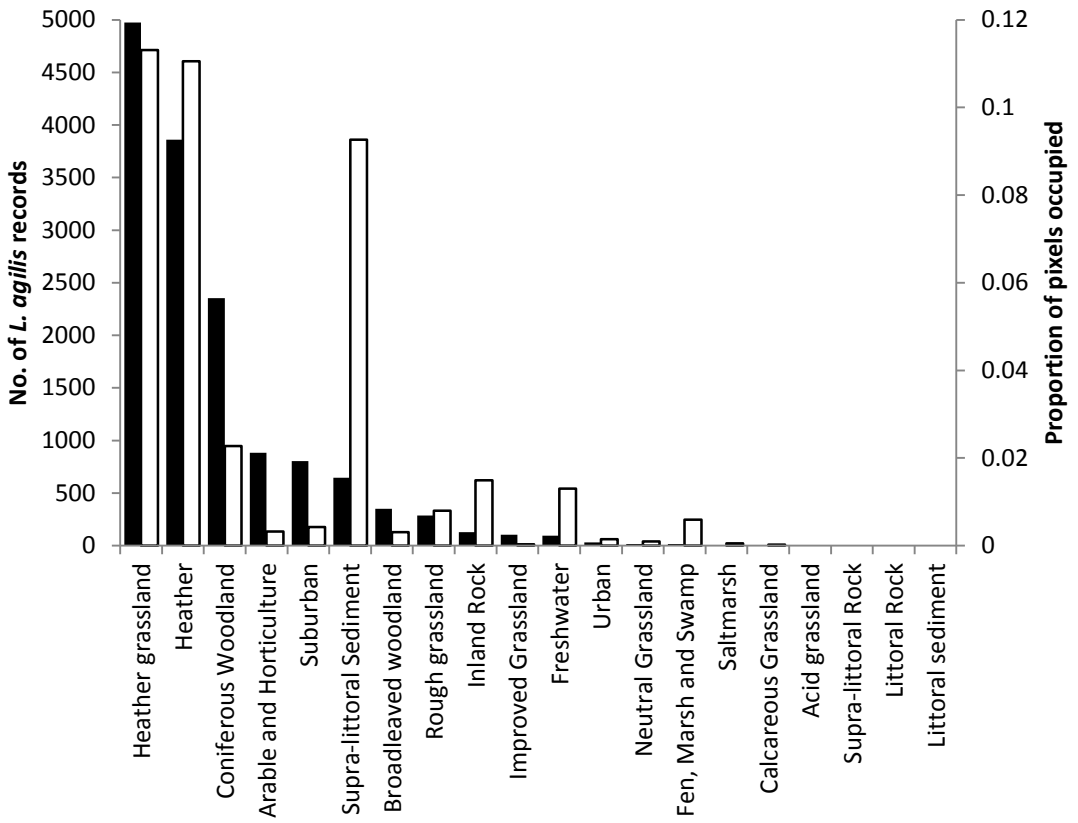

Figure 4.2. Number of *Lacerta agilis* records in each LCM2007 habitat type (filled bars) found within the study area and the proportion of pixels of each habitat type within the study area containing an *L. agilis* record (open bars).

The resistance surface was parameterised on the basis of the number of *L. agilis* records occurring within each habitat type. However, there are a number of potential sources of inaccuracy in the data which need to be considered:

1. *Absence data not recorded*. Therefore, absence of records in a particular habitat type does not necessarily mean that the habitat is not used by *L. agilis*.
2. *Spatial resolution of the data varies from 10 m to 100 m*: The spatial location of each record is stored in British National Grid format. Where data were recorded at a 100 m resolution, the co-ordinates for the bottom left corner of the 100 m x 100 m square were stored. As the resolution of the habitat data was 25 m, this could result in the record appearing in a different habitat type. For example, there were 94 *L. agilis* records in the "freshwater" habitat type which was primarily due to the presence of a lake in the bottom left corner of a 100 m x 100 m square which otherwise contained mostly heathland habitat types.

3. *Recording biased to areas close to human habitation*: The proportion of records from urban and suburban areas was likely to be artificially high. *Lacerta agilis* occurs within suitable habitats in some urban areas of Dorset and due to the high levels of human activity in such areas, these animals have a greater likelihood of being observed and recorded than those on more rural sites.
4. *Recording biased to known sites*: Much of the data were collected during routine monitoring of known *L. agilis* sites. This may result in a disproportionate number of records from the habitat types found on these sites.
5. *The proportion of each habitat type was not equal or evenly distributed*: Therefore records were more likely to occur in common habitat types regardless of *L. agilis* habitat preferences.

In order to address these concerns, three different parameterisation models were used:

1. *Total Occupancy (TO)*: Resistance values for each habitat type were assigned in direct proportion to the total number of *L. agilis* records within that habitat type (Figure 4.2). This assigned relatively low resistance to habitat types with lots of records and high resistance to habitat types with few records and therefore may result in the overestimation of resistance in habitat types with few records. This is likely to be an accurate method for parameterising the resistance surface if *L. agilis* is as dependent on heathland and sand dune habitats as believed.
2. *Rank Occupancy (RO)*: Resistance values for each habitat type were assigned on the basis of the order of habitat types ranked by the number of records within each type. The habitat type with the highest number of records was assigned a resistance value, $R = 1$, the habitat type with the second highest number of records was assigned a resistance value $R = 2$ and so on. This removed the effect of oversampling in certain habitat types; however it may underestimate resistance in habitat types with few records. This is likely to be an accurate method for parameterising the resistance surface if *L. agilis* is able to easily disperse through non-heathland habitat types.
3. *Proportional Occupancy (PO)*: Resistance values were assigned to each habitat type on the basis of the proportion of pixels of each habitat type containing a record of *L. agilis* (Figure 4.2) relative to the total number of pixels of that habitat type in the entire resistance surface. This was most likely to reflect true resistance values, for example; using the TO and RO parameterisation models “supra-littoral sediment”, the LCM2007 habitat type which includes sand dunes, had the sixth lowest resistance, whereas using

PO it had the third lowest resistance and a comparable occupancy rate to heathland habitats.

In each parameterisation model the resistance value of areas beyond the study area, including seawater, was set to "NoData" which means it formed an edge to the resistance surface beyond which LCP and IBR analysis could not proceed. The resistance value (*R*) for each pixel was determined by its relative difference from the least resistant habitat for which *R* = 1. In order to control for recording bias and low resolution records, all marine and aquatic habitats ("freshwater", fen, marsh and swamp", "saltmarsh", "littoral rock" and "littoral sediment") and the "urban and "suburban" habitat types were set to a high resistance value regardless of the number of records within that habitat type.

Table 4.1. Resistance values assigned to each LCM2007 Habitat type in each occupancy model. TO = Total Occupancy, RO = Rank Occupancy, PO = Proportional Occupancy.

LCM2007 Habitat type	**No. of pixels**	**No. of *L. agilis* records**	**Resistance (*R*)**		
			TO	**RO**	**PO**
Heather grassland	43,976	4,975	1	1	1
Heather	34,921	3,860	1,116	2	27
Coniferous woodland	103,371	2,352	2,624	3	904
Arable and horticulture	276,509	882	4,094	4	1,100
Suburban	189,397	803	4,173	12	1,132
Supra-littoral sediment	6,963	645	4,331	5	206
Broadleaved woodland	115,291	352	4,624	6	1,101
Rough grassland	36,152	288	4,688	7	1,052
Inland rock	8,650	129	4,847	8	983
Improved grassland	314,794	104	4,872	9	1,129
Freshwater	7,219	94	4,976	12	1,132
Urban	19,993	30	4,976	12	1,132
Neutral grassland	16,522	16	4,960	10	1,122
Fen, marsh and swamp	2,537	15	4,976	12	1,073
Saltmarsh	9,898	5	4,976	12	1,132
Calcareous grassland	3,856	1	4,975	11	1,129
Acid grassland	417	0	4,976	12	1,132
Supra-littoral rock	37	0	4,976	12	1,132
Littoral rock	33	0	4,976	12	1,132
Littoral sediment	782	0	4,976	12	1,132
Total	*1,191,318*	*14,551*			

Euclidean geographic distance, LCP and IBR distance metrics were then calculated between each pair of sample sites on each resistance surface. Geographic distances and LCP effective distances were calculated using a specifically designed Python-based ARCGIS toolbox (Etherington 2011). The total accumulated cost of the path was used as the distance metric. The distance metric for IBR was calculated using CIRCUITSCAPE v3.5.8 (McRae 2006) in which habitat data were specified as resistance, a four-neighbour cell joining scheme was used and the cell connection calculation was set to average resistance. If measured using a straight line, some of the geographic distances would cross areas of seawater in Poole Harbour. Therefore an equivalent Euclidean distance was calculated between each pair of populations by setting all features of the resistance surface to a resistance value of 1 and calculating an LCP which avoided seawater and other areas of the resistance surface set to "NoData".

Direct comparisons between genetic distance and effective distance in landscape genetics studies can result in spurious correlations as they do not account for any IBD effect or the effect of other landscape variables (Cushman & Landguth 2010b; Cushman *et al.* 2012). The partial Mantel test (Mantel 1967; Smouse *et al.* 1986) allows comparisons to be made between genetic data and a predictor landscape variable whilst 'partialling out' the effect of other variables. In a partial Mantel test the effect of one pair-wise matrix is controlled for before assessing the correlation between the remaining matrices by permuting the rows and columns of the residuals in a second Mantel test. The reliability of Mantel and partial Mantel tests in landscape genetics has been questioned by Balkenhol *et al.* (2009), who found that they have a higher type-I error rate than other statistical methods. However, Cushman & Landguth (2010b) found partial Mantel tests to be accurate when assessing landscape resistance hypotheses and suggested that type-I errors may be due to the confounding effect of different landscape variables. Legendre & Fortin (2010) also found Mantel tests accurate when testing hypotheses relating to distance (which is the case in this thesis) and Mantel and partial Mantel tests remain the most commonly used statistic in landscape genetics studies (Storfer *et al.* 2010). In order to address some of the concerns over partial Mantel tests, Legendre *et al.* (1994) proposed an approach where the original matrices are permuted prior to the regression, and this has also been used in landscape genetics (Holzhauer *et al.* 2006; Balkenhol *et al.* 2009).

To test the significance of the relationship between habitat type and distance, Mantel tests were undertaken between pairwise matrices of genetic distance metrics and geographic distance metrics. Partial Mantel tests were undertaken to 'partial out' any IBD effect. All Mantel and partial Mantel tests to compare the landscape and genetic data were conducted in the program PASSaGE v2 (Rosenberg & Anderson 2011). This program allows a significance

testing approach as per Legendre *et al.* (1994), where one of the original matrices is permuted prior to the regression (or multiple regression), the regression for that matrix is repeated and then the partial Mantel correlation determined. This leads to more accurate estimates of the significance of partial Mantel statistics (Legendre 2000) and addresses concerns over the use of this method in landscape genetics. The significance of each Mantel test undertaken was assessed using a permutation test with 9,999 permutations.

4.2.4 Parameterisation of High-Cost Features

The study area contained several features likely to act as significant barriers to lizard dispersal which could not be parameterised using the models outlined above. These fell into two categories: artificial barriers including high-resistance "urban" and "suburban" habitats and linear barriers such roads and natural barriers, particularly rivers. The number of *L. agilis* records falling within the "urban" and "suburban" habitat types was relatively high; however this was likely to be a result of recording effort in this habitat type rather than actual use by *L. agilis* relative to other habitat types. Urbanisation has been shown to have a particularly strong fragmentation effect in lizards (Delaney *et al.* 2010; Hamer & McDonnell 2010).

No specific data were available regarding the ability of *L. agilis* to cross roads; however they have been observed crossing open spaces such as forestry tracks (pers. obs.). The ocellated lizard

Lacerta (*Timon*) *lepidus* is closely related to *L. agilis* (Arnold *et al.* 2007) although significantly larger. A motorway passing through its habitat in Spain showed no evidence of being a significant barrier, whereas the opposite effect was observed in the smaller-bodied sympatric lacertid *Psammodromus algirus* (Telleria *et al.* 2011). Meek (2009) investigated the relationship between traffic volume and reptile roadkill for a number of species, including *Lacerta bilineata*, in western France. Roadkill numbers generally increased with traffic volume with the exception of a very high-volume road, which had lower than expected roadkill (no observations of *L. bilineata*), attributed to a lack of adjacent suitable habitat. Two of the sample sites within this study (Town Common and Ramsdown) were previously part of the same extensive area of heathland but were separated by a high-volume four-lane road in the late 1970s. In Chapter 3 of this thesis, two different Bayesian assignment methods, STRUCTURE (Pritchard *et al.* 2000) and BAPS (Corander *et al.* 2008), were used to identify true populations of *L. agilis* in the study area; both methods assigned individuals from Town Common and Ramsdown to the same population despite significant genetic distance (F_{ST} = 0.0184, *D* =

0.0318, G'_{ST} = 0.0898). However, this may be a result of a genetic time-lag (Richmond *et al.* 2009; Landguth *et al.* 2010) rather than contemporary migration across the road.

Rivers are known to act as a barrier to gene flow in a number of lizard species including *L. agilis* (Bahl *et al.* 1997). However, *L. agilis* is known to swim as an escape behaviour (Blanke 2004) and to cross small expanses of water (Gollmann & Gollmann 2008) and therefore the effectiveness of a river as a barrier is likely to depend on its size. In Chapter 3 of this thesis, the population structure of *L. agilis* from a number of sites across Dorset was investigated, many of which were separated by rivers. The majority of sample sites were identified as separate populations by both STRUCTURE and BAPS where a river occurred between them. However, this did not attempt to separate the isolation effect of the rivers from isolation effects due to IBD or other intervening unsuitable habitat types.

Koen *et al.* (2012) suggested that the parameterisation of low quality habitat features could be optimised by creating several surfaces in which high quality features (i.e. areas of low resistance) are held at a constant low resistance value whilst the resistance value of low quality features is increased. When LCP and IBR distance metrics are calculated this produces a pattern of linear increase in effective distance which can be compared with genetic distance to select the most accurate values.

Two sets of resistance surfaces were parameterised to assess the effect of high resistance features within the surface, one to test the effect of artificial barriers such as roads, suburban and urban habitat, and one to test the effect of natural barriers such as rivers and aquatic and marine habitats. The Meridian™2 dataset includes three categories each for rivers (“large”, “medium” and “small”) and roads (“A roads”, “B roads” and “minor roads”). A resistance surface was parameterised with the resistance value of all habitat types set to 1, and the resistance value of “small” rivers (*R*) = 2, “medium” rivers = 2*R* and “large” rivers = 4*R*. This process was repeated with *R* = 4, *R* = 8, *R* = 16 and so on until *R* = 1024. A similar process was undertaken for the anthropogenic features where initially all habitats were set to a resistance value of 1, the resistance value of the “suburban” habitat (*R*) was set to 2, “urban” habitat and “minor roads” = 2*R*, “B roads” = 4*R* and “A roads” = 8*R*. The resistance values of any roads could not be set lower than the value for the suburban or urban habitat types as this would result in the roads acting as corridors through higher resistance areas.

LCP and IBR distance metrics were calculated for each resistance surface and compared to genetic distance metrics using Mantel tests with a permutation test to assess the significance of the relationship. The optimum value for each barrier type was selected on the basis of the

Mantel correlation coefficient (*r*). As the resistance value of the habitats between the barriers was set to 1 these resistance surfaces effectively incorporate a distance effect and therefore it was not necessary to separately partial out IBD effects.

4.2.5 Creating and Selecting the Optimum Resistance Surface

The optimum resistance surface was selected using a series of multiple regression models based on the three different habitat occupancy models in combination with natural and artificial barriers. The optimal model was selected using Akaike's Information Criterion (AIC) (Burnham & Anderson 2002) which selects a model on the basis of goodness of fit with a penalty for high numbers of parameters, and is calculated by the formula:

$$AIC = -2\ln(likelihood) + 2K$$

where K is the number of parameters in the model. Models were compared using ΔAIC, which is calculated by subtracting the AIC score of the model with the lowest score from the AIC score of the model being tested. The significance of the individual parameters within each model supported by the AIC analysis was investigated using partial Mantel tests with a permutation test to assess the significance. This process was repeated at a local scale in fragmented (East Dorset) and unfragmented (Wareham Forest) landscape scenarios, however as the number of samples for these analyses was relatively low ($n/K < 40$) the second order AIC_C value (Burnham & Anderson 2002) was used, calculated as:

$$AIC_C = -2\ln(likelihood) + 2*K + (2*K*(K+1))/(n-K-1)$$

where K is the number of parameters and n is the number of data points, was used to select the best fitting model. All multiple regressions and AIC scores were calculated in MYSTAT v12 (Systat Software, Chicago, USA).

Once the optimum resistance surface model had been selected, a new resistance surface based on this model and containing all significant features was created. Resistance for the barriers was recalibrated so that R was relative to the average resistance of all habitat pixels across the study area. Current maps were created in CIRCUITSCAPE to visualise gene flow across the landscape. These can be used identify areas or features of the landscape which are particularly important for gene flow (McRae *et al.* 2008). LCPs were also calculated as these may indicate potential dispersal corridors between sites.

4.2.6 Bayesian Analysis of Migration

As the statistical assumptions of Mantel tests have been questioned, a second additional approach to relating genetic and geographic distances was used. Gene flow between populations was inferred using the program BIMr (Faubet *et al.* 2007; Faubet & Gaggiotti 2008) which uses a Markov chain Monte Carlo (MCMC) method to identify the environmental factors (in this case geographic or effective distance related to specific landscape features) most likely to explain observed patterns in genetic diversity using a generalised linear model estimated by a Bayesian method. In a comparison of methods for analysing simulated landscape genetics data, BIMr performed well with a good balance between type-I error rate and power to detect significant landscape features (Balkenhol *et al.* 2009). The program uses F_{ST} values and therefore the LCP and IBR distance metrics for natural and artificial barriers optimised for F_{ST} were used in the analysis. Separate analyses were performed for each habitat occupancy model using LCP and IBR distance metrics. BIMr was run in its default settings (Balkenhol *et al.* 2009) with a burn-in of 20,000 steps, MCMC chain length of 1,000,000 steps and a thinning interval of 50. Ten replicate runs were performed for each analysis and the mean posterior likelihood estimate was used to select the optimum model. The migration rates inferred by BIMr at the regional Dorset level were very low and consequently the environmental models had little support. The BIMr method can be less effective when the number of populations is relatively high (Faubet & Gaggiotti 2008) and therefore the analysis was abandoned at the regional level and undertaken at the local level within East Dorset, using the same method as above. In Chapter 3 of this thesis, 23 potential migrants were identified in this area, compared to two at the regional level at the 50% threshold using STRUCTURE. Separate analyses were carried out for each habitat occupancy model and geographic distance metric. The environmental factors included in each analysis were Euclidean geographic distance (accounting for the avoidance of marine habitats), habitat type, natural barriers and artificial barriers.

4.3 Results

4.3.1 Isolation by Distance

Mantel tests showed that the unmodified genetic distance was significantly associated with geographic distance at the regional scale (within Dorset); however there was not a similar significant relationship at the local scale in either fragmented or unfragmented landscapes using any genetic distance metric. Of the significant results, D_{PS} showed the best fit of the

three genetic distance metrics and F_{ST} the least at the regional scale (Table 4.2). Rousset (1997) recommended that a correlation of $F_{ST}/(1-F_{ST})$ and log distance better explains IBD effects in habitats other than narrow corridors than direct correlations of F_{ST} and distance and this was calculated to investigate IBD. Where a significant IBD relationship was shown, correlating genetic distance/(1-genetic distance) with log geographic distance explained more variation for all genetic distance measures. At the local level there were significant correlations between genetic distance/(1-genetic distance) and log geographic distance using D and D_{PS} in East Dorset.

Table 4.2. Isolation by Distance results at a regional scale across Dorset and at a local scale in an unfragmented (Wareham Forest) and fragmented (East Dorset) landscape. *P* values shown are for a Mantel test, significance levels have been adjusted for multiple test using a False Discovery Rate (FDR) procedure (Narum 2006) and are shown at the nominal 0.05% level (*P* = 0.009 for Dorset and *P* = 0.015 for Wareham Forest and East Dorset).

Scale		FDR α	Genetic distance x geographic distance		Genetic distance/(1-genetic distance) x log geographic distance	
			r	***P***	***r***	***P***
Dorset	F_{ST}	0.009	0.4417	<0.0001	0.6277	<0.0001
	G'_{ST}	0.009	0.5429	<0.0001	0.6646	<0.0001
	D	0.009	0.5468	<0.0001	0.6912	<0.0001
	D_{PS}	0.009	0.5769	<0.0001	0.7072	<0.0001
Wareham Forest	F_{ST}	0.015	0.5149	0.0277	0.4383	0.0640
	G'_{ST}	0.015	0.3974	0.1099	0.3369	0.1843
	D	0.015	0.3662	0.1324	0.3074	0.2135
	D_{PS}	0.015	0.4104	0.0916	0.3397	0.1592
East Dorset	F_{ST}	0.015	0.6196	0.0241	0.5971	0.0168
	G'_{ST}	0.015	0.5933	0.0277	0.5676	0.0144
	D	0.015	0.5986	0.0190	0.5613	0.0286
	D_{PS}	0.015	0.5971	0.0168	0.6393	0.0117

4.3.2 Habitat Occupancy Models

Using LCP distances, all habitat occupancy models gave an improved correlation with genetic distance over a null (IBD) model. In contrast with expectations, the RO model gave the highest Mantel's *r* value, and PO the lowest (Table 4.3). However, none of the occupancy models gave a significant correlation (using an adjusted *P* value of 0.009 to denote significance at the 5% level after a False Discovery Rate (FDR) procedure (Narum 2006) for multiple comparisons) once distance had been accounted for in a partial Mantel test. The IBR distance metric gave a higher correlation than IBD for all habitat occupancy models, with the TO model having the highest *r* value and PO the lowest (Table 4.3). IBR models always produced greater correlations and higher significance than their equivalent LCP model. Within the LCP models, D_{PS} was the

most sensitive genetic distance metric for all IBD and all habitat occupancy models, however, when the effect of distance was partialled out, F_{ST} offered an improved fit. F_{ST} was the best genetic distance metric for all IBR models with the exception of IBR_PO, where D_{PS} performed better. F_{ST} generally produced more significant results than G'_{ST} or D except in IBD models and the LCP_TO model (Table 4.3).

Table 4.3. Results of Mantel and partial Mantel tests between genetic and geographic distances for each habitat occupancy model. Dist = distance, TO = Total Occupancy, RO = Rank Occupancy, PO = Proportional Occupancy. TO|IBD indicates a partial Mantel test with IBD partialled out of a TO model. Significance was assessed after an FDR procedure where the nominal 5% significance level of P = 0.009. The best correlation for each occupancy model is shown in bold.

			Habitat occupancy model						
			Dist	**TO**	**RO**	**PO**	**TO|IBD**	**RO|IBD**	**PO|IBD**
LCP	F_{ST}	*r*	0.44168	0.59876	0.55730	0.52118	0.45574	**0.42915**	**0.47325**
		P	<0.0001	<0.0001	<0.0001	<0.0001	0.0122	**0.0239**	**0.0209**
	G'_{ST}	*r*	0.54291	0.60331	0.60602	0.59084	0.35715	0.32366	0.31334
		P	<0.0001	<0.0001	<0.0001	<0.0001	0.0404	0.0750	0.0533
	D	*r*	0.54680	0.59876	0.60444	0.58978	**0.47383**	0.30897	0.29080
		P	<0.0001	<0.0001	<0.0001	<0.0001	**0.0106**	0.1007	0.0655
	D_{PS}	*r*	**0.57692**	**0.63213**	**0.63349**	**0.62027**	0.34907	0.32089	0.30451
		P	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0488	0.0819	0.0506
IBR	F_{ST}	*r*		**0.70520**	**0.66746**	0.61123	**0.62447**	**0.56052**	**0.47325**
		P		**<0.0001**	**<0.0001**	<0.0001	**0.0019**	**0.0018**	**0.0193**
	G'_{ST}	*r*		0.68624	0.62432	0.61208	0.50199	0.44354	0.36324
		P		<0.0001	<0.0001	<0.0001	0.0068	0.0134	0.0484
	D	*r*		0.67041	0.60131	0.59302	0.47383	0.40695	0.32335
		P		<0.0001	<0.0001	<0.0001	0.0113	0.0320	0.0642
	D_{PS}	*r*		0.70166	0.63840	**0.63815**	0.50228	0.44068	0.37346
		P		<0.0001	<0.000	**<0.0001**	<0.0051	0.0133	0.0385

4.3.3 Parameterisation of High Resistance Features

The optimum value of R for natural barriers was 128 using LCP effective distances for all genetic distance metrics (F_{ST}, r = 0.48766, P < 0.0001; G'_{ST}, r = 0.57808, P < 0.0001; D, r = 0.57432, P <0.0001; D_{PS}, r = 0.61520, P < 0.0001). Mantel's r value increased until it reached a plateau at 128, at which point setting R any higher did not increase the cumulative resistance of the LCP as it circumnavigated the barriers wherever possible (Figure 4.3a). For anthropogenic barriers, the optimum value of R was 16 for F_{ST} (r = 0.51375, P < 0.0001), 8 for G'_{ST} (r = 0.56656, P < 0.0001) and D (r = 0.57833, P < 0.0001) and 4 for D_{PS} (r = 0.59519, P < 0.0001) (Figure 4.3b). Using the IBR effective distance, the optimum value of R for natural barriers was 8 for F_{ST} (r = 0.67281, P < 0.0001), G'_{ST} (r = 0.69226, P < 0.0001) and D_{PS} (r =

0.71985, $P < 0.0001$), and 4 for D ($r = 0.66678$, $P < 0.0001$) (Figure 4.3c). The optimum value of R for anthropogenic barriers was 2 for all of the genetic distance metrics (F_{ST}, $r = 0.63647$, $P < 0.0001$; G'_{ST}, $r = 0.61401$, $P < 0.0001$; D, $r = 0.59212$, $P < 0.0001$; D_{PS}, $r = 0.63088$, $P < 0.0001$) and increasing the value of R caused a rapid decline in the correlation coefficient (Figure 4.3d). All correlations at the optimum R value were significant following an FDR correction (adjusted 5% significance value, $P = 0.009$). D_{PS} was the most sensitive genetic distance metric to both natural and artificial barriers using the LCP metric and to natural barriers using the IBR distance metric. However, F_{ST} was the most sensitive to artificial barriers using the IBR metric. The full results of the barrier resistance optimisation are provided in Appendix 2.

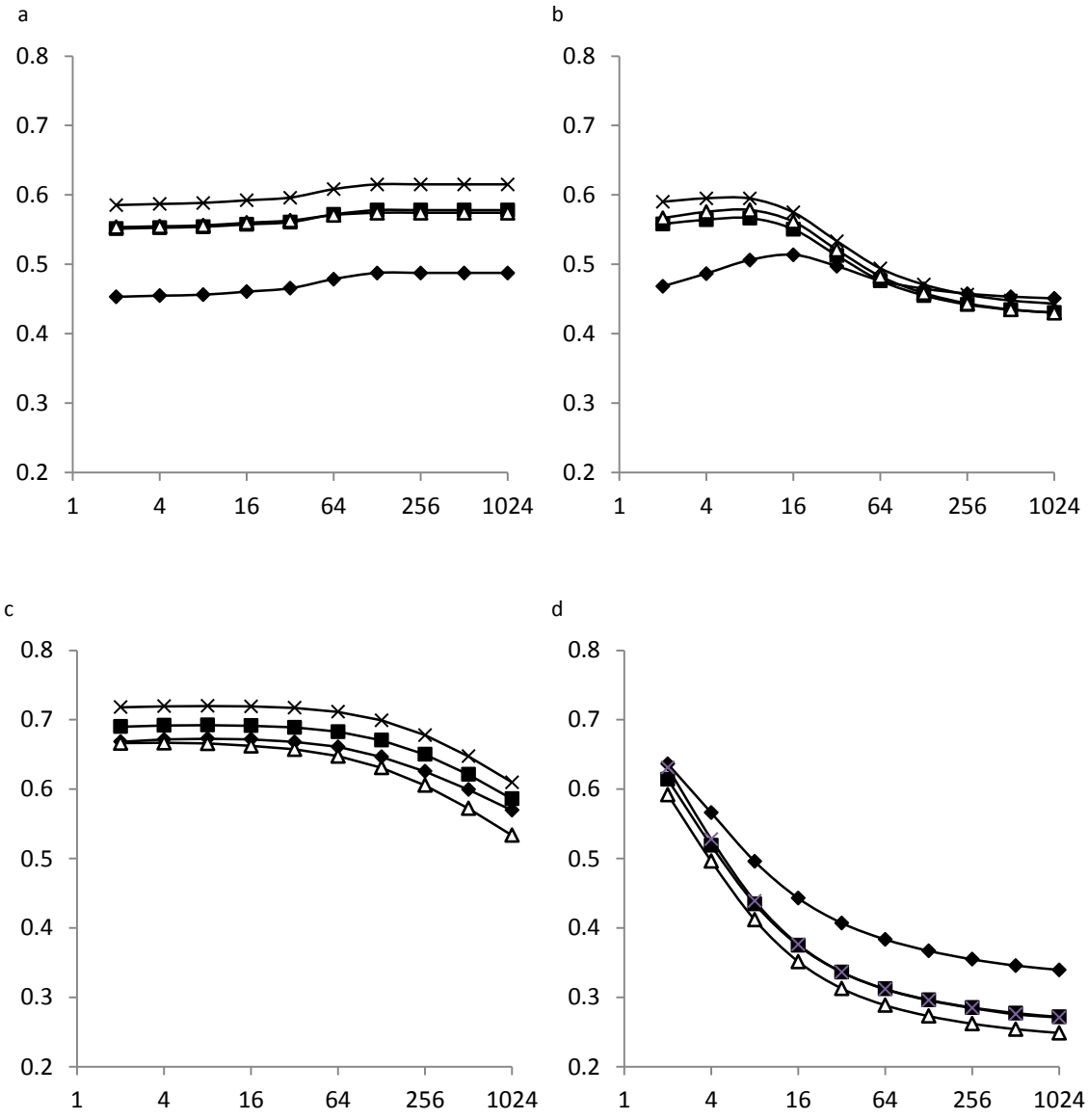

Figure 4.3. Parameterisation of high resistance features. a) natural barriers, LCP; b) artificial barriers, LCP; c) natural barriers, IBR; d) artificial barriers, IBR. X axis = resistance value (*R*), Y axis = Mantel's correlation coefficient (*r*). Solid diamonds = F_{ST}, solid squares = G'_{ST}, open triangles = D, crosses = D_{PS}.

4.3.4 Selection of the Optimal Resistance Surface

The optimum model using the multiple regression approach was F_{ST}_IBR + RO + natural barriers + artificial barriers (Table 4.4). Within this model, none of the parameters were individually significant to the nominal 5% level (P = 0.009 following an FDR procedure) following a partial Mantel test with a permutation test of significance (Table 4.5), although natural barriers were significant at a non-adjusted level. Typically, models with a ΔAIC of more than 10 compared to the best fitting model are not supported (Burnham & Anderson 2002) and therefore the only other models supported by the analysis were F_{ST}_IBR + distance + natural barriers + artificial barriers and F_{ST}_IBR + distance + natural barriers. Within these models, none of the individual parameters were significant with the exception of natural barriers in the F_{ST}_IBR + distance + natural barriers model (Table 4.5). Models including RO + natural barriers + artificial barriers were most often selected over models using the same genetic distance and geographic distance metrics. All F_{ST} models were preferentially selected over models based on G'_{ST}, D and D_{PS}, and IBR models were better than LCP (all the F_{ST} models are shown in Table 4.4 along with the best G'_{ST}, D and D_{PS} models for LCP and IBR, the complete set of G'_{ST}, D and D_{PS} models is shown in Appendix 3).

The optimum resistance surface was constructed using R values from the RO habitat occupancy model (Table 4.1). The optimised values of R for natural and artificial barriers was calculated relative to the average pixel resistance of all habitat types (R = 7). Therefore, for natural barriers, “small rivers” (8R), R = 56; and for artificial barriers, “suburban” habitat (2R), R = 14 (Figure 4.4 and Figure 4.5). The CIRCUITSCAPE current map for this resistance surface is shown in Figure 4.6.

Table 4.4. Multiple regression model selection using Akaike's Information Criterion (AIC) for all sites in Dorset. All F_{ST} models are shown and the best G'_{ST}, D and D_{PS} LCP and IBR models. All models not shown can be found in Appendix 3. LCP = Least Cost Path, IBR = Isolation by Resistance, TO = Total Occupancy, RO = Rank Occupancy, PO = Proportional Occupancy. Distance is the shortest geographical distance avoiding marine habitats and is equivalent to an Isolation by Distance model.

No.	Model	AIC	ΔAIC	Adjusted r^2	Rank
1	F_{ST}_LCP + distance	-577.929	74.959	0.189	30
2	F_{ST}_LCP + TO	-594.246	58.642	0.281	21
3	F_{ST}_LCP + RO	-598.995	53.893	0.305	19
4	F_{ST}_LCP + PO	-568.310	84.578	0.310	31
5	F_{ST}_LCP + distance + natural barriers	-588.818	64.070	0.257	26
6	F_{ST}_LCP + TO + natural barriers	-593.490	59.398	0.282	22
7	F_{ST}_LCP + RO + natural barriers	-597.676	55.212	0.304	20
8	F_{ST}_LCP + PO + natural barriers	-585.475	67.413	0.238	29
9	F_{ST}_LCP + distance + artificial barriers	-588.124	64.764	0.253	28
10	F_{ST}_LCP + TO + artificial barriers	-592.581	60.307	0.277	23
11	F_{ST}_LCP + RO + artificial barriers	-600.128	52.760	0.316	18
12	F_{ST}_LCP + PO + artificial barriers	-588.350	64.538	0.254	27
13	F_{ST}_LCP + distance + natural barriers + artificial barriers	-600.438	52.450	0.323	17
14	F_{ST}_LCP + TO + natural barriers + artificial barriers	-591.504	61.384	0.277	25
15	F_{ST}_LCP + RO + natural barriers + artificial barriers	-607.624	45.264	0.357	16
16	F_{ST}_LCP + PO + natural barriers + artificial barriers	-592.385	60.503	0.281	24
17	F_{ST}_IBR + TO	-641.952	10.936	0.494	4
18	F_{ST}_IBR + RO	-628.613	24.275	0.441	11
19	F_{ST}_IBR + PO	-612.031	40.857	0.369	15
20	F_{ST}_IBR + distance + natural barriers	-643.577	9.311	0.503	3
21	F_{ST}_IBR + TO + natural barriers	-641.691	11.197	0.496	5
22	F_{ST}_IBR + RO + natural barriers	-639.444	13.444	0.488	8
23	F_{ST}_IBR + PO + natural barriers	-628.393	24.495	0.444	12
24	F_{ST}_IBR + distance + artificial barriers	-617.062	35.826	0.396	14
25	F_{ST}_IBR + TO + artificial barriers	-641.663	11.225	0.497	6
26	F_{ST}_IBR + RO + artificial barriers	-629.669	23.219	0.450	10
27	F_{ST}_IBR + PO + artificial barriers	-619.555	33.333	0.407	13
28	F_{ST}_IBR + distance + natural barriers + artificial barriers	-644.381	8.507	0.510	2
29	F_{ST}_IBR + TO + natural barriers + artificial barriers	-641.514	11.374	0.499	7
30	**F_{ST}_IBR + RO + natural barriers + artificial barriers**	**-652.888**	**0**	**0.539**	**1**
31	F_{ST}_IBR + PO + natural barriers + artificial barriers	-632.139	20.749	0.463	9
42	G'_{ST}_LCP + RO + artificial barriers	-289.325	363.563	0.403	104
61	G'_{ST}_IBR + RO + natural barriers + artificial barriers	-313.471	339.471	0.503	94
73	D_LCP + RO + artificial barriers	-347.798	305.090	0.371	73
91	D_IBR + TO + natural barriers + artificial barriers	-369.680	283.208	0.469	51
108	D_{PS}_LCP + RO + natural barriers + artificial barriers	-378.192	274.696	0.441	42
122	D_{PS}_IBR + RO + natural barriers + artificial barriers	-402.474	250.414	0.532	32

Table 4.5. Results of a partial Mantel test to assess the significance of each parameter in the three best multiple regression models. Nominal 5% significance value following an FDR procedure, *P* = 0.009.

Model no.	Parameter	*r*	*P*
30	Habitat type (Rank Occupancy Model)	0.39038	0.0686
	Natural barriers (*R* = 16)	0.41140	0.0493
	Artificial barriers (*R* = 2)	-0.32764	0.8881
28	Distance	-0.30247	0.9148
	Natural barriers (*R* = 16)	0.44037	0.0428
	Artificial barriers (*R* = 2)	0.19519	0.2971
20	Distance	-0.30207	0.9138
	Natural barriers (*R* = 16)	0.61702	0.0014

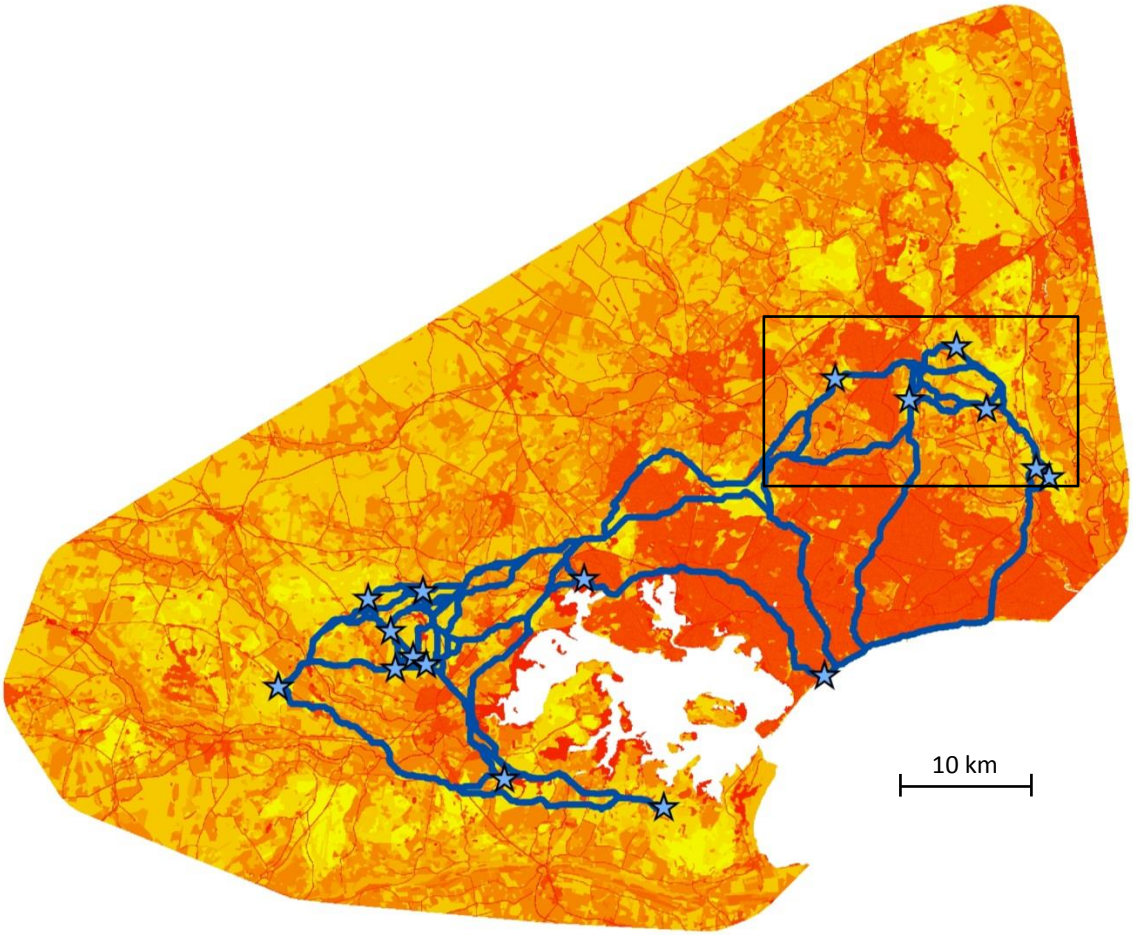

Figure 4.4. Optimised resistance surface for the whole study area. Yellow indicates areas of low resistance such as heathland and resistance increases with the shade to high resistance areas such as urban habitats and linear barriers which are coloured red. Least Cost Paths are shown in blue. A detailed section area of the resistance surface is shown below (Figure 4.5).

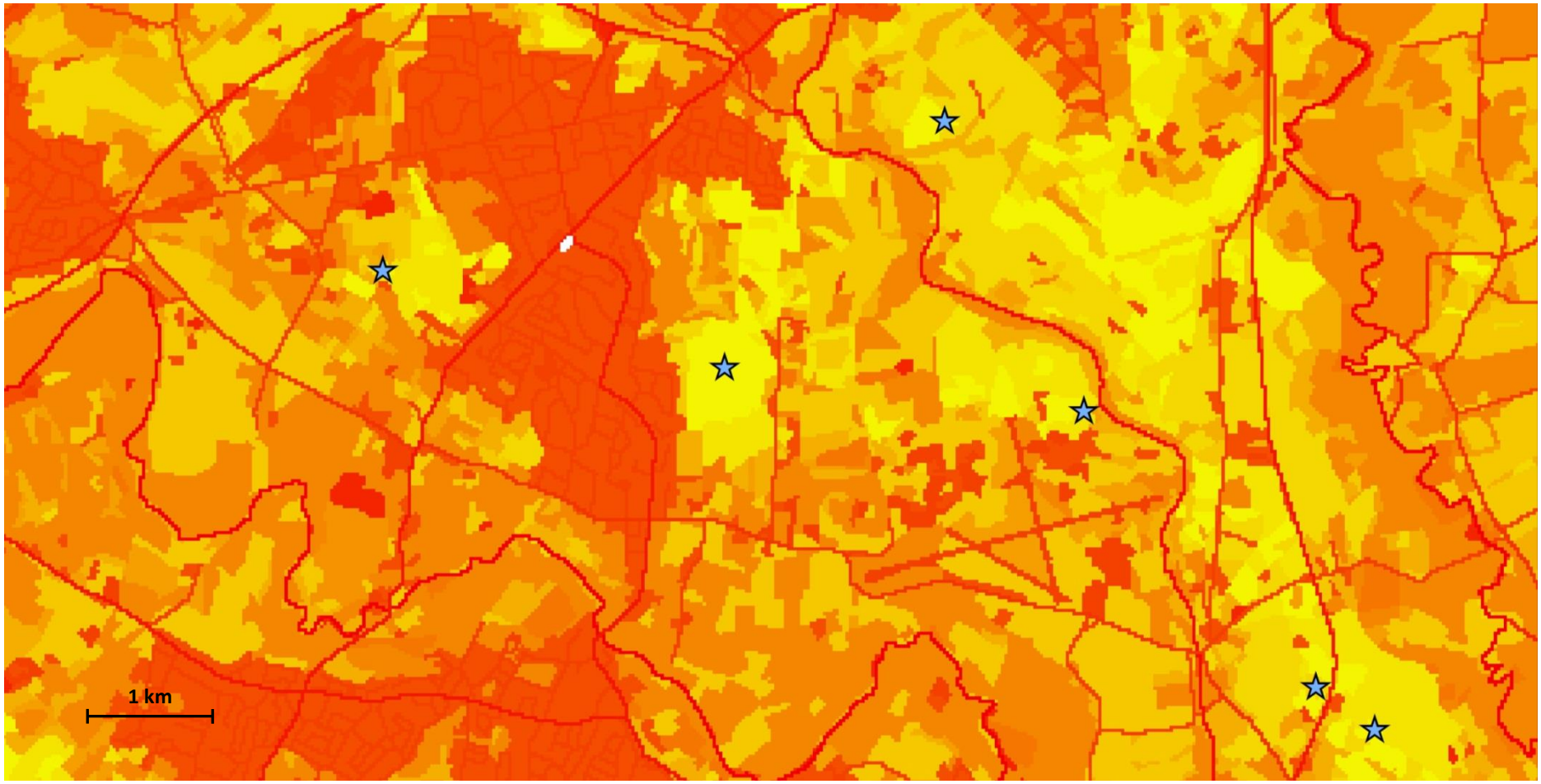

Figure 4.5. Detail of the optimised resistance surface covering the same area as in Figure 4.1. Areas of low resistance such as heathland are coloured yellow and the shade darkens as resistance increases, red areas have high resistance and generally indicate towns or linear barriers such as roads or rivers. Blue stars indicate the locations of the sample sites, from left to right: Ferndown Common, West Parley, Hurn Forest, Merritown Heath, Ramsdown and Town Common. The runway of Bournemouth Airport is visible to the bottom right of the centre, and the town of Ferndown is shown as the dark area left of the centre.

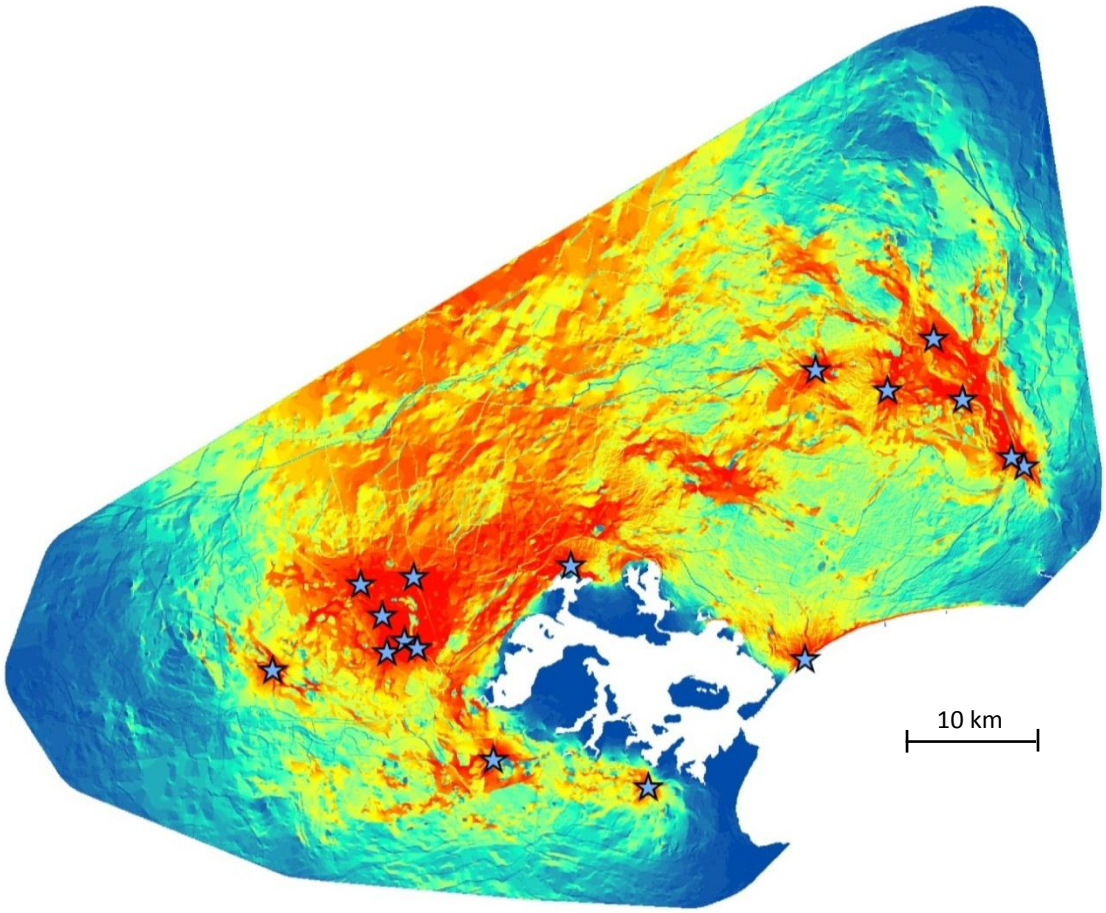

Figure 4.6. Current map produced in CIRCUITSCAPE. Warm colours indicate higher current density and therefore areas which are more important for gene flow. Cooler colours indicate a lower current density. CIRCUITSCAPE measures current between sites and therefore some areas known to be occupied by *L. agilis* show low current density as they were not important for gene flow between sampled sites.

4.3.5 Local-scale Resistance

Given its significantly greater power to predict the effect of various landscape features on patterns of genetic diversity at the regional scale, only F_{ST} based models were investigated at the local scale. In addition, the unfragmented Wareham Forest landscape does not include any mapped artificial barriers, so these were not included in any models. A small river and area of marshland were present in the area and were included as natural barriers.

Within the fragmented East Dorset landscape the most supported model was F_{ST}_LCP + RO + artificial barriers (Table 4.6) and within the unfragmented Wareham Forest landscape, the optimal model was F_{ST}_LCP + PO + natural barriers (Table 4.7). However, in both scenarios AIC_C values were typically high and many of the models had a ΔAIC_C score of less than 10 (10 of 15 models in Wareham Forest and 26 of 31 models in East Dorset) implying a little difference between them. As the relationship between F_{ST} and distance was not significant in East Dorset (Table 4.2) and the F_{ST}_distance model had a ΔAIC_C score of 6.077, implying some degree of

support (Burnham & Anderson 2002), it is not considered that any of the multiple regression models offer a significant explanation of the observed patterns of genetic diversity within the East Dorset area. Within Wareham Forest, the optimal model did offer an improvement over the null IBD model and also had a high r^2 value (0.783 compared to 0.209). Unlike the regional analysis, the top two ranked models were based on the PO occupancy model (Table 4.7).

Table 4.6. Multiple regression model selection using Akaike's Information Criterion (AIC) for East Dorset. LCP = Least Cost Path, IBR = Isolation by Resistance, TO = Total Occupancy, RO = Rank Occupancy, PO = Proportional Occupancy. Distance is the shortest geographical distance avoiding marine habitats and is equivalent to an IBD model.

No.	Model	AIC_C	ΔAIC_C	Adjusted r^2	Rank
1	F_{ST}_distance	-74.861	6.077	0.336	15
2	F_{ST}_LCP + TO	-79.741	1.197	0.521	2
3	F_{ST}_LCP + RO	-79.033	1.905	0.524	4
4	F_{ST}_LCP + PO	-79.633	1.305	0.517	3
5	F_{ST}_LCP + distance + natural barriers	-71.256	9.682	0.291	25
6	F_{ST}_LCP + TO + natural barriers	-76.336	4.602	0.495	12
7	F_{ST}_LCP + RO + natural barriers	-76.702	4.236	0.507	10
8	F_{ST}_LCP + PO + natural barriers	-76.662	4.276	0.506	11
9	F_{ST}_LCP + distance + artificial barriers	-72.222	8.716	0.336	23
10	F_{ST}_LCP + TO + artificial barriers	-77.651	3.287	0.537	6
11	**F_{ST}_LCP + RO + artificial barriers**	**-80.938**	**0**	**0.628**	**1**
12	F_{ST}_LCP + PO + artificial barriers	-76.872	4.066	0.513	8
13	F_{ST}_LCP + distance + natural barriers + artificial barriers	-68.019	12.919	0.297	31
14	F_{ST}_LCP + TO + natural barriers + artificial barriers	-73.416	7.522	0.510	20
15	F_{ST}_LCP + RO + natural barriers + artificial barriers	-77.584	3.354	0.629	7
16	F_{ST}_LCP + PO + natural barriers + artificial barriers	-73.581	7.357	0.515	18
17	F_{ST}_IBR + TO	-76.738	4.200	0.415	9
18	F_{ST}_IBR + RO	-79.003	1.935	0.497	5
19	F_{ST}_IBR + PO	-73.996	6.942	0.297	16
20	F_{ST}_IBR + distance + natural barriers	-71.051	9.887	0.282	26
21	F_{ST}_IBR + TO + natural barriers	-73.083	7.855	0.373	22
22	F_{ST}_IBR + RO + natural barriers	-75.334	5.604	0.460	13
23	F_{ST}_IBR + PO + natural barriers	-71.674	9.264	0.311	24
24	F_{ST}_IBR + distance + artificial barriers	-73.449	7.489	0.388	19
25	F_{ST}_IBR + TO + artificial barriers	-73.624	7.314	0.395	17
26	F_{ST}_IBR + RO + artificial barriers	-75.258	5.680	0.457	14
27	F_{ST}_IBR + PO + artificial barriers	-73.331	7.607	0.383	21
28	F_{ST}_IBR + distance + natural barriers + artificial barriers	-69.136	11.802	0.348	29
29	F_{ST}_IBR + TO + natural barriers + artificial barriers	-69.245	11.693	0.352	28
30	F_{ST}_IBR + RO + natural barriers + artificial barriers	-70.670	10.268	0.411	27
31	F_{ST}_IBR + PO + natural barriers + artificial barriers	-69.116	11.822	0.347	30

Table 4.7. Multiple regression model selection using Akaike's Information Criterion (AIC) for Wareham Forest. LCP = Least Cost Path, IBR = Isolation by Resistance, TO = Total Occupancy model, RO = Rank Occupancy Model, PO = Proportional Occupancy model. Distance is the shortest geographical distance avoiding marine habitats and is equivalent to an Isolation by Distance model

No.	Model	AIC_C	ΔAIC_C	Adjusted r^2	Rank
1	F_{ST}_distance	-95.173	16.760	0.209	9
2	F_{ST}_LCP + TO	-102.316	9.617	0.508	3
3	F_{ST}_LCP + RO	-97.421	14.512	0.319	5
4	F_{ST}_LCP + PO	-107.308	4.625	0.648	2
5	F_{ST}_LCP + distance + natural barriers	-92.704	19.229	0.216	13
6	F_{ST}_LCP + TO + natural barriers	-102.179	9.754	0.583	4
7	F_{ST}_LCP + RO + natural barriers	-93.882	18.051	0.276	11
8	**F_{ST}_LCP + PO + natural barriers**	**-111.933**	**0**	**0.783**	**1**
17	F_{ST}_IBR + TO	-95.826	16.107	0.242	7
18	F_{ST}_IBR + RO	-93.120	18.813	0.093	12
19	F_{ST}_IBR + PO	-95.841	16.092	0.243	6
20	F_{ST}_IBR + distance + natural barriers	-95.451	16.482	0.348	8
21	F_{ST}_IBR + TO + natural barriers	-94.125	17.808	0.287	10
22	F_{ST}_IBR + RO + natural barriers	-89.847	22.086	0.052	14
23	F_{ST}_IBR + PO + natural barriers	-65.732	46.201	0.360	15

4.3.6 Bayesian Analysis of Migration

The migration rates estimated by BIMr for East Dorset were generally low, and consequently the posterior probabilities of the environmental models to predict migration were also low (Table 4.8). However, consistent results were obtained across all the different habitat occupancy and geographic distance metric models and therefore the environmental factors identified as best predicting the genetic data are likely to be significant. The model with the highest posterior probability in all of the analyses undertaken included natural barriers as the only factor.

Table 4.8. Mean posterior probabilities for each environmental model estimated by BIMr. Dist = Euclidean distance avoiding marine habitats, Hab = LCM2007 habitat cover, NB = natural barriers, AB = artificial barriers.

Model	LCP			IBR		
	TO	**RO**	**PO**	**TO**	**RO**	**PO**
None	0.044	0.030	0.047	0.052	0.033	0.033
Dist	0.085	0.091	0.071	0.053	0.050	0.048
Hab	0.034	0.037	0.039	0.036	0.040	0.018
Dist + Hab	0.053	0.054	0.050	0.036	0.034	0.032
NB	**0.149**	**0.114**	**0.164**	**0.111**	**0.133**	**0.131**
Dist + NB	0.085	0.085	0.098	0.083	0.087	0.087
Hab + NB	0.083	0.065	0.081	0.070	0.086	0.095
Dist + Hab + NB	0.063	0.058	0.062	0.051	0.065	0.070
AB	0.028	0.023	0.033	0.083	0.063	0.065
Dist + AB	0.060	0.054	0.051	0.068	0.048	0.049
Hab + AB	0.028	0.026	0.026	0.051	0.041	0.048
Dist + Hab + AB	0.043	0.046	0.035	0.042	0.033	0.035
NB + AB	0.082	0.110	0.085	0.088	0.092	0.087
Dist + NB + AB	0.061	0.069	0.060	0.073	0.071	0.071
Hab + NB + AB	0.059	0.077	0.057	0.059	0.068	0.075
Dist + Hab + NB + AB	0.045	0.062	0.043	0.046	0.059	0.059

4.4 Discussion

4.4.1 Genetic Distance Metrics

Both G'_{ST} and *D* showed a stronger IBD effect than F_{ST}, and D_{PS} showed the strongest IBD effect of all the genetic distance metrics. D_{PS} also proved most effective at detecting the effect of barriers in the absence of other landscape features. However, when the different elements of the landscape (habitat type and both natural and artificial barriers) were combined in the same model, F_{ST} showed the best fit. Raeymaekers *et al.* (2012) found that G_{ST} (an analog of F_{ST}) was more sensitive to recent demographic events than *D*, whilst *D* better explained phylogenetic relationships due to postglacial recolonisation in the three-spined stickleback *Gasterosteus aculeatus*. Murphy *et al.* (2010b) found D_{PS} more effective than F_{ST} at predicting the effect of recent landscape change on patterns of genetic diversity in the western toad *Bufo boreas* in Yellowstone National Park and Spear & Storfer (2008) found G'_{ST} to be the most effective of several genetic distance metrics (including F_{ST} and D_{PS}) in predicting the effect of forest cover on genetic connectivity in the coastal tailed frog *Ascaphus truei*.

The comparative sensitivity of F_{ST} to landscape observed in this study supports the thesis of Whitlock (2011) who argued that F_{ST} is better at elucidating population structure and that G'_{ST} (Hedrick 2005) and D (Jost 2008) are of limited use in inferring demographic and evolutionary processes from which genetic variation arises. Whitlock (2011) highlighted that F_{ST} is affected by all evolutionary processes, it is increased by genetic drift as a result of small population size, bottlenecks or founder effects; and it is reduced by migration between populations. Mutation may result in a reduction of differentiation between populations by increasing heterozygosity or due to homoplasy (of particular relevance to microsatellites). However, mutation varies significantly between loci whilst genetic drift and migration have similar effects upon all loci and therefore spatial patterns across all loci are only significantly affected by drift and migration. This gives F_{ST} very useful properties for landscape genetics studies. By contrast, G'_{ST} and D do not share these properties and whilst they provide a good measure of differentiation between populations, they are less affected by evolutionary processes which are of importance in landscape genetics.

The relatively low power of D_{PS} to detect recent landscape change compared to F_{ST} in this study is in contrast to recent empirical landscape genetics studies (Murphy *et al.* 2010a; Murphy *et al.* 2010b) and simulations (Landguth *et al.* 2010). Landguth *et al.* (2010) simulated a variety of dispersal strategies to determine the time taken for a barrier to gene flow to become established and found F_{ST} to be much less sensitive than D_{PS} using an individual-based approach. However, Landguth *et al.* (2010) acknowledged a number of limitations in their study: firstly the simulations were based on non-overlapping generations, which would not be the case in *L. agilis* populations and is likely to have an effect on population structure. Secondly, the simulated populations had a constant size whereas the rate of genetic change in a small or fluctuating population may be significantly greater. The effective population size (N_e) of the *L. agilis* populations within the study area varied between 105 and 280 (Chapter 3 of this thesis), considerably lower than the N_e of 1,000 of the simulated populations, in addition, many of the studied populations are likely to have undergone reductions in size in recent years (Corbett 1969, 1988a). Given these considerations, the greater sensitivity of F_{ST} observed in this study may be a result of its behaviour in relation to demographic processes such as genetic drift, the response of D_{PS} to which is not yet fully understood (Landguth *et al.* 2010).

4.4.2 Isolation by Distance Effects

A significant IBD effect was observed at the regional scale across Dorset, however no significant IBD effects were recorded at the local scale in either the fragmented or unfragmented landscapes, and distance was not identified as a significant factor within East

Dorset at a local scale in the BIMr analysis. Hutchison & Templeton (1999) investigated IBD in eastern collared lizards *Crotaphytus collaris* which inhabited areas with different landscape histories and degrees of fragmentation (see section 1.3.1). In an anthropogenically fragmented landscape they observed a typical IBD relationship between genetic and geographic distance over a shorter geographic distances, whilst at larger geographic distances this relationship was not apparent. This was explained by gene flow and genetic drift being in equilibrium at short distances, whilst at larger distances gene flow was prevented by fragmentation, and genetic drift was dominant. This effect was also observed in Jerusalem crickets *Stenopelmatus* spp. in California, another species which has been affected by recent anthropogenic habitat fragmentation (Vandergast *et al.* 2007). A similar pattern was observed in this study at the regional level when genetic distance was correlated with geographic distance (Figure 4.7). When the data were transformed to genetic distance/(1-genetic distance) correlated with log geographic distance as recommended by Rousset (1997), more of the variation was explained (r = 0.628, as opposed to 0.442 in the untransformed data, Figure 4.8). Whilst still discernible, the relationship observed by Hutchison & Templeton (1999) between genetic and geographic distance observed in *C. collaris* in a fragmented landscape is not as clear in plot of the transformed data.

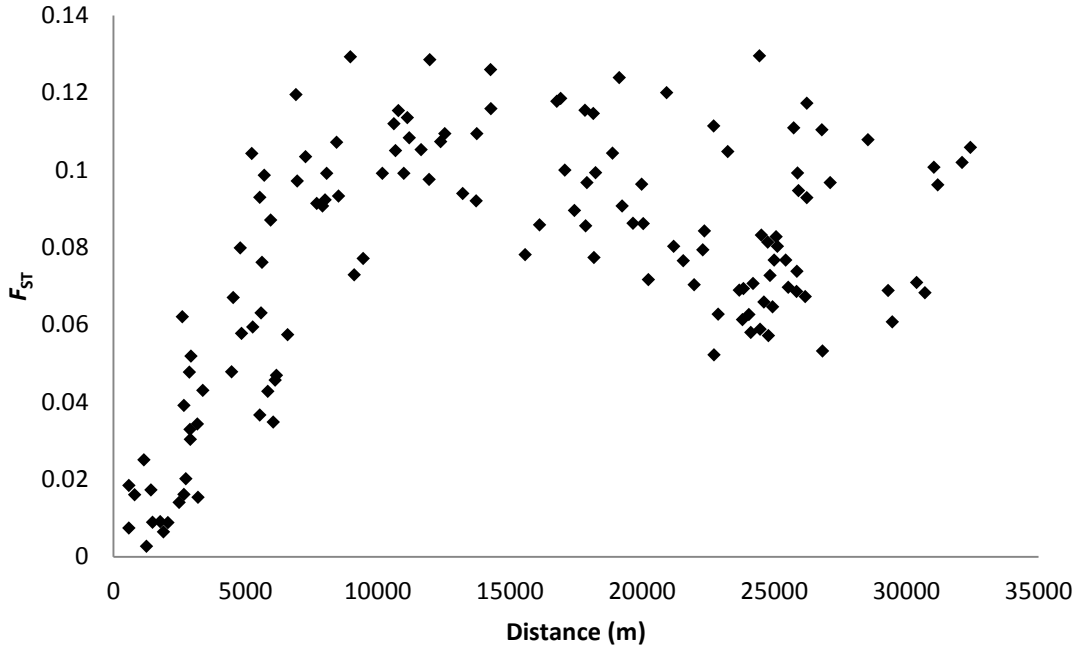

Figure 4.7. Relationship between F_{ST} and geographic distance across Dorset. This reflects the results of Hutchison & Templeton (1999) for the eastern collared lizard *Crotaphytus collaris* in a fragmented landscape which showed an IBD relationship over short distances and a more complex pattern at larger distances.

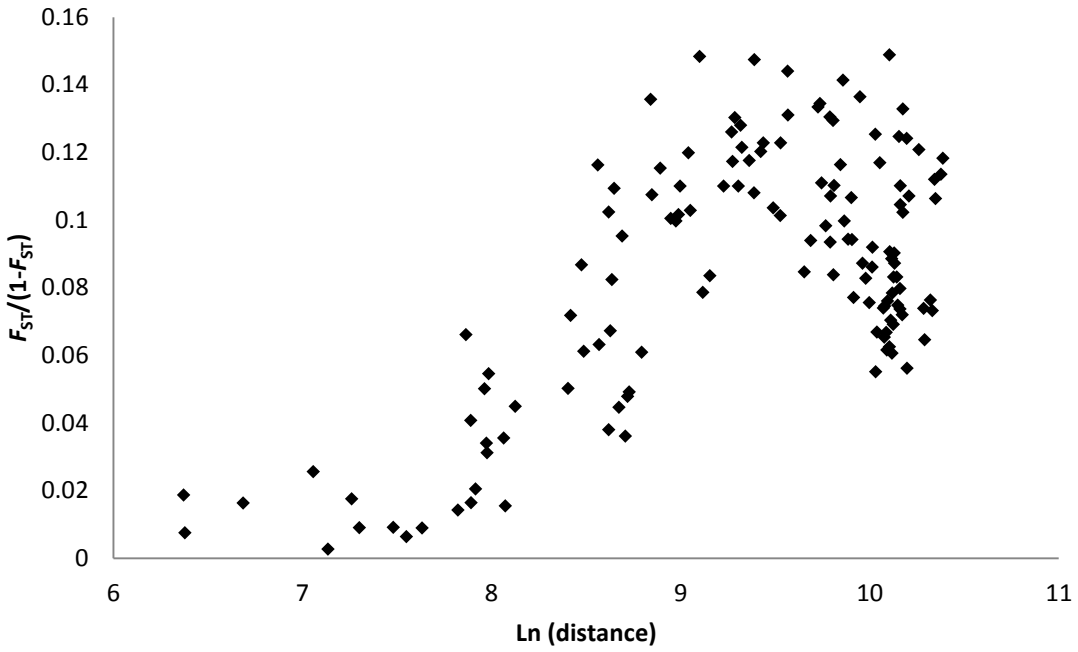

Figure 4.8. Relationship between $F_{ST}/(1-F_{ST})$ and Ln(geographic distance). The relationship between genetic and geographic distance observed by Hutchison & Templeton (1999) for the eastern collared lizard *Crotaphytus collaris* in a fragmented landscape is still apparent, although less clear than the untransformed data.

4.4.3 Relative Importance of Habitat Type

Habitat type was a significant component of the optimum multiple regression model, but not in the optimum models of the BIMr analysis. The AIC model selection procedure identified the Rank Occupancy (RO) habitat occupancy model as the closest fitting to the genetic data as a component of the optimal multiple regression model at the regional and local scales. It was also selected above the Total Occupancy (TO), Proportional Occupancy (PO) and null (distance only) models in multiple regression models which excluded other features such as barriers. These results suggest that habitat type does have a significant impact on gene flow between *L. agilis* populations. However, the RO model reduced the difference in resistance between habitat types when compared to the TO and PO models.

At the local scale within Wareham Forest, the optimal multiple regression models were based on a PO occupancy model. This landscape contains no artificial barriers and only one natural barrier (a "small" river with some associated wetland habitat). This implies that the PO model may offer the best explanation of on the relative permeability of different habitat types. However, when the study area is expanded, habitat type has less effect on patterns of genetic diversity than barriers such as roads or rivers.

In the TO model, the relative importance of heathland habitats is exaggerated compared to other habitat types. Although *L. agilis* is particularly associated with heathland habitat in Britain (Corbett 1988b; Beebee & Griffiths 2000), this association may be a result of the requirement for a sandy substrate for egg incubation in the climate of northwest Europe (Beebee & Griffiths (2000) and see Chapter 3 of this thesis). Throughout other parts of its range in Europe, *L. agilis* is found in a variety of different habitat types including grassland, forests, steppe and marginal habitat within agricultural areas (Gasc *et al.* 2004). Given its ability to utilise these habitats in other parts of its range, it is likely that they provide sufficient food and have an appropriate physical structure for thermoregulation and shelter for *L agilis* in Britain. Although successful reproduction within these habitats may not be possible, they may provide sufficient resources for dispersing lizards to move through, therefore facilitating a certain degree of migration between optimal heathland sites.

As the RO model was preferentially selected over the null model at the regional level and the PO model selected at the local level, it cannot be assumed that all habitat types are equally permeable and some, such as arable, improved grassland or dense plantation without rides, are likely to be significant barriers as they lack the structural diversity necessary for successful thermoregulatory behaviour. The PO model was based on the proportion of pixels of each habitat type which contained an *L. agilis* record (Table 4.1). This model most closely reflects the recorded habitat use of *L. agilis* within the study area compared to the TO model which exaggerates the relative resistance of habitat types with few records and the RO model which reduces the difference in resistance between all habitat types. Within the PO model, the most important habitat types were heathland (LCM habitat “heather grassland” and “heather”, with a resistance of 1 and 27 respectively) and sand dune (LCM2007 habitat “supra-littoral sediment” with a resistance of 206). Other habitat types were relatively similar in their resistance with values ranging between 904 and 1,132 for the least resistant habitat. Heathland habitats were also the least resistant in the RO model. Coniferous woodland was the third least resistant habitat in the RO model and the fourth least resistant in the PO model. Although this is not particularly suitable for *L. agilis*, many of the plantations within the study area have been managed for wildlife and contain small areas of heathland along ride edges and in small plots of land which are not commercially viable for timber production. “Arable and horticulture” was the fourth least resistant habitat in the RO model, however this likely to be a result of the high proportion of this habitat within the study area.

4.4.4 Relative Importance of Contemporary and Historical Landscape Features

Both natural barriers (rivers and other aquatic habitats) and artificial barriers (roads and development) were important components of the optimum multiple regression model. The optimisation procedure identified a higher resistance value in the natural barriers (from R = 8 for small rivers to R = 32 for large rivers) than artificial barriers (from R = 2 for suburban habitat to R = 16 for major roads). In addition, the optimum models estimated in the BIMr analysis included natural barriers only and therefore, individually, natural barriers are more significant barriers to gene flow than artificial barriers. The identification of rivers as a significant barrier to gene flow is supported by a number of studies of lizard population genetics and phylogeography which have reported similar results in the common ground skink *Scincella lateralis* in North America (Jackson & Austin 2010), the lacertids *Eremias argus* and *E. brenchleyi* in China (Zhao *et al.* 2011), the mountain lizard *Liolaemus monticola* in the Andes (Torres-Perez *et al.* 2007) and also for *L. agilis* in Europe (Bahl *et al.* 1997).

At an individual level, roads appear to have less effect on between-population gene flow than rivers; for example the resistance of a large river was identified as twice that of large road. The primary example of this within the study area is Town Common and Ramsdown, which are less than 1 km apart but separated by a large road. Genetic structuring between them as assessed by F_{ST} was significant, but both Bayesian assignment methods used in Chapter 3 (STRUCTURE and BAPS) failed to identify them as separate populations, implying significant migration between them. The road which separates the sites comprises two carriageways, each approximately 6.5 m in width, and has a mean traffic volume of 1,936 vehicles per hour over a 24 hour period (Transport Statistics Division 2012). Assuming a burst speed between 1.66 ms^{-1} (Avery *et al.* 1987) and 2.68 ms^{-1} (Vanhooydonck *et al.* 2002), recorded for the slightly larger *L. viridis* and *L. bilineata* respectively, any lizards crossing this road would be likely to encounter between three and seven vehicles (although this is likely to be significantly higher as the majority of traffic movements would occur during the day when lizards are most likely to attempt to cross), and therefore such a road is likely to be a significant barrier. The efficacy of roads as a barrier to dispersal and gene flow in lizards is less well understood than that of rivers, especially at a molecular level. Roads have been identified as a significant cause of mortality in lizards, many of which will take advantage of retained heat within the road's surface for thermoregulation (Koenig *et al.* 2002; Tanner & Perry 2007; Meek 2009). However, Telleria *et al.* (2011) found that whilst a motorway was a significant barrier to the small lacertid *Psammodromus algirus*, it was not for the larger *Lacerta* (*Timon*) *lepidus*. Roads were not found to be a significant cause of mortality in the northern alligator lizard *Elgaria coerulea* and

western skink *Eumeces skiltonianus*, as these species tend not to move over long distances (Rutherford & Gregory 2003).

The difference in the relative effect of roads and rivers on patterns of genetic diversity within the study area is likely to be a result of a genetic time-lag. Although the likelihood of an individual lizard being able to cross a major road may be similar to that of it crossing a large river, rivers are long-established features of the landscape which would have acted as barriers restricting migration for many hundreds of generations resulting in the divergence of populations either side (Piertney *et al.* 1998; Pellegrino *et al.* 2005; Gehring *et al.* 2012). By contrast, roads are relatively recent additions to the landscape and sufficient traffic to prevent animals from crossing is even more recent. For example, the road separating Town Common and Ramsdown was constructed in the late 1970s, a time period which represents less than ten *L. agilis* generations to the present day.

In a simulation exercise, Landguth *et al.* (2010) found that it may take up to 200 generations before the presence of a barrier could be detected using $F_{ST.}$ Keyghobadi *et al.* (2005) empirically demonstrated a genetic time-lag in the butterfly *Parnassius smintheus*, in which, although genetic structure as assessed by G_{ST} was highly correlated with contemporary canopy cover, genetic diversity (heterozygosity) showed a greater correlation with canopy cover 40 years before the study. The habitat of the Florida sand skink *Plestiodon reynoldsi* has suffered increasing anthropogenic fragmentation in the past 60 years, which represents approximately 15 generations (McCoy *et al.* 2010), a similar generation time to *L. agilis*. Despite this, Richmond *et al.* (2009) found no genetic evidence of isolation between sites. Due to this genetic time-lag, the pattern of genetic diversity of *L. agilis* in Dorset reflects a historical, rather than contemporary landscape. This pattern very closely mirrors that observed by Hutchison & Templeton (1999) in the eastern collared lizards *Crotaphytus collaris* in the southwestern Ozark region of Arkansas (Figure 1.1 and Figure 4.7) where large populations occupied extensive areas of habitat until recent human settlement resulted in habitat fragmentation.

Zellmer & Knowles (2009) used historical mapping to separate the effect of recent habitat fragmentation from historical genetic process in the wood frog *Rana sylvatica*. The Land Utilisation Survey (LUS) of Britain (Stamp 1931) mapped land cover in Britain during the 1930s and covered the study area in 1936. The LUS map of the study area (Figure 4.9) shows extensive areas of heathland with river valleys the only significant barriers between the sample sites. Some roads are present, however traffic numbers at this time were considerably lower

(Hicks & Allen 1999) and therefore they are unlikely to be as effective as barriers as present-day roads. This landscape appears to more closely reflect the observed patterns of genetic diversity in Dorset *L. agilis* than the contemporary landscape, however further research, such as creating a historical resistance surface from this map, is required to fully investigate this.

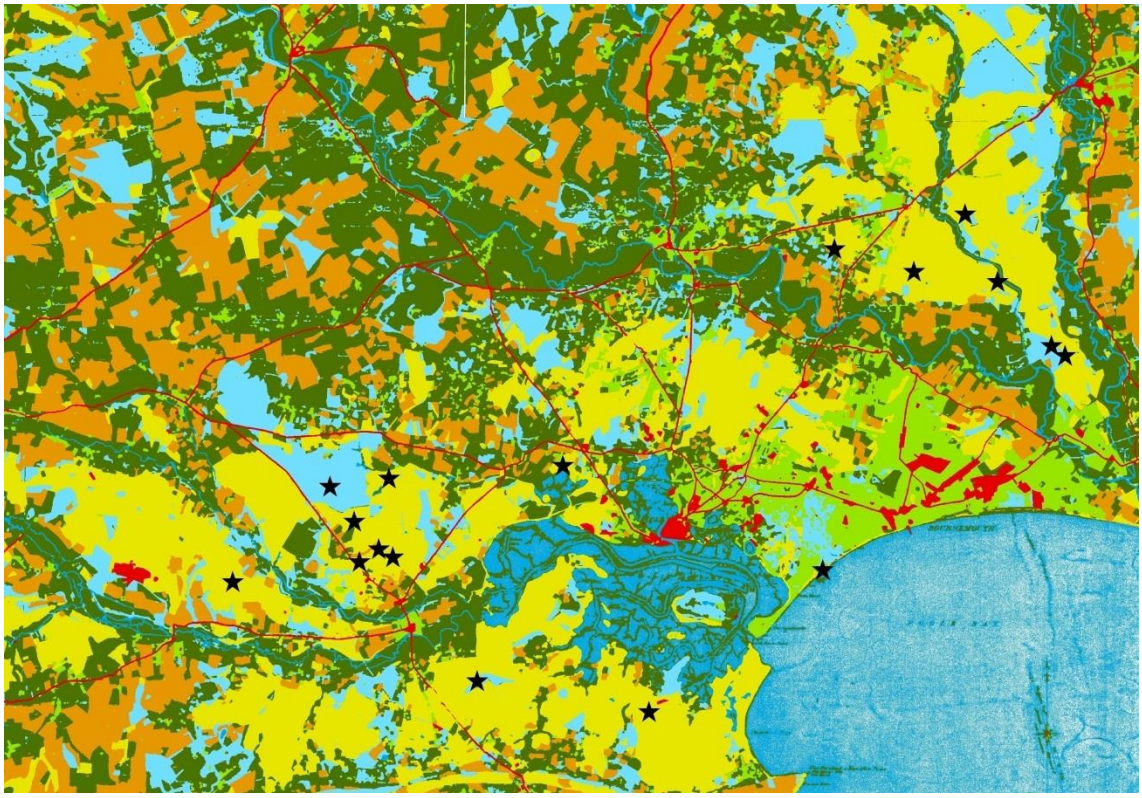

Figure 4.9. Land Utilisation Survey map (Stamp 1931) of the study area dating from 1936. Heathland is shown as yellow, roads and developed land are red, sample sites are marked by black stars.

4.4.5 Importance of Landscape Features at Different Scales

Given the low support for all models at the local scale in East Dorset it is difficult to draw firm conclusions about the relative importance of different landscape features in this scenario. However, the optimal models at the local scale in Wareham Forest differed from the optimal regional model in the habitat occupancy model with the greatest support. At the regional scale, RO-based models were selected, implying that different habitat types have relatively similar resistance, whereas at the local scale, TO and PO-based models were selected, implying more marked differences in habitat resistance. Given the well documented habitat preferences of *L. agilis* in Britain, habitat type undoubtedly is a significant factor in patterns of dispersal. However, the effect of habitat type on patterns of genetic diversity is overwhelmed by the more significant effect of barriers and in particular rivers at broader spatial scales. Similar results have been observed in other landscape genetic studies, for example habitat cover was

the most important factor influencing gene flow at a local scale in both *Bufo boreas* (Murphy *et al.* 2010b) and *Hyla arborea* (Angelone *et al.* 2011), whereas at broader scales the most significant factors were environmental gradients and large rivers respectively.

4.4.6 Gene Flow at a Landscape Level

Least cost paths for the optimum resistance model (Figure 4.4) were within areas of high gene flow identified by the IBR modelling (Figure 4.6) and these areas can be considered as particularly important for the maintenance of genetic processes within Dorset *L. agilis*. As would be expected, these mostly coincided with heathland and this therefore reinforces the importance of this habitat type for this species in Britain. Heathland in Dorset has been particularly impacted by fires which have significantly impacted *L. agilis* in some areas (Corbett 1988b; Edgar & Bird 2006). Gene flow was high within fire-affected heathland even though these may no longer support large populations (e.g. Canford Heath) and demonstrates that such sites may still be important for the conservation of *L. agilis* as a corridor between other populations. Other habitat types including some agricultural land also played a role in facilitating gene flow between the sampled populations and although no particular corridors were identified through agricultural land, current could flow fairly evenly across it. This was particularly important where heathland was rarer, and gene flow was high across predominantly agricultural land between the heathlands of Wareham Forest and Canford Heath to the north of the urban areas of Poole.

Corridors which allowed gene flow between or around urban areas were important regardless of habitat type. Within the East Dorset area, a corridor facilitating gene flow between West Parley and Ferndown Common ran through a narrow area of agricultural land and a golf course to the south of the town of Ferndown and north of the River Stour. A similarly important corridor was identified to the north of Ferndown either side of the main A31 road between the suburban areas of Ferndown and West Moors. By comparison, current density was uniformly high across Wareham Forest, although avoiding the large wetland area within the centre, and no particularly important corridors were identified.

The current map produced in CIRCUITSCAPE identified areas of relatively high gene flow to the north of the present range (Figure 4.6). Chapter 3 of this thesis examined the phylogeography of *L. agilis* in Great Britain and found evidence of range expansion during periods of climate warming followed by contraction during cooler and wetter periods. As well as thermal benefits, a warmer climate may enable *L. agilis* to occupy a broader range of habitat types and therefore these areas indicate potential routes of expansion in the event of predicted future

climate warming (Murphy *et al.* 2009). The potential effect of predicted climate change is discussed further in Chapter 6.

4.4.7 Conclusions

Habitat cover, natural and artificial barriers all significantly influence dispersal and migration between *Lacerta agilis* populations in Dorset. Patterns of genetic diversity more closely reflect the historical landscape than the present-day one with natural barriers, particularly rivers, having the most significant effect. However it is likely that roads and development are a significant barrier to dispersal and migration but their effect is not yet apparent at a molecular level due to a genetic time-lag. The IBR modelling indicated the possible areas for *L. agilis* to increase their range to the north should the climate become more suitable (see Chapter 6 for a more detailed discussion) but anthropogenic fragmentation may limit the potential for any expansion. Further genetic monitoring in future years is required to quantify the full effects of habitat fragmentation on *L. agilis* in Dorset and the implications for its conservation.

5 GEOGRAPHIC VARIATION OF COLOUR AND PATTERN IN BRITISH SAND LIZARDS

5.1 Introduction

Colour plays an important role in many aspects of the ecology of lacertid lizards including: thermoregulation (Diaz 1994; Raia *et al.* 2010); sexual selection (de Lanuza & Font 2007; Sullivan & Kwiatkowski 2007; Galan 2008; Martin & Lopez 2009); social interaction (Vercken & Clobert 2008a); crypsis and predator avoidance (Martin & Lopez 2001; Carretero *et al.* 2006; Capula *et al.* 2009; Font *et al.* 2009; Martin *et al.* 2009).

Within-sex colour variation has been recorded in a number of species of Lacertidae and different colour morphs often exhibit different social and reproductive strategies. Female viviparous lizards *Lacerta* (*Zootoca*) *vivipara* occur in three different morphs, with orange, yellow or mixed ventral colouration. Different colour morphs display differing social, reproductive and dispersal behaviour and the outcome of interactions between differently coloured females is dependent on the colour of both parties (Vercken *et al.* 2007; Vercken & Clobert 2008b; Vercken *et al.* 2010; Vercken *et al.* 2012). Both sexes of the Dalmatian wall lizard *Podarcis melisellensis* also occur in different colour morphs and males with an orange ventral colour typically have a larger head and more powerful bite (Huyghe *et al.* 2007). Orange males always dominate over yellow and white morphs regardless of body size. However, females appear to select mates on the basis of size and body condition rather than colour or dominance (Huyghe *et al.* 2012). Orange males also tend to have a lower haemogregarine (a protozoan blood parasite) burden at the end of the season, whilst white males have the highest levels of infection (Huyghe *et al.* 2010). The common wall lizard *P. muralis* shows similar difference between ventral colour morphs. Calsbeek *et al.* (2010) found that different morphs allocated resources differently, with some preferentially investing in attaining a large body size and others in immune response. Unlike *P. melisellensis,* colour is not an indicator of social dominance in *P. muralis* where body size and residency were better predictors of victory in territorial fights between males (Sacchi *et al.* 2009).

Intraspecific colour-based signalling in lacertids is often coupled with chemical signals (Bauwens *et al.* 1987; Martin & Lopez 2000; Lopez *et al.* 2002; Vercken & Clobert 2008a; Martin & Lopez 2010a; Kopena *et al.* 2011). Female Iberian rock lizards *Iberolacerta monticola* are able to distinguish between two distinct male colour morphs using chemical cues alone

(Lopez *et al.* 2009) and odour takes precedence over colour signals in mate selection in the Iberian wall lizard *P. hispanica* (Lopez & Martin 2001).

The importance of male pigment and colouration in sexual signalling and selection is well documented in the green lizards of the genus *Lacerta*. Brighter flank colouration and UV reflectivity are indicators of breeding success in male sand lizards *L. agilis* (Anderholm *et al.* 2004; de Lanuza & Font 2007; Olsson *et al.* 2011a). The importance of colouration and UV reflectivity as indicators of mate quality has been demonstrated in the green lizard *L. viridis* where the brightness of the blue throat of the male is positively correlated with body size and relative head size and negatively correlated with ectoparasite load (Vaclav *et al.* 2007; Molnar *et al.* 2012). Colour is also an indicator of health in *L. agilis* (Olsson *et al.* 2005b). The UV reflectivity of the males' throat and flanks is correlated with fighting success in *L. viridis* (Bajer *et al.* 2011) and *L. agilis* (Olsson 1994b) respectively. Ultra-violet throat reflectivity is a predictor of territorial dominance in Schreiber's green lizard *L. schreiberi* (Martin & Lopez 2009) and female *L. viridis* exhibit a preference for males with higher throat reflectivity (Bajer *et al.* 2010). Producing these pigments is energy expensive and requires males to spend more time at an elevated body temperature (Olsson 1994a; Olson & Owens 1998; Bajer *et al.* 2012). Basking is also important as UV-deprived male *L. agilis* experience lower mating success (Olsson *et al.* 2011a). Many of the colours of lacertids are carotenoid-based and have been shown to fade in response to stress (Fitze *et al.* 2009; Cote *et al.* 2010) which would provide an indication of health to prospective mates and rivals (Martin & Lopez 2010a). Olsson *et al.* (2012) demonstrated that accumulated DNA damage, an indication of age, resulted in fading breeding colouration in the painted dragon *Ctenophorus pictus*.

The significance of colour in relation to thermoregulation in lizards is comparatively less well studied. Melanism has been demonstrated to give individuals a thermoregulatory advantage in many snake species (Forsman 1993; Bittner *et al.* 2002; Tanaka 2005). However, whilst colour-related difference in thermoregulatory behaviour has been recorded in the west Canarian lizard *Gallotia galloti* (Diaz 1994), Gvozdik (1999) found no advantage to melanism as assessed by heating rates, body size and body condition in *L. vivipara.*

The colour and pattern in many lacertids represents a trade-off between conspicuous breeding colouration and crypsis (Carretero 2002). Fleeing from a potential predator may expose a lizard to increased risk of being discovered as well as having an energetic cost. Colour-related conspicuousness influences the fleeing time (the point when the risk of being predated equals the cost of fleeing) in the rock lizard *Iberolacerta cyreni*, with more conspicuous individuals

fleeing before drably-coloured conspecifics (Martin *et al.* 2009). Males of *I. monticola* and *I. cyreni* advertise their quality using blue lateral and ventral markings respectively. The more conspicuous *I. monticola* males engage in more anti-predator behaviours as assessed by readiness to flee and time spent inside refuges than the less conspicuous *I. cyreni* (Cabido *et al.* 2009). Carretero *et al.* (2006) found differences in the escape behaviour of syntopic forms of the Moroccan rock lizard *Lacerta (Tiera) perspicillata* in which striped lizards were quicker to flee than spotted forms which relied more on crypsis. The two forms also differed in microhabitat selection with spotted lizards utilising larger rocks which offered more refuges from terrestrial predators but were more susceptible to avian attacks.

Lacerta agilis occurs within three distinct and widely separated areas of Great Britain: Merseyside in the northwest, Surrey in the southeast and Dorset in the southwest. In Dorset and Surrey, sand lizards occupy lowland heathland habitat, whilst in Merseyside they are found in coastal sand dunes. (Beebee & Griffiths 2000). Lizards from these three areas are genetically distinct (Beebee & Rowe (2001b), and Chapter 3 of this thesis) and also appear to exhibit differences in colour and pattern (Simms 1970; Beebee & Griffiths 2000), although this has not been empirically demonstrated. Males from the southern heathland populations are typically a darker green with darker markings and ocelli on the flanks and overall, a more spotted appearance, whilst those from Merseyside are generally a paler green and tend to be more striped (Figure 5.1). Previous authors (Simms 1970; Beebee & Griffiths 2000) have speculated that these differences may have arisen due to camouflage in the different habitats in which the populations are found, with the spots of the southern populations blending in well with the heather *Calluna vulgaris* dominated heathland and the paler striped northern animals better suited to the marram grass *Ammophila arenaria* habitats of the coastal dunes. It should however be noted that *L. agilis* populations in the Netherlands do not appear to differ between heathland and sand dune sites (H. Strijbosch, pers. comm.).

This chapter quantifies the variation in colour and pattern in *L. agilis* populations across Britain and uses univariate and multivariate statistics to investigate the differences between the three geographical areas. A Bayesian assignment technique is then used to assign individuals to populations on the basis of colour and pattern alone and using a combination of genotypic and phenotypic data.

Figure 5.1. Examples of variation in *L. agilis* colour and pattern across the study sites. a) male from Morden Bog, Dorset; b) female from Wareham Forest, Dorset; c) male from Frensham Common, Surrey; d) female from Frensham Common, Surrey; e) male from Ainsdale, Merseyside; f) female from Ainsdale, Merseyside; g) male from Bergherbos, the Netherlands; h) female from Bergherbos, the Netherlands; i) hypomelanistic male from Ainsdale, Merseyside.

5.2 Materials and Methods

5.2.1 Introduction

The materials and methods presented here are specific to the analysis conducted in this chapter, particularly with reference to the analysis of colour and pattern. Details of materials and methods relating to the sample sites, field sampling, collection and initial analysis of DNA samples are provided in Chapter 2.

5.2.2 Data Collection

Photographs were taken of the back patterns of all the lizards sampled for this study using a Canon PowerShot A720IS digital camera in automatic settings and without using a flash. The lizards were held in the hand and all photographs were taken in natural shade. As the initial purpose of the photograph was to enable identification of individuals in order to prevent resampling, no other attempt was made to control the lighting conditions of the photograph.

The primary aim of this chapter was to investigate perceived differences in the colour and pattern of *L. agilis* from the three regions of Great Britain in which it occurs. Although both sexes appear to exhibit differences in colour and pattern, differences are more marked in the males, particularly their green breeding colouration, and therefore the analysis was restricted to male lizards. As the aim was to compare the main geographical areas, lizards from Ainsdale, Frensham Common and Bergherbos in the Netherlands were included. Merritown Heath and Wareham Forest were used to represent lizards from east and west Dorset respectively, with these sites selected on the basis of availability sufficient photos of post-slough male lizards. Twelve male lizards from each site were analysed excluding any that were younger than in their third year (as assessed by size and colouration) and therefore may not have developed their full breeding colours (Simms 1970). When sand lizards emerge from hibernation they will bask extensively and then shed their skin before attaining their full breeding colouration (Olsson & Madsen 1996). Therefore, animals which had not yet shed their skin were also excluded, however the majority of males from Frensham Common were sampled prior to their first slough and as these were the only representatives from Surrey they could not be excluded. Therefore, only pattern was characterised for these animals.

5.2.3 Characterisation of Colour and Pattern

Colour for each animal was assessed using Adobe Photoshop Elements v8 (Adobe Systems Incorporated, California), which enables the measurement of the red, green and blue (RGB) components of each pixel within the digital image (Fitze & Richner 2002). The RGB colour model is an additive colour model in which a colour is composed from differing proportions of

red, green and blue light. Two green scales on each flank were selected and the RGB colour components recorded from the middle of the scale. As RGB colour scores are dependent on the system used (in this case AdobeRGB 1998) (Adobe Systems Incorporated 2005), they were averaged and converted to a percentage so that the total percentage of red, green and blue = 100. Previous studies using this method to analyse colour have taken photographs under controlled lighting conditions (Fitze & Richner 2002). As the photographs for this study were taken under field conditions, the environmental light at the time of the photograph may influence the colour as recorded by the photograph. This was controlled for as much as possible by taking multiple measurements on both flanks so that the effect of any sources of reflected light on one side of the lizard was reduced.

Six other characteristics of the dorsal and flank pattern were also recorded (Figure 5.2 and Table 5.1). Many of these were measured using a scale encoding technique (Sacchi *et al.* 2007) where the morphology of individual scales is recorded and the number of scales with a particular characteristic is expressed as a proportion of the total. For example, the presence and completeness of a dorsal line was recorded by counting the total number of scales along the centre of each lizard's back between the pectoral and pelvic girdles. The completeness of the dorsal line was expressed as the quantity of white scales as a percentage of the total

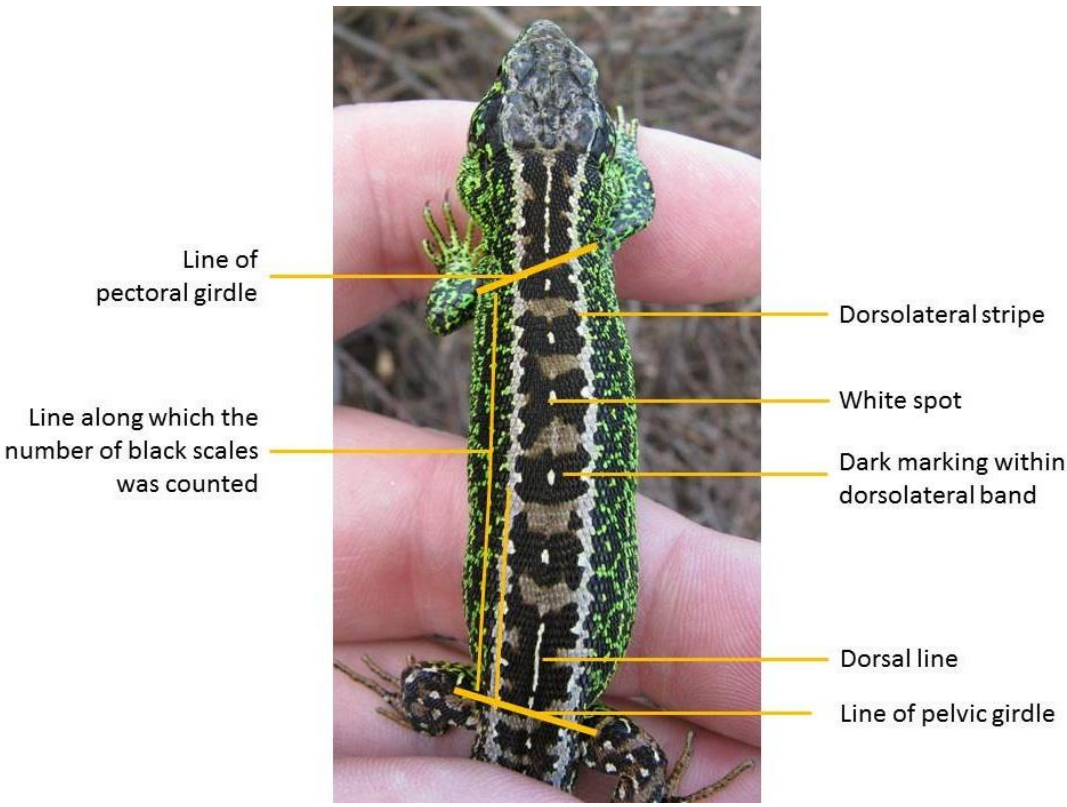

Figure 5.2. Pattern characteristics measured for each lizard, see Table 5.1 for a full explanation of each characteristic.

Table 5.1. Pattern and colour characteristics recorded in the study and how they were calculated. See Figure 5.2 for an illustration for each characteristic.

Abbreviation	Characteristic	Description
COLR	Red colour	% of red within the flank scale colour.
COLG	Green colour	% of green within the flank scale colour.
COLB	Blue colour	% of blue with the flank scale colour.
DOLN	Dorsal line	% of the dorsal line complete between the pectoral and pelvic girdles. Measured as the number of scales along the centre of the back coloured white as a proportion of the total.
DLST	Dorsolateral stripes	Width of the dorsolateral stripes. Measured as the number of light-coloured scales between the flank and dark-coloured dorsal band. Four counts were made at different locations along the stripe and an average used.
DLSM	Dorsolateral stripe markings	Number of dark spots or other markings within the dorsolateral stripes between the pectoral and pelvic girdles. Such markings may be entirely within the stripe, but also include features such as scallops. Markings were counted if they crossed the centre of the dorsolateral.
DOWS	White spots	Number of white spots or other white markings within the dorsal band between pectoral and pelvic girdles.
DOBM	Dorsal band markings	Number of dark markings within the dorsal band between pectoral and pelvic girdles.
BLKM	Black flank markings	Black (or dark-coloured) scales on the flanks, scales recorded as 1 (all black), 0.5 (partially black) or 0 (all green). Scales were counted along a single line passing through any ocelli. Therefore, this characteristic also provides a measure of the number and intensity of ocelli.

5.2.4 Data Analysis

Significant differences in each colour and pattern characteristic between sample sites were investigated for each characteristic using a Kruskal-Wallis one way analysis of variance. Principal Components Analysis (PCA) was used to assess whether it was possible to distinguish between lizards from the different sites on the basis of colour and pattern. The PCA creates new sets of variables from the dataset which explain as much of the variation in the data as possible, these can then be plotted against each other to reveal clusters of related cases. As it was not possible to quantify the colour of lizards from Frensham Common, two separate PCAs were performed, one including all sample sites in which colour was excluded as a variable and a second including colour as a variable but excluding lizards from Frensham Common.

In order to create a single variable to represent in colour (COLT) in the PCA, an initial PCA was conducted using the proportions of red, green and blue as the variables. The first Principal Component explained 79.568% of the variance in the colour data and this was used as a proxy

for colour in the subsequent PCA and Bayesian analysis. All statistical procedures were undertaken in MYSTAT v12 (Systat Software, Chicago, USA).

Pattern and colour data were also analysed using GENELAND v4 (Guillot *et al.* 2005; Guillot *et al.* 2008; Guillot *et al.* 2012), a Bayesian assignment program which uses genotypic, phenotypic and spatial data to determine the true number of populations (*K*). Three separate analyses were carried out: the first using only genotype data; the second only phenotype data; and the third using both phenotype and genotype data. Spatial data were not used. When running GENELAND, an uncorrelated alleles model was used with a Markov chain Monte Carlo (MCMC) chain length of 1,000,000 and a thinning factor of 100. Ten separate runs were carried out in each analysis and the one with the highest posterior log probability was resampled with a burn in of 20,000 to obtain the population membership probability of each individual. Previous Bayesian assignment analyses using STRUCTURE (Pritchard *et al.* 2000) and BAPS (Corander *et al.* 2008) identified each of the populations analysed within this chapter as distinct (Chapter 3 and Figure 3.2), therefore the maximum number of *K* was set to 5.

5.3 Results

5.3.1 Colour Analysis

No significant difference in the green component of the RGB colour model was observed between the sites (Kruskal-Wallis one-way test: $H = 7.526$, $P = 0.057$), however there were significant differences between sites in the proportion of red ($H = 16.594$, $P = 0.001$) and blue ($H = 13.247$, $P = 0.004$) (Figure 5.3). Lizards from Ainsdale had the lowest proportion of blue within their flank colour (and highest proportion of red) between all the sites and once the samples from Ainsdale were removed from the analysis, the difference in the proportion of red in the flank colour was significant between sites ($H = 7.518$, $P = 0.023$) and the proportion of blue was insignificant ($H = 1.653$, $P = 0.438$).

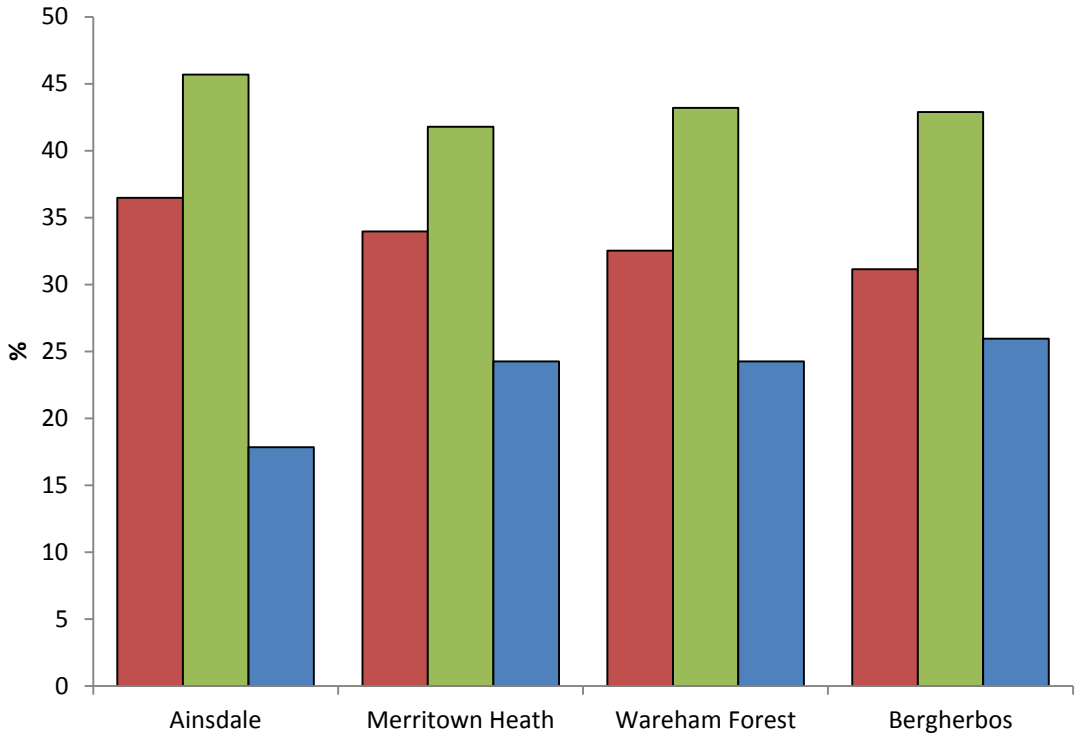

Figure 5.3. Proportion (percentage) of the red, green and blue (RGB) components in the flank colour of male *Lacerta agilis* from the sampled sites.

5.3.2 Pattern Analysis

All pattern characteristics exhibited significant differences between sites (Kruskal-Wallis one-way test: DOLN, $H = 26.08$, $P < 0.001$; DLST, $H = 27.40$, $P < 0.001$; DLSM, $H = 18.06$, $P = 0.001$; DOWS, $H = 16.87$, $P = 0.002$; DOBM, $H = 32.00$, $P < 0.001$; BLKM, $H = 38.98$, $P < 0.001$). Ainsdale lizards typically had a more complete dorsal line, wider dorsolateral stripes containing fewer dark markings, and considerably less black on their flanks than lizards from the other sites (Figure 5.4a, b, c and f). Ainsdale lizards also typically had fewer white spots than those from Frensham Common and the two Dorset sites, but a similar number to lizards from Bergherbos (Figure 5.4d). The number of dark markings within the dorsal band was less variable across the sites with the exception of Merritown Heath where the sample included a number of animals with all the dorsal band markings joined into a single dark marking covering the entire dorsal band (Figure 5.4e).

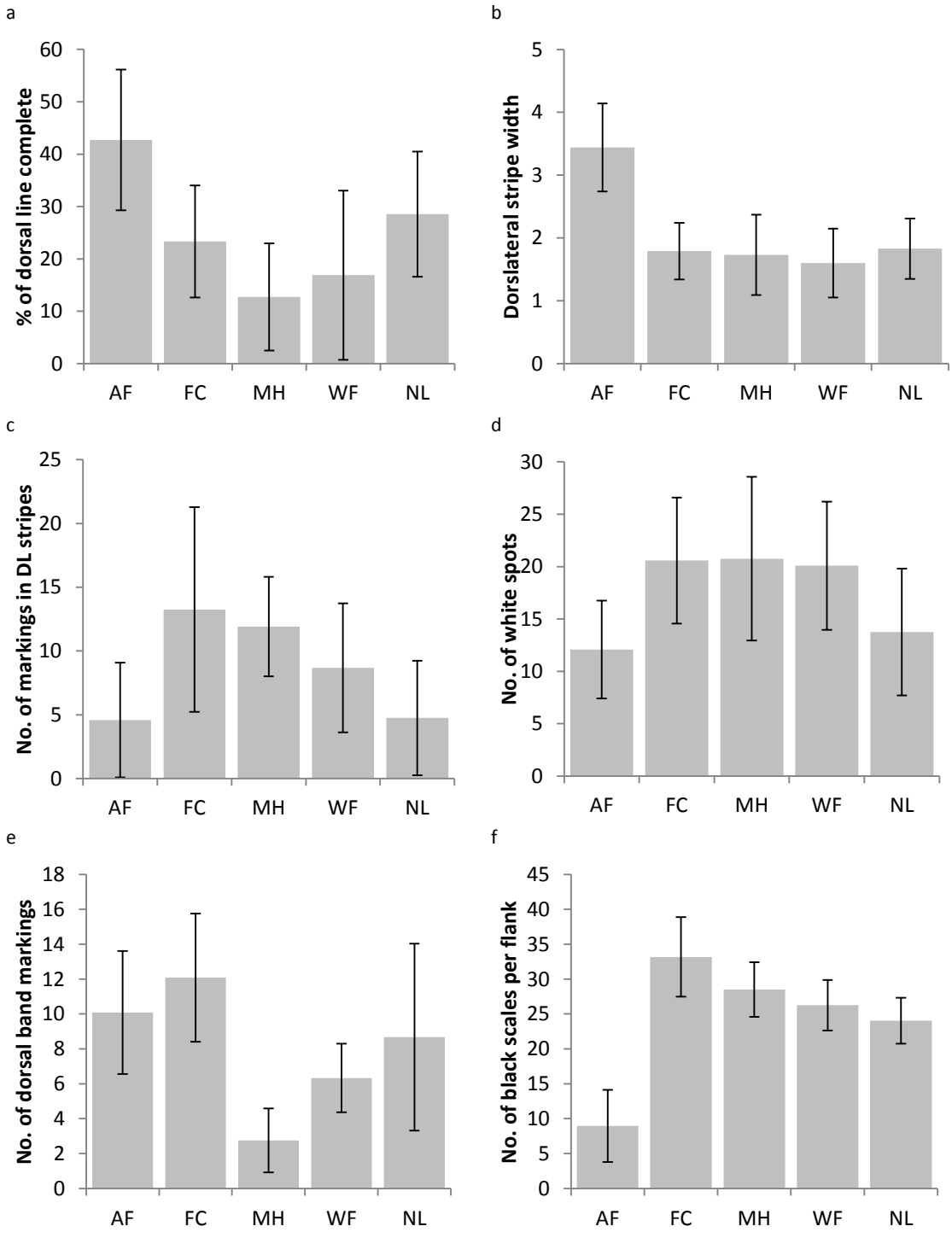

Figure 5.4. Differences in pattern characteristics between sample sites, error bars = one standard deviation. a) completeness of the dorsal line (DOLN); b) width of dorsolateral stripes (DLST); c) number of markings within the dorsolateral stripes (DLSM); d) number of white spots (DOWS); e) number of markings within the dorsal band (DOBM); f) number of black scales per flank (BLKM). AF = Ainsdale, FC = Frensham Common, MH = Merritown Heath, WF = Wareham Forest, NL = Bergherbos.

5.3.3 Multivariate Analysis

The PCA including colour as a variable (whilst excluding Frensham Common as a sample site) resulted in a reasonably clear separation of Ainsdale from the other sample sites along the first Principal Component. The two Dorset sites were also reasonably well separated from the other sites although with a slight overlap between them. BLKM was the most significant contributor to the PCA on the negative side and DOLN and DLST on the positive side (Table 5.2 and Figure 5.5), therefore the PCA separates animals with more black on their flanks on the negative side and a more complete dorsal line and wider dorsolateral stripes on the positive side. The PCA which excluded colour but included the Frensham Common samples showed a similar pattern to the first PCA, however sample sites were less clearly separated (Table 5.3 and Figure 5.6). The relative importance of each characteristic was similar in both PCAs.

Table 5.2. Results of the first PCA including colour as a variable but excluding samples from Frensham Common, the first four Principal Components are shown.

		PC1	PC2	PC3	PC4
Eigenvalue		3.198	1.289	0.906	0.563
% of explained variance		45.684	18.408	12.940	8.047
Cumulative % of explained variance		45.684	64.092	77.032	85.079
Contribution of individual variables to the factor	COLT	0.497	0.581	0.350	0.508
	DOLN	0.763	0.146	-0.071	0.019
	DLST	0.779	0.034	0.386	-0.389
	DLSM	-0.580	0.692	-0.119	-0.078
	DOWS	-0.661	0.570	0.067	-0.284
	DOBM	0.517	0.189	-0.780	0.037
	BLKM	-0.849	-0.299	0.049	0.255

Table 5.3. Results of the second PCA excluding colour as a variable but including samples from all sample sites, the first four Principal Components are shown.

		PC1	PC2	PC3	PC4
Eigenvalue		2.789	1.175	0.793	0.516
% of explained variance		46.487	19.576	13.220	8.594
Cumulative % of explained variance		46.487	66.063	79.283	87.877
Contribution of individual variables to the factor	DOLN	-0.722	0.392	0.116	0.447
	DLST	-0.807	-0.130	0.346	-0.175
	DLSM	0.675	0.452	0.324	0.309
	DOWS	0.682	0.022	0.619	-0.213
	DOBM	-0.179	0.894	-0.171	-0.374
	BLKM	0.815	0.022	-0.378	0.063

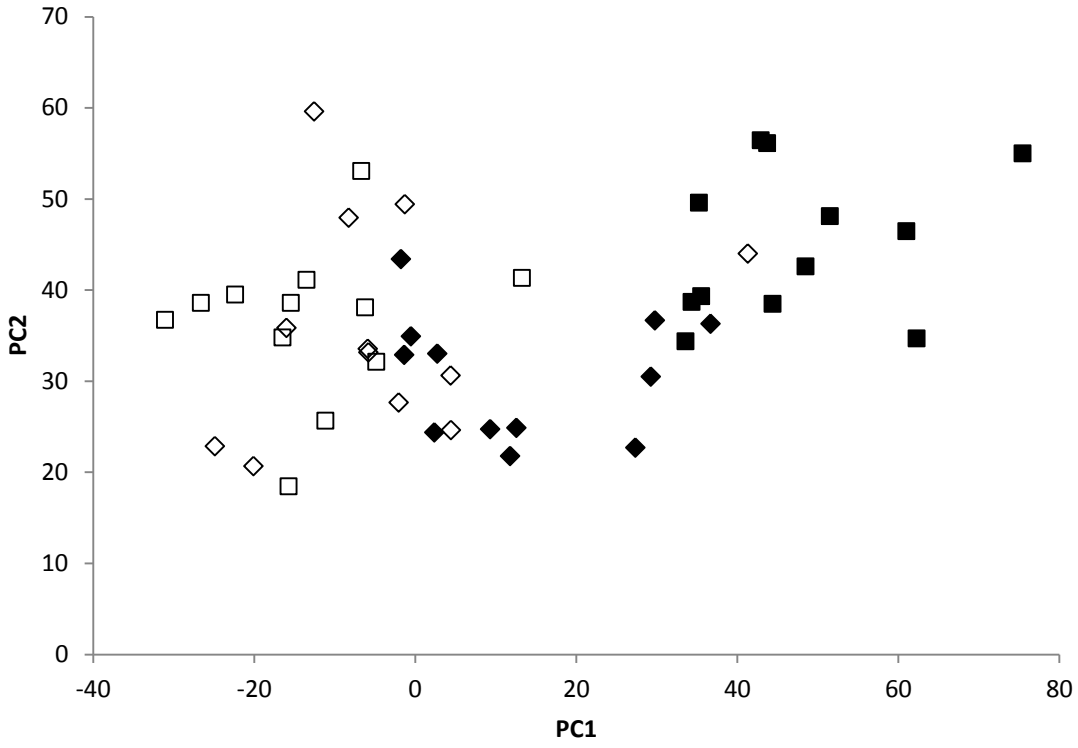

Figure 5.5. Scatterplots for the PCA including colour as a variable, but no samples from Frensham Common. PC1 x PC2 and PC1 shown. Filled squares = Ainsdale, open squares = Merritown Heath, open diamonds = Wareham Forest, filled diamonds = Bergherbos.

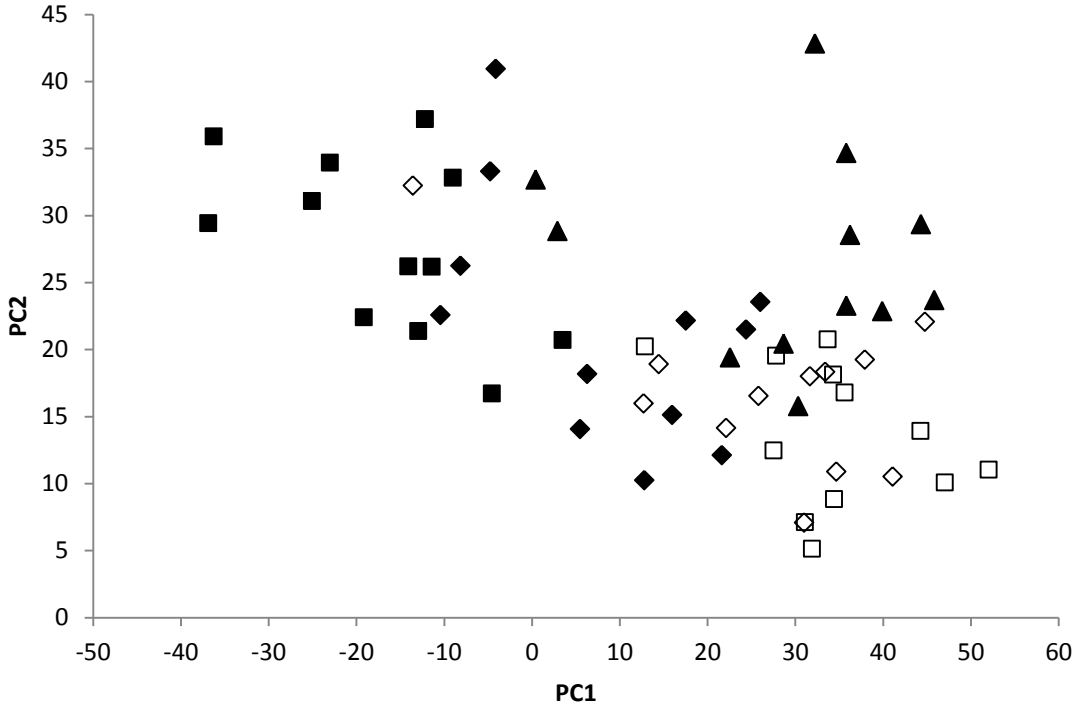

Figure 5.6. Scatterplots for the PCA excluding colour as a variable, but including all sample sites. PC1 x PC2 shown. Filled squares = Ainsdale, filled triangles = Frensham Common, open squares = Merritown Heath, open diamonds = Wareham Forest, filled diamonds = Bergherbos.

5.3.4 Bayesian Assignment

Using genotype data only, GENELAND identified five populations (Table 5.4). The assignment of each individual corresponded to its sample site with the exception of two from Merritown Heath, one of which was assigned to Frensham Common and one to Wareham Forest. Most lizards were assigned with a probability > 0.9 with the exception of two lizards each from Merritown Heath and Wareham Forest. Two populations were identified using phenotypic data; all Ainsdale lizards were assigned to one population and all the southern British animals were assigned to a second with the exception of four individuals. Bergherbos lizards were assigned with 50% in each population. The combined genotype and phenotype data identified Ainsdale and Bergherbos as well separated populations, however the three southern British populations were not well defined with most individuals having a probability of 0.49 – 0.52.

Table 5.4. GENELAND assignment of individuals to populations. No genetic data was available for samples 558 and 580.

Sample no.	Sample site	Population assignment (probability)		
		Genotype	Phenotype	Genotype + Phenotype
543	Ainsdale	1 (1)	1 (1)	1 (0.99)
545	Ainsdale	1 (1)	1 (0.99)	1 (0.99)
547	Ainsdale	1 (1)	1 (1)	1 (0.99)
551	Ainsdale	1 (1)	1 (1)	1 (0.99)
552	Ainsdale	1 (1)	1 (1)	1 (0.99)
557	Ainsdale	1 (0.99)	1 (1)	1 (0.99)
558	Ainsdale	n/a	1 (1)	1 (0.99)
563	Ainsdale	1 (1)	1 (1)	1 (0.99)
564	Ainsdale	1 (1)	1 (0.99)	1 (0.99)
566	Ainsdale	1 (1)	1 (1)	1 (0.99)
567	Ainsdale	1 (1)	1 (1)	1 (0.99)
572	Ainsdale	1 (0.99)	1 (1)	1 (0.99)
455	Frensham Common	2 (1)	2 (0.99)	2 (0.52)
456	Frensham Common	2 (1)	2 (0.85)	2 (0.52)
458	Frensham Common	2 (1)	2 (0.99)	2 (0.52)
461	Frensham Common	2 (1)	2 (0.99)	2 (0.52)
463	Frensham Common	2 (1)	1 (0.98)	2 (0.52)
464	Frensham Common	2 (1)	2 (0.99)	2 (0.52)
465	Frensham Common	2 (1)	2 (0.98)	2 (0.52)
466	Frensham Common	2 (0.99)	2 (0.99)	2 (0.52)
467	Frensham Common	2 (1)	1 (0.93)	2 (0.52)
471	Frensham Common	2 (1)	2 (0.99)	2 (0.52)
475	Frensham Common	2 (1)	2 (0.99)	2 (0.52)
616	Frensham Common	2 (1)	2 (0.99)	2 (0.52)

Table 5.4 cont.

Sample no.	Sample site	Population assignment (probability)		
		Genotype	Phenotype	Genotype + Phenotype
451	Merritown Heath	3 (0.99)	2 (0.99)	3 (0.52)
453	Merritown Heath	3 (0.92)	2 (1)	3 (0.52)
454	Merritown Heath	3 (1)	2 (1)	3 (0.52)
478	Merritown Heath	3 (0.99)	2 (0.99)	3 (0.52)
479	Merritown Heath	3 (0.98)	2 (1)	3 (0.52)
484	Merritown Heath	3 (0.91)	2 (1)	3 (0.52)
487	Merritown Heath	3 (1)	1 (0.54)	3 (0.52)
521	Merritown Heath	2 (0.73)	2 (0.99)	3 (0.52)
522	Merritown Heath	3 (1)	2 (0.98)	3 (0.52)
628	Merritown Heath	4 (0.78)	2 (0.99)	3 (0.50)
631	Merritown Heath	3 (0.99)	2 (0.99)	3 (0.52)
632	Merritown Heath	3 (0.90)	2 (0.99)	3 (0.52)
501	Wareham Forest	4 (1)	2 (0.99)	2 (0.52)
508	Wareham Forest	4 (1)	2 (1)	2 (0.52)
510	Wareham Forest	4 (1)	2 (0.99)	2 (0.52)
512	Wareham Forest	4 (0.99)	2 (0.95)	2 (0.52)
513	Wareham Forest	4 (0.99)	2 (0.98)	2 (0.52)
514	Wareham Forest	4 (0.99)	2 (0.73)	2 (0.52)
515	Wareham Forest	4 (0.99)	2 (0.99)	2 (0.52)
516	Wareham Forest	4 (1)	2 (0.99)	2 (0.52)
518	Wareham Forest	4 (0.99)	2 (0.92)	2 (0.52)
645	Wareham Forest	4 (0.60)	1 (0.99)	4 (0.97)
646	Wareham Forest	4 (0.62)	2 (0.99)	2 (0.52)
667	Wareham Forest	4 (0.95)	2 (0.99)	2 (0.52)
578	Bergherbos	5 (0.99)	2 (0.99)	4 (0.98)
580	Bergherbos	n/a	2 (0.98)	2 (0.49)
583	Bergherbos	5 (0.89)	2 (0.91)	4 (0.85)
584	Bergherbos	5 (1)	1 (0.99)	4 (0.98)
586	Bergherbos	5 (1)	1 (0.94)	4 (0.98)
591	Bergherbos	5 (1)	1 (0.97)	4 (0.98)
592	Bergherbos	5 (0.99)	1 (0.53)	4 (0.98)
596	Bergherbos	5 (1)	2 (0.98)	4 (0.98)
597	Bergherbos	5 (0.99)	2 (0.97)	4 (0.98)
601	Bergherbos	5 (1)	1 (0.98)	4 (0.98)
602	Bergherbos	5 (1)	1 (0.85)	4 (0.98)
606	Bergherbos	5 (1)	2 (0.85)	4 (0.98)

5.4 Discussion

5.4.1 Geographical Variation in Colour and Pattern

British *Lacerta agilis* exhibited significant variation in colour and pattern across their range. In particular, lizards from Merseyside were distinct from the southern populations of Dorset and Surrey as assessed by multivariate statistics and Bayesian assignment. Ainsdale lizards typically had a smaller proportion of blue in their flank colour, were more striped with fewer black scales on their flanks and fewer white spots within their dorsolateral band than lizards from the southern British populations. There were some differences between the Dorset and Surrey populations; however there was a significant overlap in their characteristics. In both the multivariate and Bayesian assignment analysis, lizards from Bergherbos in the Netherlands were intermediate between the Merseyside and southern British populations.

Lacerta agilis colonised Britain via a land bridge across the North Sea and diverged from continental European populations approximately 8,000 years BP. In Chapter 3 of this thesis, divergence times and ancestral population sizes for the British *L. agilis* populations were estimated using the program IMa2 (Hey 2010a). Since their divergence, the Bergherbos population has maintained a larger effective population size (N_e = 3522.5) compared to the British populations (Frensham Common, N_e = 387.5; Town Common (a Dorset population); N_e = 1390; no reliable estimates were obtained for Ainsdale, although using a different estimation method (Peel *et al.* 2004) it was significantly lower than the other British populations). Given its location and large, stable population size, the Bergherbos population is likely to bear a closer resemblance to the ancestral British population as it would be less affected by genetic drift and therefore would be expected to share characteristics with all the British populations.

Previous authors have suggested the difference in pattern and colouration between heathland and sand dune populations may be a result of selection for crypsis (Simms 1970; Beebee & Griffiths 2000). Sexual selection appears to be the primary driver for male flank colouration in *L. agilis* (Anderholm *et al.* 2004; Olsson *et al.* 2005b), however colouration in Lacertids often represents a trade-off between advertising fitness to mates and rivals and crypsis to reduce predation risk (Carretero 2002). Male *Lacerta bilineata* are more vulnerable than females to aerial predators due to their behaviour (Costantini *et al.* 2007) and therefore cryptic colouration may be beneficial, however Olsson (1993b) found no difference in predation risk for bright green male-patterned *L. agilis* models compared to cryptically coloured models. Characteristics such as the pattern of stripes and spots which differ between the Merseyside populations from sand dune habitat and the Dorset and Surrey populations from heathland

habitat may play a role in camouflage adapted to suit their specific habitat. However, *L. agilis* which inhabit sand dune habitat in the Netherlands are not noticeably different to those from heathland sites (H. Strijbosch, pers. comm.), and lizards from the only large sand dune population in Dorset appear to bear a closer resemblance to those from nearby heathland sites than to Merseyside lizards (pers. obs.) with heavily marked flanks, narrow dorsolateral stripes and many white spots (Figure 5.7). The Merseyside population has a very low effective population size compared to populations from Surrey, Dorset and the Netherlands (Chapter 3 of this thesis) and is also isolated from other non-sand dune populations. Therefore changes in phenotype could occur relatively quickly as a result of selection pressure. Further research is required to provide clear evidence of selection such as characterising the colour and pattern of larger sand dune populations or predation risk experiments such as those of Olsson (1993b).

Figure 5.7. Male *Lacerta agilis* from Studland, a sand dune and heathland site in Dorset. Lizards from this site appear closer in colour and pattern to those from nearby heathland sites than those from sand dune sites in Merseyside.

5.4.2 The Importance of Being Green

Olsson *et al.* (2011a) demonstrated a spectral reflectance peak at a wavelength of 540 nm in the flanks of male *L. agilis*, which is in the centre of the range of green light in the visible spectrum. The primary function of the green flank colouration is as an intraspecific indicator of fitness, which is important for signalling fighting ability to rival males and is correlated with

breeding success (Olsson 1994b; Anderholm *et al.* 2004; Olsson *et al.* 2005b; Olsson *et al.* 2011a). Consequently, should a male lizard be less 'green' than a rival, he would be at a reproductive disadvantage and there would be a significant selection pressure for 'greenness'. Whilst there was a significant difference in the flank colour of Merseyside lizards compared to the other populations assessed, the green component of this colour did not differ significantly. Therefore, despite differences in other parts of the reflectance spectra of the males' flanks, the importance of 'greenness' is consistent across all populations.

Sexually selected colour (including UV) traits as indicators of fitness are common in the Lacertidae, including orange head coloration in *Psammodromus algirus* (Martin & Forsman 1999; Salvador & Veiga 2001), blue throat colouration in *Lacerta viridis* (Bajer *et al.* 2011; Molnar *et al.* 2012) and *L. schreiberi* (Martin & Lopez 2009) and blue spots on the flanks of *Timon lepidus* (Font *et al.* 2009) and *Iberolacerta monticola* (Lopez *et al.* 2004). As with many sexual signals, bright colouration is a disadvantage as it is energetically expensive to produce (Olson & Owens 1998) and requires males to spend more time basking to maintain (Bajer *et al.* 2012), leaving them more vulnerable to predation (Costantini *et al.* 2007) and therefore is an honest indicator of fitness (Molnar *et al.* 2012).

5.4.3 Conclusions

This study confirms the observations of Simms (1970) and Beebee & Griffiths (2000) who commented on the apparent differences in colour and pattern of *Lacerta agilis* between the three geographical areas of Great Britain in which they occur. Lizards from Merseyside are particularly distinct and whilst those from Dorset and Surrey do have some differences, there is a considerable overlap in characteristics. Despite geographical variation in colour, sexual selection for 'greenness' ensures that the green component of flank colouration is constant across the different areas. Further work is required to elucidate the underlying causes of the observed differences, however the small effective size of the Merseyside population means that phenotypic changes are likely to occur more rapidly.

6 GENERAL DISCUSSION

6.1 Introduction

Patterns of genetic diversity within a species are the result of historical biogeography and contemporary genetic processes such as gene flow and genetic drift. An understanding of the influences on the population genetics of a species will aid its practical conservation and enable conservation practitioners to target resources appropriately. The research presented in this thesis gives a comprehensive overview of the population genetics of *Lacerta agilis* in Great Britain. This chapter summarises the findings of the research and considers the implications for conservation of *Lacerta agilis* in the context of current habitat fragmentation and predicted global climate change.

6.2 Summary of Thesis Aims and Results

The primary objective of this research was to increase knowledge of the conservation genetics and inform the future conservation of *L. agilis* in Britain. Specifically it aimed to quantify genetic diversity within *L. agilis* populations, establish how Britain was colonised by sand lizards and explore the factors responsible for their current disjunct distribution and identify the effect of historical and contemporary landscape configuration of population genetics. In addition, the genetics of two anthropogenic populations were assessed and differences in colour and pattern between the British populations investigated.

Dorset is the stronghold of *L. agilis* in Great Britain (Corbett 1988b, 1994; Beebee & Griffiths 2000). All of the Dorset populations sampled as part of this study were genetically diverse, even those with a small effective population size. Levels of genetic diversity were similar to European populations nearer to their glacial refugium and were considerably higher than published values for populations in Sweden and for sympatric smooth snake *Coronella austriaca* populations occupying the same sites in Dorset. However, genetic diversity within the Surrey and Merseyside populations, which have historically had smaller population sizes, was significantly lower and comparable to small, fragmented populations in Sweden. Genetic structuring was significant across Dorset, even between populations which were geographically close with few obvious barriers to dispersal. However, there was greater evidence of migration within an unfragmented landscape where individual populations could

not be separated using a variety of Bayesian assignment methods, compared to a fragmented landscape where most populations were well separated.

The divergence of British *L. agilis* from continental European populations was estimated at approximately 8,000 years BP which coincides with the last presence of a land bridge across the North Sea. Following colonisation, sand lizards underwent a range expansion during a period of climate warming which reached a peak approximately 5,000 years BP, by which time it had reached the limits of its current distribution. The timing of subsequent divergence of populations within Britain coincided with periods of cooler and wetter climate, which has restricted sand lizards to specific habitats such as heathland and sand dunes where the substrate and local climate provides suitable conditions for successful breeding and incubation of egg clutches.

In Chapter 4, a resistance surface was used to demonstrate that contemporary patterns of genetic diversity across Dorset are influenced by distance and landscape features such as habitat type, natural barriers such as rivers and anthropogenic barriers such as roads and development. Natural barriers had a significant influence on patterns of genetic diversity at a local and regional scale. Habitat type was also an important component of multiple regression models to predict genetic distance between populations but its effect was more apparent at a local scale than at a regional scale. Artificial barriers were also important at a regional scale, however their effect was not as significant as that of natural barriers. This is likely to be the result of a genetic time-lag and their full effect of these is yet to be realised. These results are consistent with previous work where recent habitat fragmentation was demonstrated to be causing a shift from equilibrium between gene flow and genetic drift to the dominance of genetic drift (Hutchison & Templeton 1999).

The colour and pattern of male *L. agilis* was shown to vary between the three geographical areas of Great Britain in which it occurs. Lizards from Merseyside were particularly distinct in colour and pattern with a smaller proportion of blue in their flank colour, more prominent stripes, fewer black scales on their flanks and fewer white spots within their dorsolateral band than lizards from the southern British populations. Some differences between the Dorset and Surrey populations were observed but there was a significant overlap in pattern characteristics. Lizards from Bergherbos in the Netherlands had shared pattern characteristics with the Merseyside and southern British populations but were more similar in colour to Dorset and Surrey animals. Green flank colouration is an important sexual signal in *L. agilis* and despite differences in the overall colour, the proportion of green in the flank colour was

constant across all populations. The distinctiveness of the Merseyside populations may be a result of an adaptation for crypsis which has occurred over a relatively short period of time due to the small effective population size.

6.3 Current Challenges for the Conservation of *Lacerta agilis*

The status of *Lacerta agilis* in Great Britain is currently considered stable, not in the least part due to the considerable effort of conservation practitioners (Edgar & Bird 2006). However, as they are dependent on a plagioclimax community for which there is no longer an economic or agricultural imperative to maintain, the conservation of sand lizards requires continuous input, particularly habitat management (Corbett & Moulton 1998; Moulton & Corbett 1999; Herpetological Conservation Trust 2009). As the majority of *L. agilis* habitat in Great Britain is now protected, provided this effort can be maintained, suitable habitat for this species is likely to persist. However, potential challenges remain.

6.3.1 Low Genetic Diversity in the Surrey and Merseyside Populations

Whilst genetic variability remains high within Dorset, populations from Surrey and particularly Merseyside are significantly less diverse and are therefore these populations are at a higher risk of genetic stochasticity. Many lizard species, including *L. agilis*, have reproductive strategies which avoid inbreeding such as the preferential selection of genetically different mates (Olsson *et al.* 1999; Olsson *et al.* 2003), and this may enable these populations to maintain current levels of genetic diversity provided environmental conditions remain favourable. However, in the event of environmental changes such as a shift to unfavourable climate conditions or habitat loss through anthropogenic activity or natural succession, these populations are less likely to possess sufficient variability to adapt.

6.3.2 The Effects of Fragmentation and Isolation

The habitat of *L. agilis* throughout Britain has become increasingly fragmented and isolated in recent years (Moore 1962; Jackson 1979; Webb 1986; Hooftman & Bullock 2012). However, the genetic effects of this appear limited at present, with populations in Dorset maintaining high levels of genetic diversity. Significant structuring was apparent between populations within Dorset and anthropogenic fragmentation has accentuated this despite significant structuring in unfragmented landscapes (Chapters 3 and 4). The full genetic effects of fragmentation may be obscured by a genetic time-lag which could become apparent in future generations (Richmond *et al.* 2009; Landguth *et al.* 2010; McCoy *et al.* 2010). Sand lizards may also be able to counter the potential consequences of fragmentation through inbreeding

avoidance mechanisms (Olsson *et al.* 1999; Olsson *et al.* 2003). The Branksome Chine population has been isolated from other large populations for approximately 100 years and may provide some indication of the effect of long-term isolation. This population shows evidence of a genetic bottleneck, a lower effective population size and slightly lower diversity than the other Dorset populations. However, genetic diversity within this population is still relatively high when compared to the Surrey and Merseyside populations and previously published estimates of diversity in Swedish populations (Gullberg *et al.* 1998; Madsen *et al.* 2000).

6.3.3 The Captive Breeding and Reintroduction Programme

One of the main approaches to *L. agilis* conservation in Britain is the captive breeding and re-introduction programme, which has been highly successful in re-establishing the species on sites within its former range (Corbett & Moulton 1998; Moulton *et al.* 2011). This study (Chapter 3) assessed the genetics of two anthropogenic populations: Crooksbury Common and Aberffraw. Although neither of these populations were created following the protocol of the captive-breeding and release programme, they do provide some insight into the genetics of translocated populations. The Crooksbury Common population in Surrey was created from animals translocated from development sites in Dorset during the 1960s and 1970s. Although genetic diversity was comparable with the local Surrey population, it was lower than for Dorset populations located close to the original donor sites and therefore genetic diversity may have been reduced as a result of the translocation. Genetic diversity within the Aberffraw population was considerably lower than all the natural populations. However, details of its origins, such as how long it has been established and the size of the founder population, are unknown. New sites within the captive breeding programme are established by the phased release of approximately 150 juvenile lizards bred from captive stock (Corbett & Moulton 1998; Moulton & Corbett 1999). Given the relatively small number of adults from which the released animals are bred, it is possible that these populations may be vulnerable to founder effects (Fitzsimmons *et al.* 1997; Miller *et al.* 2009; Miller *et al.* 2011) and have less potential for adaptation to environmental change. Some lizard species including *L. agilis* are able to avoid inbreeding through various mechanisms such as mate selection and sperm competition (Olsson *et al.* 1999; Olsson *et al.* 2003) which may compensate for low genetic diversity to a certain extent. Nevertheless, an assessment of the genetic diversity of reintroduced populations is advisable and should diversity be low, measures such as supplementary releases of juveniles from different parents and the exchange of animals between release sites should be considered.

6.4 Potential Effects of Climate Change

Global climate change is predicted to have a mixed effect on Europe's reptile populations, however many species, particularly those with a northern distribution, are predicted to benefit from warmer conditions (Araujo *et al.* 2006). Recent climate projections (based on a medium increase in CO_2 emissions) for Great Britain predict an increase in both summer and winter mean temperatures of 3.9 °C and 2.8 °C respectively (central estimate). Annual mean precipitation is predicted to remain similar to current levels. However, there will be a significant shift to wetter winters and drier summers (Murphy *et al.* 2009).

Climate is vitally important for the successful reproduction of *L. agilis*, particularly for the incubation of egg clutches (Rykena 1987; Elbing 1993). Jackson (1978) implicated low May sunshine in a decline in sand lizard number in Merseyside in the 1960s and 1970s. Although the methodology of this research was later questioned (Langton 1988), the importance of spring temperatures and sunshine in many aspects of the reproductive ecology of *L. agilis* is undeniable. For example, warm temperatures and UV light are required by males post-hibernation to produce breeding colouration (Olsson *et al.* 2011a; Bajer *et al.* 2012), warm spring temperatures lead to earlier clutches (Olsson & Shine 1997b) and more multiple paternity clutches (Olsson *et al.* 2011b) resulting in fitter hatchlings. Positive responses to warmer temperatures have been recorded in many lacertids including increased fitness (Chamaille-Jammes *et al.* 2006), improved feeding and digestion (Van Damme *et al.* 1991; Pafilis *et al.* 2007) and improved predation avoidance ability (Martin & Lopez 2010b; Steen *et al.* 2011).

Thomas *et al.* (1999) predicted that a 2-3 °C increase in temperature could result in a sizable increase in suitable habitat for *L. agilis* in Dorset in terms of total area availability and temporal availability due to a slowing of the rate of vegetation succession. In other parts of its range, *L. agilis* is able to utilise a wider variety of habitats than in Great Britain including grassland, steppe and agricultural margins (Arnold & Ovenden 2002; Gasc *et al.* 2004) and Godinho *et al.* (2005) cited a high dispersal ability as an explanation for its rapid postglacial colonisation of Europe. Therefore, in a more favourable climate, the potential exists for British *L. agilis* to expand beyond its heathland habitat as the availability of suitable habitat increases and warmer temperatures enable colonisation of other, previously unoccupied habitat types. In addition, warmer spring and summer temperatures would confer reproductive advantages including earlier clutches and the possibility of females producing two clutches per year.

In order for *L. agilis* to benefit from range expansion due to climate change, it must be able to disperse into previously unoccupied habitat. *Lacerta* (*Timon*) *lepidus* inhabits the Iberian peninsula and southern France and, like *L. agilis*, it could potentially expand its range northward in a warmer climate. However, Grillet *et al.* (2006) found that its dispersal potential was limited by anthropogenic habitat fragmentation. Barriers preventing expansion to the north resulted in reduced genetic diversity in *Lacerta schreiberi* populations as the suitability of habitat to the south was reduced due to increasing temperatures (Roedder & Schulte 2010). Although warmer temperatures may increase the variety of habitats available to some species, its effects are not predictable and a potential link between warming temperatures and reduced dispersal behaviour has been observed in *Lacerta* (*Zootoca*) *vivipara*. Phenotypically different *L. vivipara* vary in their dispersal behaviour with reticulated individuals taking longer to cross open areas than those with stripes (Zajitschek *et al.* 2012). Furthermore, Lepetz *et al.* (2009) found a positive correlation between average mean temperature and the proportion of reticulated animals in a long-term study. A separate study also linked increasing temperatures to reduced dispersal behaviour in *L. vivipara* (Massot *et al.* 2008).

The level of genetic structuring found within *L. agilis* populations in this study (Chapter 3) implies that dispersal ability in this species may be limited even within unfragmented landscapes, and rivers form a significant barrier to gene flow (Chapter 4). Despite the potential constraints to dispersal, Isolation by Resistance (IBR) modelling identified areas of low habitat resistance and high gene flow to the north of the current range (Chapter 4, Figure 4.6). Habitat in this area is primarily agricultural but contains a substantial network of hedgerows, and is within the range of habitat types in which *L. agilis* is found in continental Europe (Arnold & Ovenden 2002; Gasc *et al.* 2004; Ekner *et al.* 2008). This represents a potential area into which *L. agilis* could expand if current climate change predictions are realised. However, the IBR model may underrepresent the effectiveness of anthropogenic barriers such as development and particularly roads due to a genetic time-lag (Richmond *et al.* 2009; Landguth *et al.* 2010; McCoy *et al.* 2010). These barriers may therefore present a significant impediment to the natural range expansion of *L. agilis* in Britain.

Although the projected increase in summer temperatures is likely to be advantageous to *L. agilis*, other aspects of the climate change predictions may be less beneficial. Drought conditions result in lower hatchling survivorship (Strijbosch & Creemers 1988) and can result in more fires which damage habitat causing the loss of heather as heathland is recolonised by grasses (Wessel *et al.* 2004). Warmer, wetter winters may also have negative implications. Hibernation is important in the life-cycle of many European reptiles and warmer winter

temperatures may affect the ability of *L. agilis* to hibernate successfully. Female *L. vivipara* require a sustained period of cold temperature (< 10 °C) in order to complete vitellogenesis (Gavaud 1983, 1991), and warmer-than-average winters have been linked with poorer body condition at emergence from hibernation in common toads *Bufo bufo* (Reading 2007) Whilst many Lacertids are able to tolerate temperatures approaching freezing during hibernation (Grenot *et al.* 2000; Burke *et al.* 2002; Voituron *et al.* 2006), survivorship is lower in wet conditions (Burke *et al.* 2002). A changing climate may also have detrimental effects on the habitat of *L. agilis* in Great Britain such as the loss of coastal sand dune habitats as a result of a predicted increase in the number and intensity of Atlantic storms (Clarke & Rendell 2011).

6.5 Opportunities for Further Research

This thesis has expanded current knowledge of *Lacerta agilis* population genetics and provided an insight into the effects of natural and anthropogenic processes on patterns of genetic diversity. However, as with any scientific research it asks as many questions as it answers. Chapter 3 offers a potential explanation for the current disjunct distribution of *L. agilis* in Great Britain in terms of the climatic conditions required for successful egg incubation and linked this to the potential for range expansion in the event of climate warming. In order to accurately predict how British *L. agilis* would react to climate change, it would be necessary to conclusively determine the factors, be they climatic or otherwise, which both restrict British sand lizards to their current habitat types and may enable them to expand their range should the predicted climate changes occur. Whilst climate warming may present an opportunity for range expansion, this may be restricted by anthropogenic habitat fragmentation. Further genetic monitoring and assessment in the future and the use of historical mapping may allow the quantification of the true effect of barriers such as roads as the genetic time-lag catches up with the present-day landscape.

The sand lizard captive breeding and reintroduction programme has been successful and is highly regarded (Moulton *et al.* 2011). However, Chapter 3 highlighted the potential loss of genetic diversity in two introduced populations. Reintroductions began in the 1990s (Corbett & Moulton 1998) and to date, no assessment of the genetic diversity of introduced populations has been undertaken. Quantifying genetic diversity at reintroduction sites would provide another measure of the success of the programme and would inform ongoing population management decisions.

Gene flow across Dorset was mapped using CIRCUITSCAPE (McRae 2006) in Chapter 4 (Figure 4.6). CIRCUITSCAPE can only estimate gene flow between sampled sites and therefore some areas, particularly at the periphery of the study area, were identified as having low current density, and therefore as unimportant for gene flow. Further genetic sampling in these areas would fill in gaps in the current map and allow gene flow across Dorset to be mapped in greater detail. This may reveal other, previously unidentified, areas which are important for gene flow but were not highlighted within this study.

The existence of the Land Utilisation Survey Maps (Stamp 1931) presents an opportunity to investigate historical gene flow within the study area. Digitisation of the map would enable the creation of an historical resistance surface which would allow a detailed comparison between historical and contemporary patterns of gene flow and genetic variation.

Chapter 5 identified significant geographic variation in the colour and pattern of male *L. agilis*, particularly in the Merseyside population. It is difficult to directly attribute this to a specific driver however, given the small effective population size, phenotypic changes may occur relatively quickly in response to selection pressure, such as that for crypsis. Further research is necessary to provide greater evidence for this hypothesis and predation-risk experiments using appropriately patterned models, as per Olsson (1993b), could be used to explore this further.

6.6 Conclusion

In many respects, sand lizards in Great Britain can be considered a conservation success story as the dramatic declines in the 19th and 20th Centuries have been halted, genetic diversity remains high as a whole and the species is now stable across much of its range. However, the species' dependence on early successional habitats which require active management leaves them vulnerable to future declines and the full genetic effects of anthropogenic habitat loss and fragmentation may not yet be realised. Climate change could present opportunities for sand lizards to increase in both range and number, but their ability to colonise new areas may be limited by human modification of the landscape. This research will assist conservation practitioners in the maintenance of existing sand lizard populations and enable them to maximise any opportunities associated with climate change. Firm foundations have been laid for the conservation of British sand lizards, but ongoing management must build upon these to enable expansion and secure their future.

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APPENDIX 1: PAIRWISE D_{PS} VALUES BETWEEN DORSET SITES

Table A1.1. Pairwise D_{PS} values for all sites within Dorset. HF = Hurn Forest, LH = Lytchett Heath, WP = West Parley, WF = Wareham Forest, MP = Master's Pit, HM = Hartland Moor, NH = Newton Heath, TC = Town Common, RD = Ramsdown, MH = Merritown Heath, FC = Ferndown Common, BC = Branksome Chine.

	WP	LH	WF1	MP	HM	NH	TC	RD	MH	FC	BC	WF2	WF3	WF4	WF5	WF6
HF	0.40161	0.53139	0.44770	0.46219	0.5025	0.50264	0.33809	0.33667	0.35691	0.44136	0.45801	0.39747	0.42671	0.42771	0.40347	0.42998
WP	-	0.53412	0.48125	0.39517	0.50054	0.52343	0.36481	0.42394	0.32748	0.36322	0.53014	0.44478	0.44877	0.46432	0.43994	0.44746
LH		-	0.45625	0.48677	0.52551	0.52881	0.49693	0.51106	0.51979	0.52764	0.42237	0.42642	0.47795	0.46405	0.42968	0.44407
WF1			-	0.3726	0.45979	0.50929	0.49014	0.50148	0.47411	0.46653	0.4784	0.30620	0.28505	0.27877	0.31620	0.26239
MP				-	0.44242	0.46474	0.43076	0.43108	0.43027	0.46383	0.51687	0.39507	0.40986	0.40730	0.39477	0.40994
HM					-	0.33054	0.47283	0.52670	0.49703	0.53356	0.50788	0.46127	0.46427	0.47562	0.44488	0.49372
NH						-	0.49347	0.52647	0.49960	0.51940	0.53499	0.49471	0.50619	0.51281	0.47010	0.52686
TC							-	0.25366	0.35267	0.41731	0.44600	0.42146	0.44797	0.45292	0.39309	0.44265
RD								-	0.36715	0.47576	0.48874	0.42654	0.42741	0.45213	0.41007	0.43986
MH									-	0.34384	0.47518	0.39606	0.40693	0.45319	0.39719	0.43897
FC										-	0.52718	0.46489	0.47676	0.48706	0.48475	0.46425
BC											-	0.40764	0.44892	0.44050	0.42003	0.45396
WF2												-	0.24472	0.28972	0.24116	0.24704
WF3													-	0.23866	0.26474	0.23784
WF4														-	0.25626	0.22669
WF5															-	0.23035

APPENDIX 2: PARAMETERISATION OF HIGH RESISTANCE FEATURES

Table A2.1. Parameterisation of natural barriers in an IBR model. *R* = resistance of "small rivers", *r* = Mantel's correlation coefficient. Significance at the nominal 5% level after an FDR procedure, *P* = 0.009.

R	F_{ST}		G'_{ST}		*D*		D_{PS}	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
2	0.66833	<0.0001	0.69011	<0.0001	0.66621	<0.0001	0.71802	<0.0001
4	0.67153	<0.0001	0.69178	<0.0001	**0.66678**	**<0.0001**	0.71941	<0.0001
8	**0.67281**	**<0.0001**	**0.69226**	**<0.0001**	0.66578	<0.0001	**0.71985**	**<0.0001**
16	0.67192	<0.0001	0.69148	<0.0001	0.66239	<0.0001	0.71930	<0.0001
32	0.66815	<0.0001	0.68888	<0.0001	0.65744	<0.0001	0.71715	<0.0001
64	0.66103	<0.0001	0.68280	<0.0001	0.64758	<0.0001	0.71151	<0.0001
128	0.64648	<0.0001	0.67057	<0.0001	0.63089	<0.0001	0.69929	<0.0001
256	0.62560	<0.0001	0.65005	<0.0001	0.60567	<0.0001	0.67794	<0.0001
512	0.59935	<0.0001	0.62115	<0.0001	0.57233	<0.0001	0.64730	<0.0001
1024	0.56952	0.0003	0.58603	<0.0001	0.53349	<0.0001	0.60985	<0.0001

Table A2.2. Parameterisation of natural barriers in an LCP model. *R* = resistance of "small rivers", *r* = Mantel's correlation coefficient. Significance at the nominal 5% level after an FDR procedure, *P* = 0.009.

R	F_{ST}		G'_{ST}		*D*		D_{PS}	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
2	0.45314	0.0002	0.55134	<0.0001	0.55354	<0.0001	0.58537	<0.0001
4	0.45481	<0.0001	0.55270	<0.0001	0.55477	<0.0001	0.58693	<0.0001
8	0.45618	<0.0001	0.55404	<0.0001	0.55607	<0.0001	0.58851	<0.0001
16	0.46064	<0.0001	0.55752	<0.0001	0.55981	<0.0001	0.59225	<0.0001
32	0.46550	<0.0001	0.56070	<0.0001	0.56309	<0.0001	0.59616	<0.0001
64	0.47872	<0.0001	0.57182	<0.0001	0.57103	<0.0001	0.60834	<0.0001
128	**0.48766**	**<0.0001**	**0.57808**	**<0.0001**	**0.57432**	**<0.0001**	**0.61520**	**<0.0001**
256	0.48766	<0.0001	0.57808	<0.0001	0.57432	<0.0001	0.61520	<0.0001
512	0.48766	0.0003	0.57808	<0.0001	0.57432	<0.0001	0.61520	<0.0001
1024	0.48766	<0.0001	0.57808	<0.0001	0.57432	<0.0001	0.61520	<0.0001

Table A2.3. Parameterisation of artificial barriers in an IBR model. *R* = resistance of the "suburban" LCM2007 habitat type, *r* = Mantel's correlation coefficient. Significance at the nominal 5% level after an FDR procedure, *P* = 0.009.

R	F_{ST}		G'_{ST}		***D***		D_{PS}	
	r	***P***	***r***	***P***	***r***	***P***	***r***	***P***
2	**0.63647**	**<0.0001**	**0.61401**	**<0.0001**	**0.59212**	**<0.0001**	**0.63088**	**<0.0001**
4	0.56596	<0.0001	0.51888	<0.0001	0.49631	<0.0001	0.52800	<0.0001
8	0.49581	<0.0001	0.43465	<0.0001	0.41170	0.0019	0.43887	<0.0001
16	0.44287	0.0027	0.37477	0.0009	0.35154	0.0248	0.37630	0.0018
32	0.40710	0.0117	0.33620	0.0105	0.31279	0.0564	0.33637	0.0106
64	0.38346	0.0286	0.31209	0.0328	0.28863	0.0920	0.31164	0.0282
128	0.36700	0.0433	0.29639	0.0354	0.27298	0.0859	0.29571	0.0485
256	0.35481	0.0573	0.28534	0.0771	0.26203	0.1048	0.28458	0.0667
512	0.34580	0.0655	0.27733	0.0912	0.25415	0.1097	0.27563	0.0737
1024	0.33958	0.0724	0.27180	0.0965	0.24873	0.1171	0.27097	0.0758

Table A2.4. Parameterisation of artificial barriers in an LCP model. *R* = resistance of the "suburban" LCM2007 habitat type, *r* = Mantel's correlation coefficient. Significance at the nominal 5% level after an FDR procedure, *P* = 0.009.

R	F_{ST}		G'_{ST}		***D***		D_{PS}	
	r	***P***	***r***	***P***	***r***	***P***	***r***	***P***
2	0.46833	<0.0001	0.55811	<0.0001	0.56666	<0.0001	0.59015	<0.0001
4	0.48674	<0.0001	0.56434	<0.0001	0.57560	<0.0001	**0.59519**	**<0.0001**
8	0.50629	0.0002	**0.56656**	**<0.0001**	**0.57833**	**<0.0001**	0.59485	<0.0001
16	**0.51375**	**0.0002**	0.55060	<0.0001	0.56156	<0.0001	0.57487	<0.0001
32	0.49738	<0.0001	0.51277	<0.0001	0.52091	<0.0001	0.53343	<0.0001
64	0.47643	0.0004	0.47653	0.0003	0.48273	0.0002	0.49419	<0.0001
128	0.46487	0.0008	0.45518	<0.0001	0.45861	0.0004	0.47080	0.0002
256	0.45775	0.0016	0.44203	0.0004	0.44362	0.0007	0.45630	<0.0001
512	0.45340	0.0015	0.43443	0.0005	0.43490	0.0005	0.44781	0.0003
1024	0.45102	0.0027	0.43037	0.0008	0.43026	0.0006	0.44329	0.0005

APPENDIX 3: G'_{ST}, D AND D_{PS} MULTIPLE REGRESSION MODELS

Table A3.1. Full list of all G'_{ST} based multiple regression models. r^2 = is the adjusted r^2 value for the entire model. ΔAIC and rank for all models includes the F_{ST} models (Table 4.4), D models (Table A3.2) and D_{PS} models (A3.3).

No.	Model	r^2	AIC	G'_{ST} models		all models	
				ΔAIC	rank	ΔAIC	rank
32	LCP + distance	0.289	-266.633	46.784	30	386.255	123
33	LCP + TO	0.359	-280.687	32.730	19	372.201	112
34	LCP + RO	0.363	-281.386	32.031	18	371.502	111
35	LCP + PO	0.189	-248.585	64.832	31	404.303	124
36	LCP + distance + natural barriers	0.337	-275.014	38.403	27	377.874	120
37	LCP + TO + natural barriers	0.354	-278.691	34.726	24	374.197	117
38	LCP + RO + natural barriers	0.361	-280.063	33.354	21	372.825	114
39	LCP + PO + natural barriers	0.339	-275.488	37.929	26	377.400	119
40	LCP + distance + artificial barriers	0.313	-270.281	43.136	29	382.607	122
41	LCP + TO + artificial barriers	0.363	-280.562	32.855	20	372.326	113
42	LCP + RO + artificial barriers	0.403	-289.325	24.092	11	363.563	104
43	LCP + PO + artificial barriers	0.314	-270.343	43.074	28	382.545	121
44	LCP + distance + natural barriers + artificial barriers	0.362	-279.272	34.145	22	373.616	115
45	LCP + TO + natural barriers + artificial barriers	0.360	-278.840	34.577	23	374.048	116
46	LCP + RO + natural barriers + artificial barriers	0.401	-287.915	25.502	12	364.973	105
47	LCP + PO + natural barriers + artificial barriers	0.352	-277.150	36.267	25	375.738	118
48	IBR + TO	0.467	-305.727	7.690	10	347.161	103
49	IBR + RO	0.385	-286.321	27.096	15	366.567	108
50	IBR + PO	0.370	-282.986	30.431	17	369.902	110
51	IBR + distance + natural barriers	0.478	-307.614	5.803	5	345.274	98
52	IBR + TO + natural barriers	0.491	-311.049	2.368	3	341.839	96
53	IBR + RO + natural barriers	0.483	-308.814	4.603	4	344.074	97
54	IBR + PO + natural barriers	0.474	-306.458	6.959	7	346.430	100
55	IBR + distance + artificial barriers	0.396	-287.819	25.598	13	365.069	106
56	IBR + TO + artificial barriers	0.473	-306.312	7.105	8	346.576	101
57	IBR + RO + artificial barriers	0.381	-284.321	29.096	16	368.567	109
58	IBR + PO + artificial barriers	0.390	-286.401	27.016	14	366.487	107
59	IBR + distance + natural barriers + artificial barriers	0.476	-306.137	7.280	9	346.751	102
60	IBR + TO + natural barriers + artificial barriers	0.496	-311.254	2.163	2	341.634	95
61	**IBR + RO + natural barriers + artificial barriers**	**0.503**	**-313.417**	**0**	**1**	**339.471**	**94**
62	IBR + PO + natural barriers + artificial barriers	0.478	-306.602	6.815	6	346.286	99

Table A3.2. Full list of all *D* based multiple regression models. r^2 = is the adjusted r^2 value for the entire model. ΔAIC and rank for all models includes the F_{ST} models (Table 4.4), G'_{ST} models (Table A3.1) and D_{PS} models (Table A3.3).

No.	Model	r^2	AIC	*D* models		all models	
				ΔAIC	rank	ΔAIC	rank
63	LCP + distance	0.294	-332.932	36.748	30	319.956	92
64	LCP + TO	0.354	-345.001	24.679	17	307.887	78
65	LCP + RO	0.361	-346.459	23.221	13	306.429	74
66	LCP + PO	0.195	-315.181	54.499	31	337.707	93
67	LCP + distance + natural barriers	0.325	-338.184	31.496	29	314.704	90
68	LCP + TO + natural barriers	0.349	-343.010	26.670	23	309.878	84
69	LCP + RO + natural barriers	0.358	-345.000	24.680	18	307.888	79
70	LCP + PO + natural barriers	0.330	-339.085	30.595	26	313.803	87
71	LCP + distance + artificial barriers	0.325	-338.194	31.486	28	314.694	89
72	LCP + TO + artificial barriers	0.349	-343.101	26.579	22	309.787	83
73	LCP + RO + artificial barriers	0.371	-347.798	21.882	12	305.090	73
74	LCP + PO + artificial barriers	0.328	-338.658	31.022	27	314.230	88
75	LCP + distance + natural barriers + artificial barriers	0.358	-343.997	25.683	19	308.891	80
76	LCP + TO + natural barriers + artificial barriers	0.344	-341.102	28.578	25	311.786	86
77	LCP + RO + natural barriers + artificial barriers	0.369	-346.283	23.397	14	306.605	75
78	LCP + PO + natural barriers + artificial barriers	0.352	-342.691	26.989	24	310.197	85
79	IBR + TO	0.445	-365.789	3.891	5	287.099	63
80	IBR + RO	0.357	-345.651	24.029	16	307.237	77
81	IBR + PO	0.347	-343.558	26.122	21	309.330	82
82	IBR + distance + natural barriers	0.438	-363.022	6.658	8	289.866	66
83	IBR + TO + natural barriers	0.459	-368.160	1.520	3	284.728	57
84	IBR + RO + natural barriers	0.444	-364.568	5.112	6	288.320	64
85	IBR + PO + natural barriers	0.439	-363.208	6.472	7	289.680	65
86	IBR + distance + artificial barriers	0.379	-349.460	20.220	11	303.428	72
87	IBR + TO + artificial barriers	0.456	-367.437	2.243	4	285.451	59
88	IBR + RO + artificial barriers	0.352	-343.654	26.026	20	309.234	81
89	IBR + PO + artificial barriers	0.363	-346.071	23.609	15	306.817	76
90	IBR + distance + natural barriers + artificial barriers	0.435	-361.253	8.427	10	291.635	68
91	**IBR + TO + natural barriers + artificial barriers**	**0.469**	**-369.680**	**0**	**1**	**283.208**	**51**
92	IBR + RO + natural barriers + artificial barriers	0.046	-368.315	1.365	2	284.573	56
93	IBR + PO + natural barriers + artificial barriers	0.439	-362.379	7.301	9	290.509	67

Table A3.3. Full list of all D_{PS} based multiple regression models. r^2 = is the adjusted r^2 value for the entire model. ΔAIC and rank for all models includes the F_{ST} models (Table 4.4) and G'_{ST} (Table A3.1) and D models (Table A3.2).

No.	Model	r^2	AIC	D_{PS} models		all models	
				ΔAIC	rank	ΔAIC	rank
94	LCP + distance	0.328	-355.171	47.303	30	297.717	71
95	LCP + TO	0.395	-369.507	32.967	21	283.381	53
96	LCP + RO	0.397	-369.898	32.576	19	282.990	50
97	LCP + PO	0.210	-333.222	69.252	31	319.666	91
98	LCP + distance + natural barriers	0.384	-366.132	36.342	27	286.756	62
99	LCP + TO + natural barriers	0.392	-367.819	34.655	24	285.069	58
100	LCP + RO + natural barriers	0.400	-369.585	32.889	20	283.303	52
101	LCP + PO + natural barriers	0.389	-367.067	35.407	25	285.821	60
102	LCP + distance + artificial barriers	0.345	-357.608	44.866	29	295.280	70
103	LCP + TO + artificial barriers	0.420	-374.203	28.271	13	278.685	44
104	LCP + RO + artificial barriers	0.406	-371.028	31.446	17	281.860	48
105	LCP + PO + artificial barriers	0.355	-359.700	42.774	28	293.188	69
106	LCP + distance + natural barriers + artificial barriers	0.404	-369.498	32.976	22	283.390	54
107	LCP + TO + natural barriers + artificial barriers	0.418	-372.842	29.632	15	280.046	46
108	LCP + RO + natural barriers + artificial barriers	0.441	-378.192	24.282	11	274.696	42
109	LCP + PO + natural barriers + artificial barriers	0.390	-366.516	35.958	26	286.372	61
110	IBR + TO	0.489	-392.321	10.153	10	260.567	41
111	IBR + RO	0.399	-370.569	31.905	18	282.319	49
112	IBR + PO	0.403	-371.248	31.226	16	281.640	47
113	IBR + distance + natural barriers	0.515	-398.488	3.986	5	254.400	36
114	IBR + TO + natural barriers	0.525	-401.331	1.143	2	251.557	33
115	IBR + RO + natural barriers	0.518	-399.479	2.995	4	253.409	35
116	IBR + PO + natural barriers	0.523	-397.996	4.478	6	254.892	37
117	IBR + distance + artificial barriers	0.429	-376.401	26.073	12	276.487	43
118	IBR + TO + artificial barriers	0.493	-392.648	9.826	9	260.240	40
119	IBR + RO + artificial barriers	0.395	-368.566	33.908	23	284.322	55
120	IBR + PO + artificial barriers	0.419	-374.026	28.448	14	278.862	45
121	IBR + distance + natural barriers + artificial barriers	0.512	-396.827	5.647	8	256.061	39
122	**IBR + RO + natural barriers + artificial barriers**	**0.532**	**-402.474**	**0**	**1**	**250.414**	**32**
123	IBR + PO + natural barriers + artificial barriers	0.515	-397.583	4.891	7	255.305	38