Investigating the Movements of Migratory Thrushes *Turdus merula* and *T. iliacus* using Intrinsic Markers and Morphology

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Redwing *Turdus iliacus* Photo by Chris Redfern

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ABSTRACT

Stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and hydrogen ($\delta^{2}H$) and microsatellite markers were used in conjunction with morphological data, to test whether breeding origins of two migratory passerines the Blackbird *Turdus merula*, and Redwing *T. iliacus*, overwintering in the UK & Ireland could be predicted, based on data collected at European breeding sites. Geographical trends of feather $\delta^{2}H$ and $\delta^{13}C$ values of birds sampled at European breeding sites were useful for determining breeding origins of Blackbirds at a broad geographical scale. Genetic markers were useful for discriminating between the two Redwing races *iliacus* and *coburni.* Conversely, no genetic structure was found within the nominate race *iliacus* and there was only weak genetic structure in Blackbird populations, suggesting relatively high gene flow. These results indicate that genetic markers are of limited value for population assignment of either species. Mean wing length of breeding Blackbirds differed significantly between breeding regions, and was positively correlated with latitude, suggesting that wing length is potentially a useful variable for discriminating between Blackbirds of different breeding origin.

It was predicted that combining techniques would prove substantially more useful for assigning individuals to their most likely origin, than any one technique used in isolation. For Blackbirds, the combination of $\delta^{2}H$ and $\delta^{13}C$ values with wing length proved to be the most effective combination of variables (and was more effective than using either stable isotopes or wing length alone), allowing 72.2% to 76.3% of breeding Blackbirds to be correctly and consistently assigned to one of three broad geographic regions (UK, Fennoscandia and Continent). For Redwings, using DNA markers alone, 94.5% of birds were correctly assigned to either the *coburni* or *iliacus*; the addition of stable isotopes produced only a marginal improvement.

Useful insights into the contrasting migratory strategies of Blackbirds and Redwings were revealed. The lack of genetic variation within the nominate Redwing race indicates a lack of migratory connectivity. In contrast, weak genetic population structure in the Blackbird, and differences in stable isotope

ratios and wing length between birds sampled at different wintering sites, suggests that different parts of the UK & Ireland may receive differing proportions of migrant Blackbirds originating from different breeding sites. This suggests that migratory connectivity might be stronger in this species.

The approach of using multiple techniques may prove useful for other species about which less is known regarding breeding origins, which may be particularly relevant for species of conservation concern. However, the most useful variable/combination of different variables for a study of migratory connectivity will vary, both according to the species, its geographical range, and the scale of resolution required, and a clear understanding of the ecology and physiology of the study species is essential

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CHAPTER 1: GENERAL INTRODUCTION

Background

Migration is one of the most astounding of all natural phenomena, and a fundamental aspect of the life history of many avian species (Kelly & Finch, 1998). Many of the migratory passerine species in Europe and Asia weigh less than 25g, yet may travel hundreds, or even thousands of kilometres during the course of each seasonal migration. The ability to link geographic regions used by a migratory species throughout its annual cycle, forms the basis of our understanding of the ecology and evolution of migration (Hobson, 1999a; Hobson, 2005a). Furthermore, without an adequate knowledge of the link between breeding and wintering areas, it is difficult to determine at which points in the annual cycle avian populations are most vulnerable. Thus, it may be difficult to develop effective management strategies, particularly for species of conservation concern (e.g. Myers et al., 1987; Hobson, 1999a). Moreover, events occurring at one stage of the annual cycle are likely to have an impact on populations in subsequent stages (e.g. Marra et al., 1998; Sillett et al., 2000; Bearhop et al., 2004). In addition, the recent trend in global climate change is predicted to have a profound impact on the extent and distribution of biomes (Huntley, 1995). This is likely to affect summer breeding areas or winter foraging grounds of many avian species, leading to range constrictions or expansions. For example, Zöckler & Lysenko (2001) modelled the potential impact of climate change on waterbirds breeding in the Arctic. From their findings, the authors predict major habitat shifts in the Arctic, resulting in an environment less suitable for the birds that currently breed there.

Kelly & Finch (1998) identify a number of long-standing questions relating to avian migration that, in most species, have yet to be fully explored. These include the following: "Are particular stopover sites used more frequently by birds that breed in particular latitudinal bands? Do more northerly breeding populations winter in more northerly areas? Do migrants follow the same flyway in both spring and autumn? How common is differential migration among sex

and age classes?" To these could be added: To what extents do individuals from the same breeding area migrate to the same wintering area? And perhaps most fundamentally: How will migratory species be able to respond to changes in the environment, particularly to a profoundly changing climate?

Migratory connectivity

Whilst there is a considerable amount of information available concerning migration at the species level, far less is known at the population (and intra-population) level (Bairlein, 2001). Esler (2000) suggests that identifying demographically independent subpopulations is of key importance for understanding population structure, particularly for species of conservation concern. It is also necessary to understand the mechanisms leading to demographic independence or panmixia of subpopulations (e.g. philopatry and dispersal). This is especially challenging for migratory species; subpopulations cannot be defined as synonymous with geographic areas, since they move during the annual cycle, and may co-occur with other subpopulations. Groups of birds may be spatially distinct at some stages of the annual cycle (e.g. on breeding or wintering grounds), yet may occur together at others (Esler, 2000). Thus, another important (but largely unresolved) issue when considering avian migration is the extent to which individuals from the same breeding area migrate to the same wintering area, and vice versa. This has been termed 'migratory connectivity' (Webster et al., 2002) (Figure 1.1).

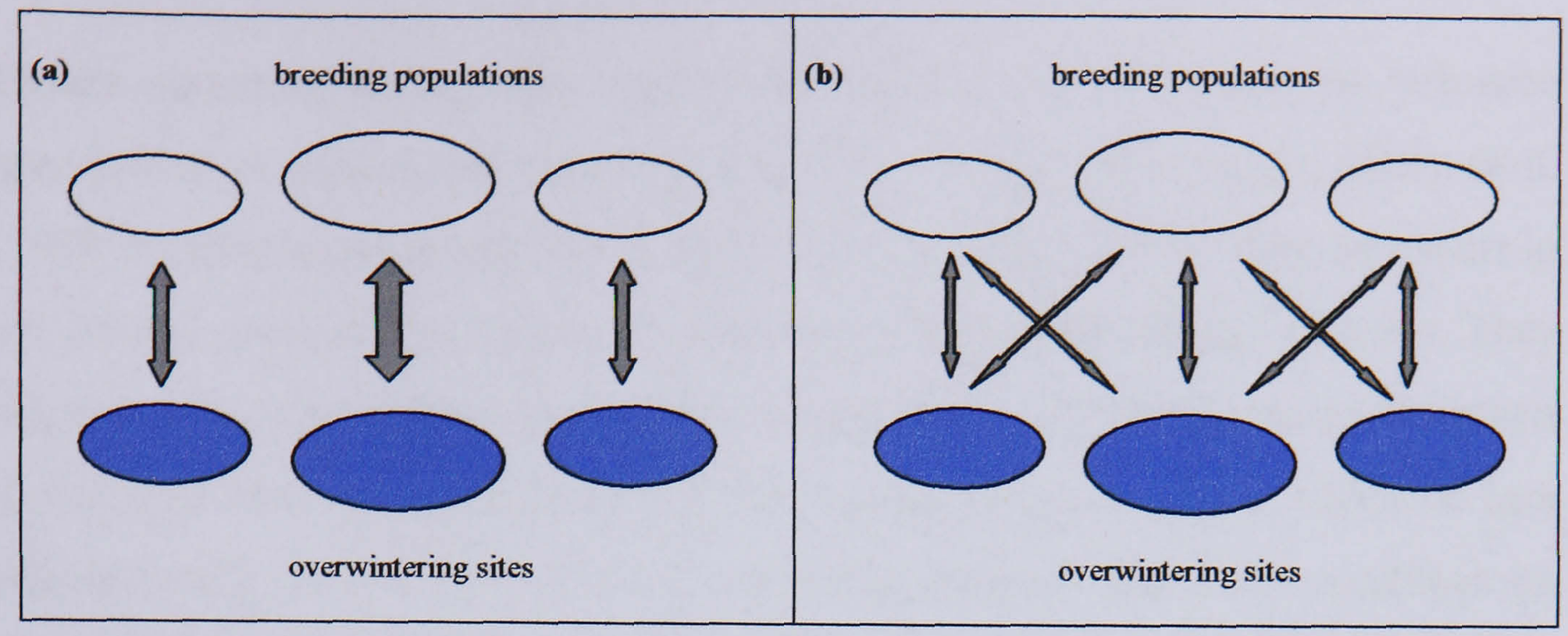


Figure 1.1 Simplified representation of differing extents of migratory connectivity between breeding populations (white ovals) and wintering quarters (blue ovals). Two extreme situations are illustrated, with (a) representing strong migratory connectivity, and (b) representing no connectivity. Arrows represent movements of individuals between sites. Modified from Boulet & Norris (2006).

In their reviews, Robbins et al. (1989) and Rappole & McDonald (1994) discuss the importance of understanding when and how bird populations are constrained, in the light of population declines. However, without knowledge of how populations are spatially distributed between different periods of the annual cycle, it is very difficult to understand how events occurring in these different periods influence abundance (Boulet & Norris, 2006). For example, the endangered Kirtland's Warbler (*Dendroica kirtlandii*) forms a single breeding population (in Michigan), but is widely dispersed throughout the islands of the Bahaman Archipelago in winter (Sykes & Clench, 1998). The population thus appears to be structured as a distinct breeding population, with winter panmixia, which could be interpreted as weak connectivity. Although negative factors such as drought and predation may affect birds wintering on some islands, Sykes & Clench (1998) suggest that the lack of concentration of wintering birds should buffer the population (as a whole) against these problems. However, migratory populations with restricted ranges and high site fidelity, i.e. *strong* migratory connectivity (at any stage of the annual cycle), are especially vulnerable to environmental changes (Pain et al., 2004).

Seasonal interactions

Events occurring during one stage of the annual cycle are likely to influence populations in subsequent stages (Marra et al., 1998). For example, Marra et al. (1998) used habitat-specific stable carbon isotope ratios to link seasonal events in the annual cycle of the migratory American Redstart *Setophaga ruticilla*. They were able to demonstrate that winter habitats occupied by American Redstarts determined their physical condition and spring departure dates, which in turn affected both time of arrival on the breeding grounds and body condition on arrival. Marra et al. (1998) suggest that this is driven by intraspecific competition for optimal winter habitat. Norris et al. (2004) went on to show that the reproductive success of American Redstarts is affected by habitat quality on the wintering grounds. Using a winter-habitat model, Norris et al. (2004) predicted that females occupying high-quality winter habitat would produce more young and fledge offspring earlier, than females wintering in poor-quality habitat.

In a study similar to that of Marra et al. (1998), Bearhop et al. (2004) used stable carbon isotope ratios of claw material of another migratory bird, the Black-throated Blue Warbler (*Dendroica caerulescens*), to infer their wintering habitats. This then allowed Bearhop et al. (2004) to investigate whether winter habitat quality was linked to body condition during migration. They found that migrant birds with low $\delta^{13}C$, which in this case is indicative of more mesic (i.e. high-quality) habitats, were in better body condition than conspecifics with higher $\delta^{13}C$ values (i.e. those wintering in poorer, drier sites). Dominance-mediated habitat segregation again appeared to be the mechanism driving this event.

Partial and differential migration

In many bird species, migration may vary within and between populations, age groups and/or sexes, as well as between years within individuals (Bairlein & Coppack, 2006). For example, populations of migratory species breeding at higher latitudes are often more migratory than those occupying lower latitudes

(Berthold, 1978). This may lead to geographical clines in morphology, possibly as a result of selection pressures exerted by climatic variables (Jones et al., 2005). Jones et al. (2005) suggest that the influence of selection pressures on migrants could be enhanced by the extent of migratory connectivity in a particular species. For example, Jones et al. (2005) argue that where a species forms a single breeding population, but overwinters in several different sites (as appears to be the case with Kirtland's Warbler, discussed above), disparate selection pressures during the non-breeding season might be more influential than the more homogeneous effects of selection pressures on the breeding ground. Geographical variation in patterns of migratory behaviour is related to the phenomenon of partial migration, where some individuals of a species migrate whilst others do not. This has been well documented in a number of species (e.g. Lack, 1944; Berthold, 1978; Lundberg, 1985, 1988; Adriaensen & Dhondt, 1990; Chan, 2001; Martell et al., 2004; Nilsson et al., 2006). Identifying patterns of intraspecific migration, and their underlying causes, facilitates elucidation of issues concerning the role and evolution of migration in avian life histories.

Several studies have demonstrated that some avian species show differential migration (e.g. Prescott, 1991; Jenkins & Cristol, 2002). Ketterson & Nolan (1988) define differential migration as "the situation in which all individuals of a population migrate, but distance travelled varies according to sex and/or age". In addition, sex and age classes are often segregated, either latitudinally or by habitat across their non-breeding range (Humple et al., 2001). For example, Stouffer & Dwyer (2003) found that Hermit Thrushes (*Catharus guttatus faxoni*) wintering in the eastern United States and northeastern Mexico showed a sex-biased latitudinal distribution, with females tending to winter further south than males. According to Catry et al. (2004), the underlying theory most often evoked is that of social dominance and competitive exclusion, whereby subordinate birds (females, juveniles and smaller individuals) are competitively excluded by more dominant conspecifics. An alternative hypothesis is that of habitat specialisation, whereby differences in foraging ecology between social groups lead to segregation. Differential migration may thus result in differences in the extent of

migratory connectivity between different groups of birds, e.g. connectivity may be strong in adults, but weak in juveniles.

Conservation of migratory species

During the last two decades, declines in the abundance of many avian species, particularly passerines, have been documented. For example, in Central Europe, approximately 30% of avian species are declining to an extent that threatens their continued existence (Berthold et al., 1998). Although populations of large birds (e.g. raptors and waders) can be determined accurately, for species that are harder to observe, such as passerines, only rough estimates are possible, and trends in the numbers of many such species have remained largely unclear. In an effort to address this, the Max Planck Institute for Ornithology in Germany began a passerine census programme in 1972, by trapping passage migrants during their autumn migration. This provided data on 35 species, including long-, intermediate-, and short-distance migrants, over a period of 25 years. During this time, the number of trapped birds fell slowly but continuously, by an average of about 1% per year. The decrease was greatest for long-distance migrants (significant negative trends were produced for 12 out of 21 species). Most of these birds came from Central Europe, although some were from northern, eastern, and western Europe and a small proportion were local breeding birds (Berthold et al., 1998). A more recent review of European breeding birds (Sanderson et al., 2006) revealed similar patterns. Analyses of trends, between the periods 1970 to 2000, of 30 closely related pairs of species, one a long-distance migrant and the other non-migrant, indicated significantly more negative trends in the former. The negative trends appeared to be largely due to declines in Afro-Palearctic species that winter in dry, open habitats in Africa.

There are a number of reasons why long-distance migrants may show different population trends from residents or short-distance migrants. Resident species are only affected in a single, year-round habitat; migratory species can decline on their breeding grounds, stopover sites, and winter quarters. Human encroachment on breeding grounds is thought to be contributing to declines in many resident

European bird species. Migratory species are similarly affected, but are also known to be declining in their winter quarters and stopover sites (Sanderson et al., 2006). Species affected include passerines, such as the Redstart, *Phoenicurus phoenicurus*. Possible causes of declines on the winter sites include drought in the Sahel region of West Africa, habitat destruction, biocides and hunting. Thus, it is not surprising that long-distance migrants are particularly vulnerable (Berthold et al., 1998). In addition to the increase in threats posed to migratory species as a result of their utilisation of multiple sites, Myers et al. (1987) identify several aspects of the natural history of migratory birds that raise concern. These include the concentration of birds into small stopover and wintering sites, the precise energy requirements and timing of migratory movements, and the competition of migratory species with man. Although Myers et al. (1987) focus on shorebirds (Charadriiformes), many of these considerations are applicable to other migratory bird taxa.

Responses to global climate change

Since the late 19th century, the global average temperature has risen by approximately 0.6 °C ± 0.2 °C. This increase is projected to continue at a rapid rate (IPCC, 2001), of a magnitude unprecedented in the Quaternary record (IPCC, 1990, 2001). As a consequence of global climate change, the extent and distribution of biomes will be altered, and inevitably this will impact on many species (Huntley, 1995). The potential changes in species' traits that may occur are numerous. At given locations, the density of species may change, and species ranges may shift either polewards in latitude or upwards in elevation. Some currently rare species may become widespread in the future; conversely others that are currently common may become rarer if their habitats are reduced (reviewed by Root et al., 2003). For example, reduced extent of boreal forest and tundra will likely result in range constrictions of species associated with these habitats, whereas the potential increase in warm-temperate forests may allow southwestern species to expand their ranges (Huntley, 1995).

Shifts in species ranges are a key prediction of global warming (Parmesan, 1996), and have already been documented for many species, especially plants (e.g. Grabherr et al., 1994), butterflies (e.g. Parmesan, 1996; Parmesan et al., 1999) and birds (e.g. Thomas & Lennon, 1999). In addition, since many life history traits are triggered by temperature-related cues, changes in phenology may occur (Root et al., 2003). Earlier onset of spring may advance flowering and leafing dates (Fitter & Carey, 1995; Sparks et al., 1995), which in turn are likely to have pronounced effects on the availability of arthropod food supplies (Crick et al., 1997b). Some bird species have advanced their egg-laying date, which is thought to be a response to changes in food supply (Crick et al., 1997b; Crick & Sparks, 1999). However, other avian species may not cope with climate change because their response differs from the response of vegetation and prey items, leading to a mismatch between the timing of reproduction and the main food supply (e.g. Visser et al., 1998; Visser & Holleman, 2001; Visser et al., 2004; Both et al., 2006). The effects of such changes on subsequent events of the annual cycle of migratory birds are not well understood (Jenni & Kéry, 2003).

Through their long-term studies of the migratory Black-throated Blue Warbler, Sillett et al. (2000) demonstrated how fluctuations in the El Niño Southern Oscillation (ENSO) had an impact on the demographic rates in both the breeding and non-breeding seasons. Adult survival and fecundity were lower in El Niño years and higher in La Niña years. Furthermore, they found that fecundity was positively correlated with subsequent recruitment of new individuals into both wintering and breeding populations. These findings thus reveal links in the population dynamics of this species in different stages of its annual cycle, and illustrate the susceptibility of migratory birds to shifts in global climate patterns.

Long-distance migrants may be particularly vulnerable to changes in global climate. As climate change leads towards milder winters, as well as earlier springs, winter mortality of birds resident at higher latitudes may be reduced. Migrants may also return to the breeding grounds earlier (e.g. Jenni & Kéry, 2003), and the start of breeding may be advanced. High Arctic breeders may also have to travel further if the tundra retreats. Residents, partial migrants, and short-distance migrants could thus be at an advantage over long-distance migrants, by

having a greater capacity to adapt rapidly to such changes. This may increase competition and lead to a progressive loss of breeding sites for long-distance migrants, which arrive at the breeding grounds last (Berthold et al., 1998). According to Berthold et al. (1998), this heralds profound changes in the Central European avifauna, and may result in a gradual restructuring in favour of the permanent residents, partial and short-distance migrants, to the detriment of long-distance migrants.

Tracking migratory movements of birds

Conventional techniques used to track movements of birds have proved limited. Despite intensive mark-recapture efforts in which millions of birds have been ringed in an effort to track their movements, relatively few are ever recovered (Webster et al., 2002). According to Chamberlain et al. (2000), the probability of obtaining intercontinental recoveries of most small birds is just one out of 10^3-10^5 individuals ringed. Active methods of tracking individuals, such as radio and satellite telemetry, still have severe limitations when applied to some species, especially small birds travelling long distances (Kelly et al., 2002). However, recent advances in the development of molecular genetic markers, and novel applications of chemical markers (stable isotopes and trace elements) have opened up exciting new avenues for ecological research. The use of these *intrinsic* markers offers the potential for illuminating some of the key questions concerning avian migration. Conventional methods remain invaluable; new techniques should not be regarded as superseding them, but rather considered as complementary. Ringing recoveries provide irrefutable evidence of movements of individuals, and should thus assist interpretation of genetic and chemical data (Hobson & Wassenaar, 2001). The crucial advantage of intrinsic markers is that these methods do not require recapturing or resighting of individuals (Rubenstein & Hobson, 2004).

DNA analyses

Since the early 1990's, new molecular genetic techniques and markers have found increasing application within the fields of ecology, evolution and conservation (Morin et al., 2004). In animals, genetic markers occur in the nuclear and mitochondrial genomes. These differ in their evolutionary characteristics, for example, in their mode of inheritance, and sequence and structural mutation rates. Furthermore, within each type of genome, the coding and non-coding regions evolve at different rates (Lowe et al., 2005). Table 1.1 provides a summary of the important features of some of the most useful genetic markers used in ecological studies.

Table 1.1 Useful genetic markers. Modified from Gillet (1999) and Lowe et al. (2005).

Marker type	Mode of inheritance	Level of genetic variability	Main advantage(s)	Main disadvantage
Mitochondrial intron marker (mtDNA)	Uniparental (maternal); each cytotype is expressed	Low variation within populations; large differentiation between regions.	Maternal mode of inheritance results in a smaller effective population size than microsatellites, resulting in fast population divergence.	Often only informative at a broad geographical scale.
Nuclear microsatellites	Biparental nuclear; codominant, with the exception of null alleles at some loci	Large variation within populations; low differentiation between populations.	Mutation rates are high compared to other DNA markers, making them highly polymorphic.	Initial identification of microsatellite loci is expensive and time-consuming.
Random amplified polymorphic DNA (RAPD)	Biparental nuclear; dominant.	Variable – dependant on primers.	Fast, simple and inexpensive; A large number of putative loci may be screened; Only small amounts of tissue required.	Many criticisms, including problems with reproducibility.
Amplified fragment length polymorphism (AFLP)	Biparental nuclear; dominant at some loci, codominant at others.	Variable – dependant on primers	Fast, simple and inexpensive; reliable and robust compared to other techniques that use randomly amplified primers.	Requires high degree of technical skill, and relatively large amounts of high quality DNA.

Application of molecular markers to identifying migratory origins

If genetic variation of populations is structured geographically on the breeding grounds, individuals subsequently sampled at overwintering sites can potentially be assigned back to the appropriate breeding locations (Webster et al., 2002; Clegg et al., 2003). A variety of genetic markers, particularly mtDNA, have already been used for tracing the origins of migratory birds, although levels of

success have been variable. The most commonly used markers for this type of study are discussed below, and relevant studies are summarised in Table 1.2.

Mitochondrial DNA

Mitochondrial DNA (mtDNA) haplotypes have often been selected as suitable markers for investigating phylogeography and population analyses (Webster et al., 2002). MtDNA has a number of features that make it useful for studies of this kind: it has a rapid rate of sequence evolution (though not as high as microsatellites), does not undergo recombination, and has an almost strictly maternal mode of inheritance (Wenink et al., 1994). The latter feature means that it has a smaller effective population size than microsatellites (since the effective number of mitochondrial genes in a population, will be approximately equal to the number of females, rather than twice the effective population size, as occurs with nuclear genes). This means that geographical sorting of variation is more rapid, leading to rapid population differentiation, relative to microsatellites (Birky et al., 1989; Clegg et al., 2003).

One of the first studies to suggest the potential for genetic markers as a tool for tracking migratory movements was that of Wenink & Baker (1996). They found that mtDNA control-region sequences of 52 migratory and wintering Dunlins (*Calidris alpina*) from around the world were identical to those found previously in a larger sample of breeding Dunlins sampled from their northern circumpolar range (Wenink et al., 1993; Wenink et al., 1996). Since mtDNA sequences confirmed the existence of five major phylogeographic groups within the breeding range, Wenink et al. (1996) suggested that mtDNA could be useful for studies investigating migratory connectivity of this species. Several studies followed on from this early work (Tiedemann, 1999; Wennerberg et al., 1999; Wennerberg, 2001; Lopes et al., 2006), and were successful in using mtDNA haplotypes of migrating and wintering Dunlins to predict their breeding origins. In contrast, a comparable study by Wennerberg et al. (2002) found little mtDNA differentiation between breeding populations of White-rumped Sandpiper (*Calidris fusicollis*).

A number of recent studies, focussing on Neotropical (North - South America) passerine species, have also attempted to elucidate patterns of migration using mtDNA haplotypes (Kimura et al., 2002; Ruegg & Smith, 2002; Lovette et al., 2004). The results of these studies are strikingly similar: genetic structure was found only at the broadest continental scale in all five species investigated, so that assignment of individuals to eastern versus western (or coastal versus inland) breeding lineages was possible, but assignment to breeding populations of origins was not. Thus, although mtDNA markers are often regionally-specific, they are not necessarily population-specific, and may only be useful for tracking migratory movements at a broad scale (Webster et al., 2002).

Microsatellites

Microsatellites have become one of the most popular molecular markers (Zane et al., 2002), and are often used for genetic studies both at the level of individuals and populations, mainly because they are highly polymorphic (Webster et al., 2002). The major drawback of this type of marker is that they often require considerable development time (Zane et al., 2002). A number of studies have employed microsatellites to examine genetic structuring of avian populations, and have found varying levels of genetic differentiation. To date, however, the only published study that has used microsatellites to investigate migratory connectivity is that of Clegg et al. (2003), who used eight microsatellites (along with stable isotope ratios of hydrogen) to characterise the population structure and migration patterns of Wilson's Warbler (*Wilsonia pusilla*). Clegg et al. (2003) found broadscale genetic variation between populations breeding in North America, with those from the west differing significantly from those in the east. However, minimal genetic structure was observed among western sites, and it was not possible to define any clear geographic clustering.

Arbitrary nuclear DNA primers

A different approach is to use fingerprinting techniques, such as random amplified polymorphic DNA (RAPD) (developed independently by Welsh & McClelland, 1990 and Williams et al., 1990), or amplified fragment length polymorphisms (AFLPs) (developed by Vos et al., 1995), which are based on the amplification of random genomic DNA fragments by arbitrarily selected primers.

These methods are fast, simple and inexpensive, although a number of drawbacks to this type of marker have been identified (Table 1.1). Few studies have used these methods in animals, despite some very promising results. For example, Haig et al. (1997) used the RAPD technique to search for markers specific to shorebird species that are difficult to differentiate. Not only were they able to successfully differentiate between all species, they also identified population-specific markers for three species. Consequently, Haig et al. (1997) were able to estimate the likelihood that an individual had come from an easterly versus westerly population. They were also able to predict whether individuals in a migrant group originated from a similar breeding location. Although not directly investigating migratory connectivity, (Bensch et al., 2002a) used the AFLP method to obtain genetic markers distinguishing two subspecies of willow warblers *Phylloscopus trochilus* with different migratory behaviour. The authors had already tried using mtDNA and microsatellite markers for this purpose, but were unsuccessful (Bensch et al., 1999).

Table 1.2 Examples of studies utilising genetic markers to investigate connectivity between breeding and non-breeding subpopulations of migratory birds.

Authors	Type of marker	Bird species	Brief summary of results
(Haig et al., 1997)	RAPDs	Nine shorebird species (Suborder: Charadrii)	Found that, within species, all populations were significantly differentiated, although levels of differentiation were variable. Able to estimate likelihood that an individual had come from an easterly versus westerly population, and whether individuals in a migrant group originated from a similar breeding location.
(Wenink et al., 1993) (Wenink & Baker, 1996) (Wenink et al., 1996) (Tiedemann, 1999) (Wennerberg et al., 1999) (Wennerberg, 2001) (Lopes et al., 2006)	MtDNA (and morphology)	Dunlin (*Calidris alpina*)	Initial studies found five lineages of mtDNA control-region haplotypes of Dunlins sampled across the Palearctic breeding range. Subsequent studies showed that this information could be used to assign migrating and wintering birds to their breeding origin.
(Kimura et al., 2002)		Wilson's warbler (*Wilsonia pusilla*)	Found a complex pattern of population differentiation in mtDNA variation among populations across the breeding range, with no population-specific haplotypes. Were able to discriminate between eastern and western haplotypes, thus assignment of wintering individuals was only possible at a broad geographic scale.
(Lovette et al., 2004)		Three species of Neotropical passerines	Found genetic structure only at the broadest continental scale, in all three species. Assignment of individuals to eastern or western breeding lineages was possible, but assignment to breeding populations of origins was not.
(Wennerberg et al., 2002)	MtDNA (and stable isotopes)	White-rumped Sandpiper (*Calidris fusicollis*)	MtDNA sequences showed no significant differentiation between sites, and no pattern of isolation-by-distance was found.
(Ruegg & Smith, 2002) (Kelly et al., 2005)		Swainson's Thrush (*Catharus ustulatus*)	MtDNA allowed differentiation into two distinct clades (coastal and continental), with nearly complete segregation of migratory routes and overwintering locations.
(Scribner et al., 2003)	Microsatellites and MtDNA (plus satellite telemetry)	Canada Geese (*Branta canadensis*)	Used likelihood-based assignment tests to determine that multilocus genotypes of Greenland Canada Geese originated from birds of Atlantic Population breeding in Quebec, Canada. Based on microsatellite allele and mtDNA haplotype frequencies, found that this population was genetically differentiated from that of the North Atlantic.
(Clegg et al., 2003)	Microsatellites (and stable isotopes)	Wilson's warbler (*Wilsonia pusilla*)	Using 8 microsatellites, found that breeding populations in the west differed significantly from those in the east; minimal genetic structure was observed among western sites. Failed to define any clear geographic clustering.
(Bensch et al., 2002a)	AFLPs	Willow warbler (*Phylloscopus trochilus*)	Used AFLPs to distinguish two subspecies of Willow warblers that have different migratory behaviours, but are not differentiated in mtDNA or at several microsatellite loci.

Chemical markers

Stable isotopes

Isotopes are atoms of the same element with different numbers of neutrons, and therefore unique atomic masses. Stable isotopes are those isotopes of an element that do not undergo radioactive decay. For example, hydrogen has two stable isotopes: ^{2}H (often called deuterium) and ^{1}H, with the lighter isotope (^{1}H) being more abundant (West et al., 2006). Stable isotopes are measured as the ratio of the two most abundant isotopes of a given element (Buttle, 1998). Ratios of stable isotopes of naturally occurring elements such as carbon (C), nitrogen (N) and strontium (Sr) vary across the environment, often in systematic ways. Stable isotope ratios in the local environment are incorporated into plants during nutrient uptake (Webster et al., 2002). For example, the most frequently studied and well-known stable isotope pattern in nature is that of stable carbon isotope ratios (Hobson, 2005a). Plants assimilate carbon by one of three photosynthetic pathways, the C_3, C_4, and CAM (crassulacean acid metabolism) pathways (Ehleringer et al., 1991). The rate at which stable isotopes of carbon (^{13}C and ^{12}C) are fixed by plants differs among photosynthetic pathways (Bender, 1971; Smith & Epstein, 1971). Plants with a C_3 pathway have more depleted carbon-isotope values than those with a C_4 or CAM pathway (Hobson, 2005a). C_3 plants predominate at high latitudes and C_4 plants are more common at low latitudes. There is therefore a latitudinal gradient in the ratio of stable isotopes of carbon in plant communities (Körner et al., 1991; Kelly & Finch, 1998). In addition, marine C_3 plants have stable-isotope ratios of carbon that are intermediate between terrestrial C_3 and C_4 plants (Kelly, 2000).

Similarly, in North America and in Europe, there is a latitudinal gradient in the hydrogen isotopic ratio in precipitation and groundwater, which is reflected in plants (Schiegl & Vogel, 1970; Kelly & Finch, 1998). Stable isotope ratios of oxygen and hydrogen in precipitation are altered mainly by evaporation and condensation in the atmosphere. These processes result in fractionation, the partitioning of isotopes into liquid and vapour phases. The liquid phase (which falls as rain) is enriched in the heavier isotopes (^{18}O and ^{2}H) relative to the vapour phase. Condensation occurs when the temperature of an air mass falls

below the dew point, where humidity reaches 100%. At this point raindrops will form, enriched in the heavier isotopes, while the remaining vapour will be depleted in the heavier isotopes. Further rainfall will only occur from the remaining vapour if the temperature again reaches 100% humidity. A similar split between isotopically-enriched rain and depleted vapour occurs, but this time from a more depleted air mass. Consequently, any process resulting in temperature loss in a moist air mass leads to rainfall that is progressively more depleted in the heavier isotopes. There are two processes that can cause this: movement of the air mass over land -the 'continental effect', and passage of the air mass over upland barriers - the 'altitude effect'. Most rainfall originates from evaporation of the ocean in tropical latitudes, and compared to oceanic waters, the meteoric waters (i.e. the atmospheric moisture, the precipitation, and ground- and surface water derived from them) are generally relatively depleted in the heavy isotopes (e.g. Gat, 1980; Kendall & Caldwell, 1998; Darling & Talbot, 2003).

Application of stable isotopes to ecological studies

In their laboratory experiments, DeNiro & Epstein (1978; 1981) appear to have been the first authors to establish that both the whole body, and individual tissues of an animal reflect the carbon and nitrogen isotopic composition of its diet. In a more recent laboratory experiment, Hobson et al. (1999a) showed that hydrogen in animal tissues was derived from drinking water as well as diet. However, the isotopic composition of an animal's diet is not directly transferred in a simple 1:1 relationship, because there is a fractionation factor between diet and tissue. This can be expressed as:

$$\delta X_t = \delta X_d + \delta \Delta_{dt}$$

where X is the isotope in question, $_t$ is the tissue in question, $_d$ is the diet, and $\delta\Delta_{dt}$ is the isotope fractionation factor between diet and tissue. The stable isotope value is expressed as the ratio of the rarer, heavy form to that of the more common, lighter form (Hobson, 2005a).

This transfer of stable isotope patterns in the environment through the food webs that depend on them means that stable isotopes can offer useful insights into the ecology of animals, providing important clues about the foraging behaviour and environments of animals in the field (Kelly & Finch, 1998). Moreover, animals that move between isotopically distinct foodwebs, carry with them information about the location of previous feeding sites (Hobson, 1999a). Thus, stable isotopes in the tissues of animals can provide naturally occurring markers reflecting the isotopic composition of the local environment in which the tissues were grown (Kelly & Finch, 1998; Webster et al., 2002).

The basis of this, and any other tracking technique utilising intrinsic markers, is that the (isotopic) 'signature' acquired where a particular tissue (such as feathers, hair, or claws) is grown, differs from that of the location where the tissue is subsequently sampled (Hobson, 2005a). Contrasting with genetic markers, stable isotope ratios are independent of genetic and population processes and have been used to identify the origin of migratory individuals (Webster et al., 2002). According to Webster et al. (2002), the use of stable isotopes to track animal movements requires the following: (i) predictable patterns of geographical variation in isotopic ratios; (ii) information about where the animal in question incorporates isotopes into its tissues and their turnover times, and (iii) tissue samples from animals at different locations, e.g. breeding and wintering grounds.

In the past decade, an increasing number of studies have employed stable isotopes to examine seasonal dietary shifts (particularly in the marine environment, e.g. Hobson et al., 1994; Abend & Smith, 1995; Hobson & Schell, 1998; Forero & Hobson, 2003; Quillfeldt et al., 2005), and for tracking movements of a wide range of migratory organisms. These include mammals (e.g. Fleming et al., 1993; Best & Schell, 1996; Burton & Koch, 1999; Darimont & Reimchen, 2002; Cerling et al., 2006), fish (e.g. Kennedy et al., 1997; Maruyama et al., 2001; Thorrold et al., 2001; Dempson & Power, 2004; Kennedy et al., 2005), insects (e.g. Hobson et al., 1999b), and most notably birds (Appendix 1). The choice of tissue is of fundamental importance because different tissues may have different isotopic turnover rates (i.e. the rate at which isotopic ratios of the diet are incorporated into the tissues), and in metabolically

active tissues, such as blood, the diet-tissue isotopic fractionation factor is not static (Hobson & Clark, 1992; Hobson, 2005a). For this reason, metabolically inert tissues such as hair, feathers, and claws are typically used for tracking migratory movements.

Trace elements

Another type of chemical marker with potential for tracing origins of breeding birds is trace elements. These are somewhat analogous to stable isotope ratios, in that patterns of trace elements in feathers are ultimately derived from diet, which in turn is strongly influenced by surficial geology (Hobson, 2005a). A number of studies have demonstrated their potential for identifying regions in which feathers were grown (e.g. Parrish et al., 1983; Bortolotti & Barlow, 1988), and recent developments in analytical techniques allow numerous trace elements in feathers (including As, Mn, Mg, Cu, Zn, Pb, Fe, S, V, Cd, Mo, Se, Sr, Ba, Hg, Ni, Co, Li, P, Ti, Ag and, Cr) to be measured routinely (Hobson, 2005a). For example, Parrish et al. (1983) measured concentrations of 14 trace elements in nestling Peregrine Falcons *Falco peregrinus* in three areas of Alaska and western Greenland, in order to determine whether these markers could be used to identify the geographic origins of the birds sampled. Discriminant function analyses placed birds in their correct natal locale with 100% predictability.

More recently, Szép et al. (2003) analysed feathers from Sand Martins *Riparia riparia* collected from a range of different breeding sites across Europe. It was found that differences in elemental composition among nestlings from the same nest and the same colony were small, and that moult site had a large effect on the elemental composition of feathers. However, because trace element profiles are so sensitive to microgeographical differences between sites, Szép et al. (2003) suggested that this approach might be best suited to elucidating similarity among groups of individuals in their use of moulting areas. In contrast, Donovan et al. (2006) were unable to identify natal locations with high accuracy, on the basis of trace elements of feathers, nor were they able to produce an effective map for predicting feather-element values at sample sites. According to Hobson (2005b), currently the major drawback to using trace element profiles is the generally poor understanding of landscape profiles; for this technique to be of use depends on

whether the species of interest is composed of subpopulations showing unique profiles. Work is currently in progress to further elucidate the utility of this technique for studies of bird migration (Farmer et al., 2004; Szép et al., 2007).

Combining techniques

In addition to the likely improved resolution resulting from the use of multiple stable isotope ratios or trace elements, and more than one type of genetic marker, combining both approaches should further allow migratory movements to be traced at multiple scales, both at the regional and population level (Webster et al., 2002). Clegg et al. (2003) combined microsatellite DNA analysis and stable isotopes of hydrogen to infer migratory connectivity between breeding and overwintering areas of Wilson's Warbler. The authors found that the genetic markers revealed structure in an east-west orientation, whereas isotopic analysis revealed structure in a north-south orientation. Findings of the δ^2H analyses indicated that this species followed a leapfrog pattern of migration. The authors concluded that using a combination of genetic and isotopic approaches should be useful for identifying patterns of migration and population connectivity, and may potentially contribute to investigations into the evolution of migration. More recently, Kelly et al. (2005) combined mtDNA and stable isotope ratios to examine seasonal movements in the Swainson's Thrush (*Catharus ustulatus*), and were able to predict the site-specific origin of Thrushes with 76-80% accuracy. Kelly et al. (2005) argue that further integration of methodologies will refine the scale at which links between different phases of the life cycle can be quantified.

Much of the existing research exploring the utility of intrinsic markers, particularly stable isotopes, for tracing migratory origins has taken place in the Americas; important questions remain concerning the applicability of this type of approach for birds breeding in Europe. In an effort to address this, migratory movements of two species of thrushes the Blackbird *Turdus merula* and Redwing *T. iliacus* were investigated in the present study. These species were selected because populations of both spend at least part of their annual cycle in Britain,

and because relatively large numbers are caught by bird ringers, facilitating sample collection. Ringing data have already revealed some interesting and variable migration patterns in both species.

Study Species

Blackbirds

The Blackbird occurs widely throughout Europe, where it is one of the most abundant breeding species (Tucker, 1994; Chamberlain, 2002). During the first half of the 20th century, the nominate race *Turdus merula* gradually extended its breeding range to outlying Scottish islands such as Shetland, as well as the Faeroes, and spread westward within Ireland. This coincided with an expansion into more northerly parts of Scandinavia (Kalela, 1949; Spencer, 1975; Sharrock, 1976). Kalela (1949) speculated that this breeding range expansion might have been attributable to an amelioration of climate. However, Venables & Venables (1952) found no evidence that Shetland experienced a similar climatic improvement, but suggest that the increasing numbers of Blackbirds in northern Europe may have facilitated its colonisation of Shetland. *T. merula* currently breeds throughout Europe, except for northernmost Fennoscandia, the Kola Peninsula and the Russian lowlands northeast of Moscow (Mulsow & Tomialojc, 1997) (Figure 1.2). The species is more abundant in west and central Europe, and least numerous in the southeast, east and northeast, apparently because either the climate is harsher, or food is scarcer in the latter regions. It has also been introduced to New Zealand and southeast Australia. Approximately 14 additional races occur in more southerly pockets of distribution, from the Azores and the Canaries eastward to China and Sri Lanka (Cramp & Simmons, 1988b; Chamberlain & Main, 2002). The Blackbird is the most adaptable of the *Turdus* thrushes, and is found in a diverse range of habitats. Thought to have originated as a bird of woodland, or woodland edge, the species is now more often associated with man-made habitats, particularly parks and gardens (Snow, 1993; Chamberlain & Main, 2002).

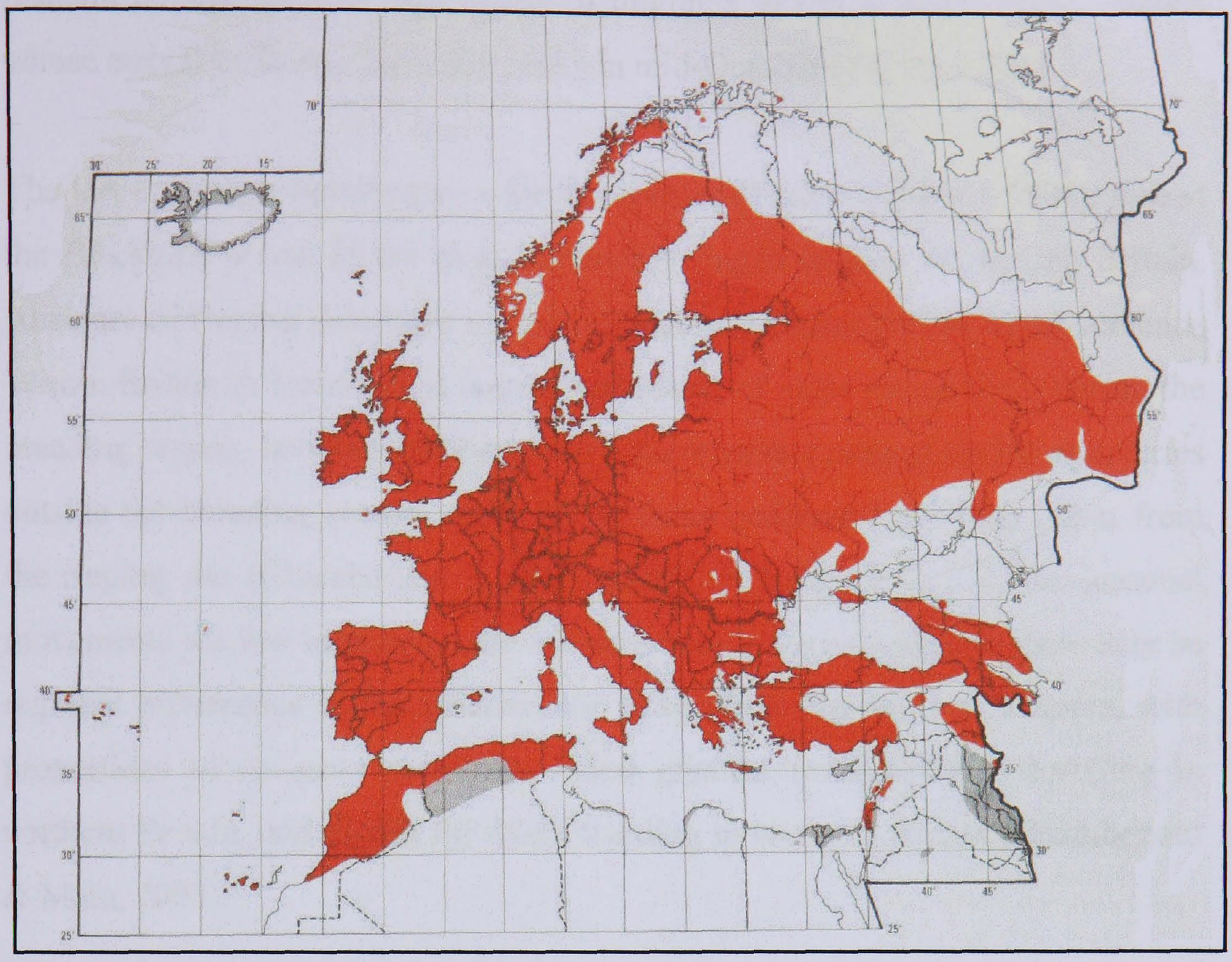


Figure 1.2 Distribution of the Blackbird Turdus merula. *From Cramp (1988).*

Over much of its range, the Blackbird is largely sedentary (especially urban individuals). However, populations from northern Europe migrate south or southwest, as may some birds breeding further south (such as those from Scandinavia, Poland and Germany) (Cramp & Simmons, 1988b; Chamberlain & Main, 2002). The reasons underlying variation in migratory behaviour between individuals from partially migrant populations remain unclear. Factors that may be involved include genetically inherited degree of migratory behaviour, phenotypic environmental influences, and dominance hierarchies within populations (Mulsow & Tomialojc, 1997). These autumn migratory movements take place almost entirely within the species breeding range, although some birds winter further south in North Africa and Mesopotamia (Mulsow & Tomialojc, 1997; Chamberlain & Main, 2002). Superimposed on the British and Irish

resident population is a huge influx of migrants in the autumn (Snow, 1986a), whose arrival in Britain typically peaks in mid-October (Taylor, 1984).

The UK is a major breeding area for this species (Tucker & Heath, 1994); indeed the Blackbird is one of the most commonly ringed species throughout Britain. Analyses of ringing data have revealed a highly complex pattern of movements. Within Britain & Ireland it is largely sedentary; of birds ringed there during the breeding season, between 1909 and 1997, there were a total of 13,385 recoveries outside the breeding season. Only 4% of these occurred more than 20km from the ringing site (Chamberlain & Main, 2002). Although long distance seasonal movements are few in number, the overseas recoveries indicate that there may be regional differences in the tendency of British-breeding birds to migrate, with proportions of overseas recoveries being greatest for Blackbirds breeding in northern Britain, and lowest for those breeding in southern Britain (Chamberlain & Main, 2002).

In a much earlier study, Lack (1944) examined data from several partially migrant species ringed in Britain until 1936, and identified a general (i.e. involving a number of species) *Zugscheide* or 'migratory divide' within Britain, whereby some individuals migrated south to France, Spain and Portugal, while others moved west to Ireland. However, although his examination of Blackbird data revealed a number of recoveries in Ireland (mostly birds ringed in Scotland and northern England), there was only a single recovery (of a southern-ringed bird) from France. Later investigations carried out by Snow (1966, 1978) suggested that migrant first-year Blackbirds from the north and west of Britain displayed a tendency to cross to Ireland for their first winter, while those from the south and east migrated south to France. Snow (1978) went on to suggest that the birthplaces of these two migratory classes (based on recoveries up to 1977) occupied almost mutually exclusive areas, but that there may have been a zone in eastern England from which birds may have migrated in either direction. Lack (1944) proposed that adoption of the migration route to Ireland was "relatively recent", originating from westerly hard-weather movements.

However, a more recent exploration of recoveries of Blackbirds ringed in Britain as nestlings or juveniles between 1909 and 1994 has revealed a steady decline in the proportion recovered overseas (including those recovered in Ireland). For example, for the years 1960-1968, 0.94% of recoveries were from overseas, compared with just 0.04% for the years 1980-1994 (despite similar numbers of total recoveries for the two periods) (Main, 2000). A comparison of recoveries from Ireland and the Continent reveals that the Irish migratory route appears to have been gradually abandoned (Table 1.3). This is thought to be at least partially due to milder winters in recent years, making weather-induced facultative movements less necessary (which would support Lack's 1944 hypothesis that this route may have originated from hard-weather movements) (Chamberlain & Main, 2002). However, according to Snow, (1966; 1986a), Blackbirds appear to be less prone to make cold weather movements than other thrushes. Moreover, it is possible that environmental changes act through natural selection to change the proportions of *obligate* migratory individuals in a partially migrant population. Experimental work (summarised by Berthold, 1999) has shown that most, if not all, of the physiological characteristics required for a migratory lifestyle are genetically controlled. Furthermore, some of these characteristics are inherited together as 'migratory syndromes', which may greatly facilitate microevolutionary changes from migratoriness to sedentariness and vice versa (Berthold, 1999; Main, 2000).

Table 1.3 Total numbers of recoveries, and numbers of overseas recoveries, of Blackbirds from the British breeding population (classified as birds ringed in Britain as nestlings, adults ringed during the breeding season, or juveniles before 1st September). From Chamberlain & Main (2002).

Years ringed	**Totals**	**Irish recoveries**	**Continental recoveries**
1909-1959	3,274	61	6
1960-1968	3,717	23	12
1969-1979	4,085	15	11
1980-1997*	4,143	1	6

*1980-1994 for adults

Partial migration and site fidelity are issues warranting further study. For example, ringing data indicate that the majority of Norwegian Blackbirds that move to Britain & Ireland do so every year (Main, 2000; Chamberlain & Main, 2002). For individuals belonging to the partially migratory populations further south, however, a North Sea crossing does not appear to be an annual event (Chamberlain & Main, 2002). Main (2000) suggests that it may be useful to distinguish between these patterns, by classifying the former as obligate partial migrants, and the latter as facultative partial migrants. He goes on to suggest that the control mechanisms for obligate partial migration are likely to be innate, whereas facultative partial migration may be a response to environmental conditions, such as weather and food availability. Given that there is evidence for regional differences between Blackbird populations, both in tendency to migrate and migratory direction, one could speculate that population responses to future climate change are likely to vary, and may enhance regional variation in migratoriness.

Increasing evidence of differential migration and habitat segregation based on age, sex and/or size of birds suggests that these may be widespread phenomena (Catry et al., 2004). There is some indication that (in some populations at least) females and juveniles show a greater tendency to migrate than males. Recently, Chamberlain & Main (2002) compared the percentage of the different ages and sexes of Blackbirds ringed in Britain & Ireland outside the breeding season, that were recovered overseas, with those recovered in Britain & Ireland. The results suggested that there were more female migrants from the continent (both adult and juvenile birds) than there were males, wintering in Britain & Ireland, although the differences were small, Table 1.4. However, it should be noted that these estimations cannot be precise, because they are derived from data for a number of different populations, which may show different patterns in migratory behaviour.

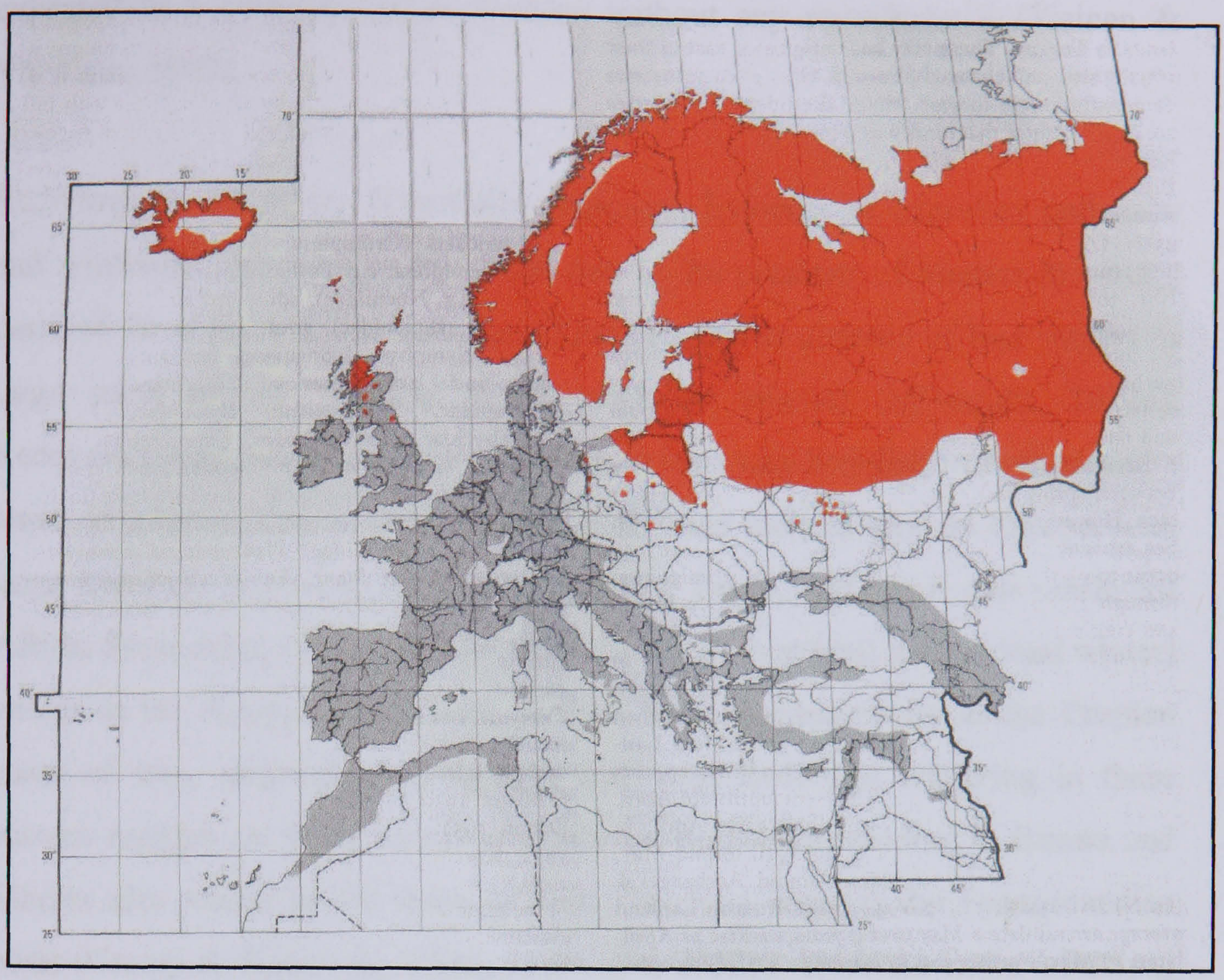
Table 1.4 The estimated percentages of Blackbirds of continental origin amongst different age and sex classes wintering in Britain & Ireland (percentages recovered abroad of birds ringed in Britain & Ireland outside the breeding season). Only birds of known sex and age are included. From Chamberlain & Main (2002).

Age	**Male** **% (*n*)**	**Female** **% (*n*)**	**Both sexes** **% (*n*)**
Juvenile	10.5 (8,724)	12.8 (5,107)	11.4 (13,831)
Adult	11.6 (4,324)	16.5 (3,044)	13.6 (7,368)
All	10.9 (13,048)	14.2 (8,151)	12.2 (21,199)

Between the early 1970s and late 1990s, the British Blackbird population declined by approximately 30% and was listed as being of medium conservation concern (a Medium BTO Alert) (Crick et al., 1997a). This coincided with agricultural intensification in Britain, which may have contributed to declines in several avian species that began in the same period (Fuller et al., 1995). However, numbers fell in woodland as well as farmland, suggesting that additional causes may have contributed to the decline (Baillie et al., 2002). A gradual decline in Blackbird numbers was also recorded in the Netherlands during roughly the same period. The cause(s) of the decline are unknown, although it could not be attributed to reduced breeding success (Dix et al., 1998). In addition, there is some evidence to suggest that numbers of Blackbirds recovered in northeast Europe have decreased in recent years (Chamberlain & Main, 2002). Although this could have arisen through population declines in the source areas, there is no evidence for recent declines in European countries other than Britain, the Netherlands and Albania (Tucker & Heath, 1994; Chamberlain & Main, 2002). An alternative explanation is that the decline in overseas recoveries may be associated with less severe winters in recent years, so that the generally milder climate reduces the tendency for birds to migrate (Chamberlain & Main, 2002). Recent surveys administrated by the British Trust for Ornithology (BTO) suggest that the British population may be starting to recover (Baillie et al., 2002).

Redwings

The breeding range of Redwings *Turdus iliacus* covers a vast range, from Iceland to eastern Siberia, mainly in the boreal taiga, but also in the subarctic and alpine zones, becoming scattered towards its southern breeding range limits (Tiainen & Väisänen, 1997) (Figure 1.3). Two races are known: the nominate race *iliacus*, which breeds from Scotland and Scandinavia eastwards, and the Icelandic race *coburni*, which nests in Iceland and the Faeroes. The two races differ markedly in several aspects of their migration (Cramp & Simmons, 1988a; Milwright, 2002b). The Redwing is a forest generalist, preferring mosaic environments. Typical boreal breeding habitats used by the Redwing are shore thickets, forest edges and upright sapling stands, but it often uses scrubby, semi-open cultivated sites, including parks and gardens. In Iceland, and in mountainous areas, it may breed in rocky areas almost lacking scrub (Tiainen & Väisänen, 1997). According to Bjerke & Bjerke (1981), populations form annually constant song dialect areas, with clear boundaries.



present all year

breeding range

overwintering range

Figure 1.3 Distribution of the Redwing Turdus iliacus. *From Cramp (1988).*

In the 1940s, the Finnish Redwing population was biased to the north, but by the 1970s breeding density below the Arctic Circle increased fivefold. The Swedish Redwing population varied similarly during this period. Although it is thought that the extremely hard winters of the early 1940s had previously decreased densities to an exceptional extent, this factor alone does not explain either the geographical pattern, or the magnitude of the increase. Modern forestry techniques, applied from 1950 onwards in Fennoscandia, leading for example to an increase in bushy habitats, appear to have been the main reason for the changes. Since around 1930, the Redwing also expanded its breeding range southwards in Lithuania, Belarus and Ukraine, which may be a consequence of population pressure further north. The growth phase began to level off in 1975, and since that time, most European populations have fluctuated (as would be

expected in a cold-susceptible species) without any regular trend (Tiainen & Väisänen, 1997).

Redwings are migratory or partially migratory. Evidence of migratory pathways and wintering areas is heavily biased due to the presence of hunters in certain parts of the wintering area (southern Europe), and an absence of bird ringers in large parts of the breeding range (such as Siberia) (Milwright, 2002a). Recoveries suggest that Redwings of the nominate race breeding in Fennoscandia leave on a broad front in autumn, and winter over most of western Europe, with large numbers migrating as far as southwest Iberia, and some into northwest Africa. Some adult Fennoscandian birds (i.e. those in at least their second winter) winter in the Aegean, the Levant, and the Transcaucasus as far as the Caspian coast of Iran, although it is thought that most Redwings wintering in these eastern regions are from Russia and Siberia. Redwings breeding in Russia and Siberia also winter in the same western European areas as the Fennoscandian birds (Cramp & Simmons, 1988a; Milwright, 2002b). Since the entire wintering range of Redwings extends to just outside the western Palearctic, Siberian-breeding birds must migrate at least 6,500km west-southwest to reach its winter quarters (Cramp & Simmons, 1988a).

The Icelandic and Eurasian races overlap to some extent on their wintering grounds (Snow, 1986a). *T. i. coburni* has a relatively small population, and the movements of this race are better understood than those of the nominate race (Milwright, 2002b). *T. i. coburni* winters mainly in Ireland and northwest Iberia, although many late-autumn recoveries are from Scotland or western France. Birds from the eastern half of Iceland are more likely to winter in Scotland and Ireland than those from the western half (Milwright, 2002a). Icelandic-ringed individuals have never been recovered anywhere in England or Wales, although the total number of recoveries is rather small ($n \sim 140$) (Milwright, 2002a).

Analyses of ringing recoveries suggest that Britain & Ireland are moderately important wintering areas for the nominate race, with juveniles most likely to overwinter in England or Wales, and adults in Scotland or Ireland (Milwright, 2002a). Typically, large influxes of Redwings into Britain are associated with

high pressure over Fennoscandia and/or Russia, which results in clear skies and easterly winds, facilitating a North Sea crossing (Roberts & White, 2006). The peak month for catching Redwings in the UK is October, when many birds are caught at ringing stations on the east coast and the Scottish northern isles (although few birds overwinter at these coastal sites, but instead disperse throughout Britain). Redwings ringed in Britain & Ireland have been recovered between May and July from Norway to Russia, just east of the Urals. More than 60% of these recoveries were from Finland, compared with just 14% for Sweden, and 5% from Norway. In Russia, the sparse human population means that few recoveries can be expected, so the 15% that have been found there is a surprisingly high figure. It therefore seems likely that the majority of birds wintering in Britain & Ireland are from Finland and the huge populations of central and Eastern Siberia (Milwright, 2002b).

In 2004, an unprecedented movement of Redwings was observed in northwest England. More than 145,000 thrushes, the majority of them Redwings, were reported, more than 90% of them from just three sites. Prior to this event, the largest day total of migrating Redwings observed in northwest England was around 6,000 individuals. It is thought that these migrants were displaced further south than usual by strong northerly winds, causing them to overshoot their usual east-coast landfall sites. Given the huge numbers involved, and a known build-up and departure of thrushes from Norway and the Low Countries, it seems likely that these birds originated from Fennoscandia and/or Eastern Europe (Roberts & White, 2006).

Redwings of the nominate race have bred sporadically in Britain, as far south as Kent, since 1925. A small Scottish breeding population of *T. iliacus* has also been maintained since around 1967 (Cramp & Simmons, 1988a; Milwright, 2002b; Reid, 1993). Reid (1993) aptly describes the species as "somewhat eruptive", and comments on the unpredictable nature of its between and within winter movements. The species tends to be nomadic, making cold weather movements within its winter range (Snow, 1986b; Reid, 1993), and Reid (1993) postulates that cold weather dispersal may have accounted for its colonisation of Scotland, by birds originating from Scandinavia. The species is not thought to

display strong site fidelity; although there have been a few recoveries of individuals at the same wintering site between years, most reveal that birds may winter in widely different localities in different winters. For example, Redwings ringed in Britain have been recovered in Italy, Greece, and eastern regions in subsequent winters (Zink, 1981, cited in Cramp & Simmons, 1988a).

Ringing recoveries suggest that almost all first year Redwings of the nominate race migrate west or southwest to western Europe or the western Mediterranean, where vast numbers concentrate into relatively small areas of western and southern France, and western Italy. Perhaps surprisingly, there appears to be no evidence that first year birds are guided to these relatively concentrated areas by adult birds. The mean distance from breeding to wintering areas of the nominate race *iliacus* is less in their first winter than in later winters, and the most distant destinations are occupied mainly by older birds (Milwright, 2002a). Milwright (2002a) speculates that this may be an adaptation allowing the adults to avoid the high density of juvenile redwings and other thrushes in Belgium, southern France and northern Italy. In contrast, among the Icelandic race *coburni*, the reverse situation appears to occur with first-winter birds tending to migrate further than the adults (Milwright, 2002a; b).

Hunting and trapping are the main causes of recovery of ringed Redwings and these factors undoubtedly exert a major control on the population sizes of both races (Milwright, 2002b). However, according to Milwright (2002b) the huge population fluctuations that Redwings undergo are more probably due to annual weather differences in the breeding area. Milwright (2002b) also postulates that the biggest long-term challenge to the species may be the effect of global warming on its breeding biology.

Aims & Objectives

The overall aim of the present study was to explore the effectiveness of genetic and stable isotope markers, coupled with morphological data, for assigning the migratory status and most probable breeding origin of a bird sampled on its UK or Irish wintering grounds. This would allow insight to be gained into the relative extent of migratory connectivity in the two species. To meet this aim, the objectives of the study were as follows:

- Identification of microsatellite loci suitable for use in Blackbirds and Redwings to allow levels of genetic structuring in breeding populations to be assessed.
- Investigation of patterns of hydrogen stable isotope ratios (δ^2H) in precipitation, and whether this is correlated with ratios in contour feathers of breeding birds.
- Assessment of levels of isotopic (δ^2H, δ13C and $\delta^{15}N$) variation in feathers between breeding sites.
- Assessment of within-site variation (i.e. age-, sex- and species-specific differences).
- Analysis of BTO Blackbird ringing data, to determine whether there are seasonal changes in mean wing length, which would be indicative of morphological differences between resident and migrant birds.
- Integration of genetic, stable isotope and morphological data to determine whether birds from particular geographic subdivisions of the breeding grounds can be characterised
- Investigation of the effectiveness of this approach for assigning wintering/migrating individuals caught in the UK and Ireland back to their breeding origins.
- Assessment of variation (in all three data types) between different non-breeding sites to address questions relating to extent of migratory connectivity.

In addressing these objectives the following hypotheses were tested:

- Genetic population structure exists within the breeding ranges of both Blackbirds and Redwings, but is weaker in Redwings, because they are nomadic, and are not thought to be strongly site-faithful.
- Isotope ratios of feathers can be used to assign wintering individuals to breeding populations.
- δ^2H varies with both longitude and latitude, and is the most useful isotope for discriminating between breeding populations of both species.
- Mean wing length of Blackbird populations change seasonally in relation to the arrival of longer-winged migrants.
- A combination of different data types will allow the two Redwing subspecies, *iliacus* and *coburni*, to be identified with a high level of confidence.
- Combining genetic and isotopic data, along with morphology (in the case of Blackbirds) will allow birds to be assigned to their region of breeding origin, on a broad geographical scale, with a high degree of confidence.

CHAPTER 2: IDENTIFICATION OF POLYMORPHIC MICROSATELLITE LOCI IN BLACKBIRDS *TURDUS MERULA* AND REDWINGS *T. ILIACUS*

Introduction

Microsatellites, also known as simple sequence or short tandem repeats (SSRs or STRs), are tandem repeats of short nucleotide motifs (1-6 bases long), common in eukaryote DNA and widely distributed throughout the genome (Hamada et al., 1982; Tautz & Renz, 1984; Queller et al., 1993). They are usually characterised by a high level of length polymorphism, which is due to an increased rate of mutation compared to other neutral regions of DNA. This is thought to be most likely due to slippage events during DNA replication (Tautz & Renz, 1984; Schlötterer & Tautz, 1992; Zane et al., 2002). Microsatellites can be amplified for identification via Polymerase Chain Reaction (PCR), using templates of flanking regions (primers), which gives them the further advantage that they can be isolated from degraded and old DNA samples, as well as samples containing only a low concentration of DNA, because all that is required is sufficient substrate for amplification of short fragments via PCR (Queller et al., 1993). These properties, along with their co-dominant mode of inheritance have made microsatellites a powerful marker, often used for genetic studies at the level of both individuals and populations (e.g. for determining paternity and for population genetic studies) (Queller et al., 1993; Jarne & Lagoda, 1996; Webster et al., 2002).

Use of microsatellite markers for avian population studies

Genetic markers may provide useful insights into migratory movements. For example, if there is genetic variation between breeding populations, then this information may be useful for ascribing likely breeding origins of overwintering birds. Several different types of genetic marker have been used in this context, and their relative advantages and drawbacks are compared in Chapter 1. It was decided that microsatellites would be an appropriate genetic marker to use for examining population genetic structure in Blackbirds *T. merula* and Redwings *T.*

iliacus (family Turdidae). Several studies have employed microsatellites to examine genetic structuring of avian populations (e.g. Dawson et al., 1997; Nesje et al., 2000; Lee et al., 2001), although to date few have used them to directly address questions concerning migration (but see Clegg et al., 2003 and Scribner, 2003). As a result, polymorphic microsatellites have already been characterised for a large number of passerine species (Lee et al., 2001), and in many cases, loci identified for individual species have also been found to be informative in other passerine families (e.g. Gibbs et al., 1999; Dawson et al., 2000; Richardson et al., 2000).

Caveats

Microsatellites do have some limitations: they appear to be relatively rare (compared with mammals) in the avian genome (Primmer et al., 1997; Zane et al., 2002), and often reveal only weak genetic differentiation between populations. In addition, point mutations in the primer annealing sites may lead to the occurrence of 'null alleles', where microsatellites fail to amplify in PCR reactions (Jarne & Lagoda, 1996). It could be considered that the major drawback of microsatellites is that they often need to be isolated *de novo* from a species being examined for the first time, which often requires considerable development time (Webster et al., 2002; Zane et al., 2002). However, highly conserved flanking regions have been identified for some microsatellite loci, allowing successful cross-species amplification, particularly where species are closely related (e.g. Schlötterer et al., 1991; Fitzsimmons et al., 1995; Rico et al., 1996; Primmer et al., 1996).

Microsatellite isolation

Novel microsatellite primers can be isolated using standard cloning methodologies. Most techniques are derived from a protocol described by Rassmann et al. (1991). The general procedure begins with construction of a partial genomic library for the focal species, through digestion by a restriction enzyme. The digested DNA fragments are ligated into a plasmid or phage vector. The vector is then inserted (transformed) into an appropriate *Escherichia coli* bacterial strain. The transformed cells are then grown on agar containing an appropriate selective medium (*e.g.* ampicillin). Subsamples of the resulting

microsatellite 'library' are then screened with repeat-containing probes. Positive clones (*i.e.* those containing inserts) are then sequenced, and primers are chosen from sequences flanking microsatellites, thus determining a specific locus.

Although relatively straightforward, this traditional approach can be inefficient for taxa such as birds that have a relatively low frequency of microsatellites in their genome. Several new strategies have therefore been devised to improve the efficiency of microsatellite isolation (Zane et al., 2002). For example, the number of positive clones can be increased through library enrichment procedures. (Queller et al., 1993; Scribner & Pierce, 2000). Several methods of enriching for specific microsatellite repeats have been described (e.g. Ostrander et al., 1992; Karagyozov et al., 1993; Armour et al., 1994; Paetkau, 1999), but the general procedure involves hybridisation to a repeat-containing probe (*before* cloning and transformation), and the subsequent recovery of microsatellite-rich DNA fragments via PCR (Hammond et al., 1998). This chapter describes the procedures used to identify suitable microsatellite primers for *Turdus merula* and *T. iliacus*.

Aims

The aim of this part of the study was to identify microsatellite primers for Blackbirds *Turdus merula* and Redwings *T. iliacus* via a dual approach:

(i) cloning and sequencing *T. merula* and *T. iliacus* DNA in order to isolate novel microsatellite loci;

(ii) screening primers characterised for other passerine species for suitability in *T. merula* and *T. iliacus*.

Methods

DNA extraction

DNA was extracted from tissue samples of four *T. merula* and four *T. iliacus* carcasses (all natural casualties, stored at –20°C until required) using a standard sodium acetate (NaAc) protocol. A subsample (approximately 25mg) of thigh muscle was dissected from each specimen, and digested with GuHCl extraction

buffer (800mM guanidine hydrochloride, 3mM EDTA, 3mM Tris, 5% v/v Tween 20^{TM}, 0.5% v/v Triton X-100) and 10µg proteinase K. Solutions were incubated at 55°C for approximately 16h, until the tissue was fully lysed. Proteins were precipitated by the addition of 100µl 3M NaAc and removed. 300µl isopropanol was added to precipitate the DNA, which was then washed in 70% ethanol and redissolved in 50-100µl TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). Samples were stored at -20°C until required.

Preparation of DNA: digestion and ligation

The following describes the procedure per sample. Approximately 13ng DNA was used to construct a dinucleotide library, enriched for $[CA]_{15}$ and $[GA]_{15}$ repeats. The protocol is based on that of Edwards et al. (1996), but with substantial modifications (Hale et al., 2001; 2002). Prior to enrichment, 1µg genomic DNA from each species was digested with 5U *Mbo*I, 1 x *Mbo*I buffer (10mM Tris-HCl, 50mM NaCl, 10mM $MgCl_2$, 1mM DTT), 100mg/ml BSA and 400mg/ml spermidine, in a total volume of 50µl, at 37°C for 2h. The digested DNA was then concentrated and purified with NaAc and ethanol, and dissolved in 25µl distilled H_2O. All the digested DNA was ligated to 500ng of *Sau*LA/*Sau*LB linkers (Armour et al., 1994) (prepared by annealing equimolar amounts of two oligonucleotides, *Sau*LA 5'GCGGTACCCGGGAAG-CTTGG3' and *Sau*LB 5'GATCCCAAGCTTCCCGGGTACCGC3', in the presence of NaCl), using 5U ligase (Promega), and 1 x ligase buffer (30mM Tris-HCl, 10mM $MgCl_2$, 10Mm DTT, 1mM ATP) in a total volume of 50µl, incubated at 16°C for 2h. 2µl of ligated DNA was amplified in 4 x 50µl reactions, each consisting of 1 x *Taq* buffer [$(NH_4)_2$ SO_4, 67mM Tris-HCl, 0.01% Tween-20], 2mM $MgCl_2$, 0.1mM dNTPs, 0.4µM *Sau*LA and 1.5U *Taq* (Bioline). The following amplification conditions were used: 72°C for 2min, 94°C for 3min, then 20 cycles of 94°C for 30s, 57°C for 30s, and 72°C for 90s, with a final extension step of 72°C for 4min. This, and all subsequent reactions, was performed in a GeneAmp PCR system 9700 thermocycler (Applied Biosystems). The amplified ligated DNA was pooled (per sample), then concentrated and purified with NaAc and ethanol, and dissolved in 25µl distilled H_2O.

DNA enrichment

The following DNA enrichment procedure was carried out twice (to increase the efficiency of the library) using a nylon membrane enriched with $[CA]_{15}$ and $[GA]_{15}$ dinucleotides for each species. To prepare the membrane, 10µg of each oligonucleotide in 3 x SSC (45mM sodium citrate, pH 7.0 and 450mM NaCl) were mixed in a total volume of 80ml, which was then spotted onto a piece of Hybond® N+ nylon membrane (Amersham). The membrane was UV-treated for 30s using a UV transilluminator, then baked for 1hr at 60°C. Weakly-bound oligonucleotides were removed from the membrane by washing twice in 10ml of hybridisation buffer, consisting of 2 x SSC + 0.5% SDS (sodium dodecyl sulfate), at 45°C in a rotating hybridisation oven for two days. The membranes were cut into $1cm^2$ pieces, each containing a spot, and stored at -20°C until required (Edwards et al., 1996).

The hybridisation procedure began with pre-hybridisation of each enriched membrane in 1ml 2 x SSC + 0.5% SDS at 45°C for 4h. The entire amplified, ligated DNA was denatured at 98°C for 5min, then cooled on ice. This denatured DNA was hybridised to an enriched membrane in 500µl 2 x SSC + 0.5% SDS. 1µg *Sau*LA was added, and the membranes were incubated in a rotating hybridisation oven for 18h at 45°C. After hybridisation, the hybridisation solution was discarded, and the membranes washed with wash solutions preheated to 45°C. This was carried out as follows: 5 x 5min washes in a low-stringency solution (2 x SSC + 0.01% SDS), followed by 3 x 5min washes in a high-stringency solution (0.5 x SSC + 0.01% SDS). The bound DNA was then eluted (separately for each membrane) into 200µl distilled H_2O by heating at 98°C for 5min (with occasional mixing).

After the first enrichment, the eluted DNA was amplified in 4 x 50µl reactions, using the same procedure as before, but without the initial 72°C step. Reaction products were pooled, then concentrated and purified with NaAc and ethanol, and dissolved in 25µl distilled H_2O. The second enrichment followed, using the same procedure as before. Again, the eluted DNA was amplified using the same conditions as used previously (i.e. without the initial 72°C step), but with the length of the final extension step of (72°C for 4min) increased to 30min.

Reaction products were pooled and concentrated/purified as before. The linkers were removed from the twice-enriched DNA by digestion with 5U *Mbo*I, 1 x *Mbo*I buffer (as above) and 100mg/ml^{-1} BSA, in a total volume of 50µl. Digestion was allowed to proceed for 2h at 37°C. The DNA was purified on columns using a QIAprep® Spin Miniprep kit plasmid (Qiagen), following the manufacturer's instructions.

Cloning and transformation

Approximately 13ng of enriched DNA was cloned into 100ng BAP dephosphorylated and *Bam* HI-digested Ready-To-Go™ pUC18 vector (Qbiogene) according to the manufacturer's instructions. 2µl of the vector were transformed into 50µl QAComp-C01 competent *Escherichia coli* cells (Qbiogene). The entire 50µl cells were then added to 950µl Luria Bertani (LB) broth and incubated for 90min at 37°C in a shaking incubator (150rpm). 100µl cells were then plated out onto individual LB agar plates containing 50µg/ml ampicillin and incubated for approximately 16h at 37°C, to allow *E. coli* colony growth. Subsamples of the colonies were amplified, using M13 sequencing primers (forward: TGTAAAACGACGGCCAGT; reverse: CAGGAAACAGCT-ATGACC) (PE Applied Biosystems), to estimate insert length. 10µl reactions containing 1 x *Taq* buffer [$(NH_4)_2$ SO_4, 67mM Tris-HCl, 0.01% Tween-20], 2mM $MgCl_2$, 0.1mM dNTPs, 0.2µM M13F, 0.2µM M13R and 2.5U *Taq* (Bioline) were amplified. The following amplification conditions were used: 94°C for 5min, then 35 cycles of 94°C for 30s, 50°C for 30s, and 72°C for 60s, with a final extension step of 72°C for 5min. The products were run on 1.4% agarose gels, and screened for insert length; those between 300 and 700bp were selected for sequencing.

Sequencing

Selected colonies were transferred to individual tubes containing LB broth with 25µg/ml ampicillin and incubated for approximately 19h at 37°C in a shaking incubator (200rpm), to allow further colony growth. Plasmid DNA was extracted from the growth solutions using a QIAprep Spin Miniprep Kit (Qiagen) and dissolved in distilled water. DNA was sequenced using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems). 20µl reactions were sequenced

using the following conditions: 25 cycles of 96°C for 10s, 50°C for 5s, and 60°C for 4min. The sequencing products were then purified with NaAc and ethanol, and dried sequencing products were stored at -20°C until required. For sequence analysis, 20µl template suppressant reagent (TSR) (Applied Biosystems) was added to the dried DNA, which was then denatured at 95°C for 2min. Samples were analysed on an ABI Prism®310 automated sequencer.

Primer Design

Primers were designed using the PRIMER3 software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) (Rozen & Skaletsky, 2000). To test primers for amplification and polymorphism, microsatellite fragments were labelled with fluorescent dCTPs (either R110 or R6G, Perkin-Elmer) and amplified in 10µl reactions containing 1 x *Taq* buffer, 2.5mM $MgCl_2$, 0.2mM dNTPs, 0.4mM of each primer pair (forward and reverse), 0.5U *Taq* (Bioline) and 1mM [F]dCTP, with 1µl genomic DNA. The following reaction conditions were used: 95°C for 12 minutes, 10 cycles of 94°C for 15s, 50°C for 15s, 72°C for 15s, followed by 30 cycles of 89°C for 15s, 50°C for 15s, 72°C for 15s, then a final extension step of 72°C for 30min. Fluorescently-labelled fragments were detected on an ABI Prism®310 automated sequencer, with ROX500 internal size standard, and analysed and sized using GENESCAN® software (Applied Biosystems). All primers were tested with both species.

The first library contained fewer microsatellites than expected, and therefore, the procedure was repeated from the hybridisation step, using the two ligated DNA samples (one per species) from the original library.

Screening of available passerine primers

The results of previous screening to find polymorphic loci for Blackbirds and other passerines were provided by Dawson, D.A. & Gentle, L. unpublished data (see BIRDMARKER database at http://www.shef.ac.uk/misc/groups/molecol/deborah-dawson-birdmarkers.html). From these, 35 loci isolated from 12 bird families were screened for suitability in each species (details are provided in the Results section).

Primer Testing

All microsatellite primers (i.e. both novel primers, and those previously-characterised for other species) were tested for utility in relatively large numbers ($n > 200$ Blackbirds; $n > 75$ Redwings) of DNA samples extracted from contour feathers (the extraction methodology is described in the following chapter). Annealing temperature was optimised for each primer pair.

Results

Isolation of novel Turdus *primers*

In the initial library, approximately 246 individual colonies (~25% total for the two species) contained inserts of 300 to 700bp. Of these, 34 (17 from each of the two species) were selected for sequencing, to test the success of the library. Only four (11.8% of those sequenced) contained microsatellites (three *T. merula* colonies; one *T. iliacus*), one of which (from *T. merula*) was suitable for primer design. The criteria for selection of suitable sequences (for primer design) were that the size of the microsatellites should be between eight and ~25 repeats, and that the flanking sequence should be good quality, and of sufficient size (a minimum of 20bp between the microsatellite and the cloning site) to accommodate primers.

In the second library, approximately 187 individual colonies (~28%) contained inserts of 300 to 700bp. Of these, 29 Blackbird and 46 Redwing clones were sequenced. Approximately half of these (51% in Blackbirds; 49% in Redwings) contained microsatellites. After discarding unsuitable sequences, 19 contained a microsatellite repeat with good flanking regions for primer design (nine from Blackbirds; 10 from Redwings). Of the total 19 primers tested, only two amplified *Turdus* DNA from both species (there were no species-specific primers) and were polymorphic. The results are summarised in Table 2.1.

Table 2.1 List of polymorphic primers developed from T. merula *and* T. iliacus *showing the sequence, repeat motif, label, number and size range of alleles, annealing temperature, and number of birds genotyped.*

Locus	Primer sequences (5′-3′)	Repeat	Label	No. of alleles	Allelic size range (bp)	Tm (°C)	No. of birds genotyped
BB3	F: GAAGTACCATGGGAGGCTGA	$[GT]_5TTAT[GT]_6$	HEX	5*	191-199*	48	314*
	R: AGGAACAAAAAGGGGTTTGC			6†	†189-199		228†
RW4	F: CTGCTGGTTTTCTTGCTGTG	$[CA]_{12}TG[CA]_4$	FAM	10*	160-180*	50	305*
	R: AACTGCCTGCATAGCATGTG			29†	150-226†		220†
RW9	F: ATGCTTTGGCTGACGACTCT	$[TG]_{10}$	FAM	9*	200-216*	48	252*
	R: GAGCAAACATCTTTATGGCAAA			7†	194-208†		225†

** T. merula; †T. iliacus*

Screening of available passerine primers

Of the 35 previously-characterised primers screened for use in *Turdus* species, seven were polymorphic and produced clearly defined peaks with baseline separation. These results are summarised in Table 2.2. Primer sequence, label, number and size range of alleles, and annealing temperature for successful primers are provided in Table 2.3.

Table 2.2 Previously-characterised microsatellite primers screened for utility (amplification and polymorphism) in Turdus merula *and* T. iliacus, *listed by family. Information about linkage groups and predicted position on passerine genome (where available) is also provided.*

Family†	Locus	Species latin name†	Predicted position on passerine genome‡	GenBank® accession number	Reference	Results for *T. merula*	Results for *T. iliacus*
Corvidae	ApCo 46**	*Aphelocoma coerulescens*	Gga 1	AF520885	Stenzler & Fitzpatrick (2002)	Did not amplify	Did not amplify
	ApCo 80**		Not mapped	AF520879		No polymorphism	No polymorphism
	ApCo 104		Gga 5	AF520900		Difficult to score due to stutter•	Difficult to score due to stutter•
	MJG1	*Aphelocoma ultramarine*	Not mapped	U82673	Li et al, (1997)	Difficult to score due to stutter•	Difficult to score due to stutter•
Fringillidae	Asμ15**	*Ammodramus savannarum*	Not mapped	AY172993	Bulgin et al. (2003)	Did not amplify	Did not amplify
	Dki B119**	*Dendroica kirtlandii*	Gga 18	AY769677	King et al. (2005)	Difficult to score due to stutter•	Difficult to score due to stutter•
	LOX1	*Loxia scotica*	Gga 2; LG2	Y16820	Piertney et al. (1998)	Difficult to score due to stutter•	Difficult to score due to stutter•
	Tc.11 B4E**	*Telespiza cantans*	Gga 11	AF036266	Tarr et al. (1998)	Did not amplify	Did not amplify
Muscicapidae	Cuμ02	*Catharus ustulatus*	Gga 24; LG4	AF122890	Gibbs et al. (1999)	Did not amplify	Did not amplify
	Cuμ04		Gga 5; LG6	AF122891		Did not amplify	Did not amplify
	Cuμ28		Gga 7; LG11	AF122894		Polymorphic	Difficult to score due to stutter•
	Cuμ32		Not mapped	AF122895		Polymorphic	Difficult to score due to stutter•

Family†	Locus	Species latin name†	Predicted position on passerine genome‡	GenBank® accession number	Reference	Results for *T. merula*	Results for *T. iliacus*
Paridae	PAT MP2-43§	*Poecile atricapillus*	LG3	DQ179380	Otter et al. (1998)	High level of polymorphism	High level of polymorphism
	Pca7	*Parus caeruleus*	Not mapped	AJ279809	Dawson et al. (2000)	No polymorphism	No polymorphism
Passeridae	Pdo47	*Passer domesticus*	Not mapped	AM159027	Dawson et al., (unpubl. data)	Did not amplify	Did not amplify
	TGU 03***	*Taeniopygia guttata*	Gga 7	DV955012	Slate et al. (2007)	No polymorphism	No polymorphism
	TGU 05***		Gga 1	DV946651		Did not amplify	Did not amplify
	TGU 06***		Not mapped	CK307697		Difficult to score due to stutter•	Polymorphic
	TGU 07***		Gga 6	DV948303		Polymorphic	Polymorphic
Pipridae	Man13	*Manacus manacus*	Not mapped	AF515782	Piertney et al. (2002)	No polymorphism	No polymorphism
Maluridae	Mcyμ4	*Malurus cyaneus*	Gga 5; LG6	U823588	Double et al. (1997)	No polymorphism	Low level of polymorphism
Sylviidae	Ase19	*Acrocephalus sechellensis*	Not mapped	AJ276376	Richardson et al. (2000)	Weak amplification	Difficult to score due to stutter•
	Ase40		Not mapped	AJ276642		High level of polymorphism	High level of polymorphism
	Ase50		CHD-Z	AJ276779		Did not amplify	Did not amplify
	Ase55**		Gga 3; LG1	AJ276784		Did not amplify	Did not amplify
	Ase56		Not mapped	AJ276785		Did not amplify	Did not amplify
	MSLP 2	*Locustella pryeri*	LG6	AB031374	Ishibashi et al. (2000)	No polymorphism	No polymorphism
	MSLP 4**		Not mapped	AB031376		Difficult to score due to stutter•	Difficult to score due to stutter•
	POCC8	*Phylloscopus occipitalis*	Gga 5	U59119	Bensch et al. (1997)	No polymorphism	No polymorphism
Timaliidae	Pte24**	*Pomatostomus temporalis*	Not mapped	DQ234870	Blackmore et al. (2006)	Did not amplify	Weak amplification
Tyrannidae	SAP 47**	*Sayornis phoebe*	Not mapped	AY823673	Beheler et al. (2007)	Weak amplification	Weak amplification
Zosteropidae	ZL04	*Zosterops lateralis*	CHD-Z	AF076665	(Degnan et al., 1999)	Did not amplify	Did not amplify
Charadriidae*	Calex 01	*Charadrius alexandrinus*	-	AM072445 AM072446 AM072447	Küpper et al. (2007)	No polymorphism	No polymorphism
	Calex 05		-	AM072453		No polymorphism	No polymorphism
	Calex 08**		-	AM072456		Difficult to score due to stutter•	Difficult to score due to stutter•

†Based on Sibley & Monroe (1990). ‡Based on Dawson et al. (2006) and/or Slate et al. (2007), where each chromosome is labelled by its chicken nomenclature (e.g. Gga 1 = *Gallus gallus* chromosome 1), and/or Hansson et al. (2005), where each linkage group is labelled LG. All Gga chromosomes are autosomal, with the exception of the CHD-Z (chromodomain-helicase-DNA-binding protein) gene, which is sex chromosome-linked.

§Otter et al did not submit sequence data for this locus, however Dawson et al. (2006) subsequently sequenced this locus in Great Reed Warbler (*Acrocephalus arundinaceus*).

•Stutter or shadow bands are secondary products (that may be either shorter or longer than the expected product) formed during PCR, the production of which makes scoring of the correct-length product difficult (e.g. Tombline et al., 1996; Klebe, 1999).

*The *Charadrius* loci (Calex01, Calex02 and Calex08) are not passerine loci but have been found to be of utility in a wide range of species including passerines (D.A. Dawson & C. Küpper unpublished data). **Alternative conserved primer sets, developed by Dawson et al. (unpublished data), were used. *** Primer sequences published, but locus names not provided (Deborah Dawson, *pers. comm.*).

Also note that while many of these previously-characterised primers proved unsuitable for this particular study, the problem was often attributable to the quality of the DNA samples, rather than with the primers. It is expected that many of the primers not used in the present study (e.g. Ase19, LOX1 and MJG1) will be useful for future studies of *Turdus* species where good quality DNA samples are available.

Table 2.3 List of previously-characterised passerine primers that amplified well and were polymorphic in Turdus merula *and* T. iliacus, *showing the sequence, repeat motif, number and size range of alleles, annealing temperature, and number of birds genotyped.*

Locus	Primer sequences (5′-3′)	Repeat	Label	No. of alleles	Allelic size range (bp)	Tm (°C)	No. of birds genotyped
Ase40	F: CACTGCTCCAGGCACTCTG R: TCCAAGGCACACAAAGGTG	$(GT)_{10}$	FAM	22* 19†	224-266* 218-262†	50	298* 220†
Cuμ28	F: GAGGCACAGAAATGTGAATT R: TAAGTAGAAGGACTTGATGGCT	$(CA)_{12}$	HEX	14*	157-187*	50	297*
Cuμ32	F: AGGAGAGTGAAAGAAAAGGG R: GAATTCTCAGCATGACAAATC	$(GT)TT(GT)_9$	FAM	6*	115-125*	50	295*
Mcyμ4	F: ATAAGATGACTAAGGTCTCTGGTG R: TAGCAATTGTCTATCATGGTTTG	$(GT)_{26}AT(GT)_3$	FAM	5†	123-131†	50	222†
PAT MP2-43	F: ACAGGTAGTCAGAAATGGAAAG R: GTATCCAGAGTCTTTGCTGATG	$(TC)_9$	HEX	22* 21†	132-176* 124-234†	50	291* 228†
TGU06	F: CGAGTAGCGTATTTGTAGCGA R: AGGAGCGGTGATTGTTCAGT	$(AG)_{21}$	HEX	9†	175-193†	54	212†
TGU07	F: CTTCCTGCTATAAGGCACAGG R: AAGTGATCACATTTATTTGAATAT	$(AC)_{22}$	HEX	11* 10†	92-112* 90-108†	54	302* 226†

* *T. merula*; † *T. iliacus*

Discussion

Novel primers

When developing a microsatellite-enriched library, it is inevitable that of those inserts that do contain microsatellites, many are unsuitable, due to their size (only fragments of 300–700bp are suitable), position (if they are located close to the cloning site, this may leave insufficient length to accommodate flanking primers), or number of repeats (8~25). Many more are unsuitable due to their lack of polymorphism. In addition, a recent review by Zane et al. (2002) revealed that birds appear to have a much lower frequency of microsatellites than all other taxa surveyed. Despite these factors, the isolation of only three novel microsatellite primers was a disappointing result, given the associated effort and

cost. Using an enrichment protocol is thought to be the best approach (particularly important in birds, since they have such a low frequency of microsatellites), because this should increase the number of positive clones. However, any isolation protocol requires a number of steps, any of which can produce less than optimal results, which will affect subsequent stages. For example, cloning may be difficult for inexperienced researchers, because the bacterial cells are very temperature-sensitive. In addition, certain factors may require optimisation, such as hybridisation wash temperature (Golo Maurer *pers. comm.*). In addition, alternative microsatellite isolation procedures may be more efficient. For example, Zane et al. (2002) describe an alternative microsatellite isolation procedure Fast Isolation by AFLP of Sequences Containing repeats (FIASCO), which was tested in a number of different organisms, including birds. This protocol is based on the digestion-ligation reaction of the amplified fragment length polymorphism (AFLP) procedure by Vos et al. (1995), and was found to produce a high percentage (50-95%) of clones containing dinucleotide repeats. However, testing a new protocol was beyond the scope of the current study.

Previously-characterised primers

Seven of the screened primers that amplified successfully, consistently produced baseline separation of peaks and were polymorphic for the two *Turdus* species (four for *T. merula*, two for *T. iliacus*). Of all the previously-characterised primers that were tested, the Cuμ primers from the family Muscicapidae were from the family most closely related to Turdidae. Two of these worked well for the *T. merula* samples, but perhaps surprisingly, did not produce good results for *T. iliacus*. The other primers that worked well for both species were Ase40, TGU07, and PAT MP 2-43, from Sylviidae, Passeridae and Paridae respectively. None of these families is closely related to that of Turdidae. A few studies have suggested guidelines for selecting primers likely to amplify across species. Primmer (1996) tested primers developed for the Barn Swallow *Hirundo rustica* and the Pied Flycatcher *Ficedula hypoleuca* on 48 different bird species. Galbusera et al. (2000) tested 40 microsatellite primers in 13 passerine species. Both studies found an increase in probability of amplification and polymorphism with decreasing phylogenetic distance. Unfortunately, the majority of primers

that produced a good amplification in one or both *Turdus* species were not found to be polymorphic (e.g. TGU03 and Man13). Others (such as ZL04 and LOX1), that had already been tested and found to be polymorphic in a number of other species (Galbusera et al., 2000), either failed to amplify, or produced poor amplifications that were difficult to score.

More recently, Primmer at al. (2005) reported that genetic distance, estimated by pairwise cytochrome b (cytb) divergence, provided accurate estimates of cross-species microsatellite amplification/polymorphism success rates. This applied not only within avian taxa, but also in taxa where microsatellites cross-amplified across contrasting levels of taxonomic classification (frogs and cetaceans). Additional features found to affect cross-species amplification/polymorphism success included decreasing the PCR annealing temperature (an approach used in the present study), and a significant positive association between polymorphism in the source species and the proportion of target species in which a locus revealed polymorphism. No association between cross-species amplification and repeat motif (di-, tri-, or tetranucelotide) or repeat structure (perfect, imperfect, or compound) was found. Based on their observations, Primmer at al. (2005) were able to identify a set of nine (previously-characterised) microsatellite loci that cross-amplified across an "unusually broad" range of passerine species. Although this study was not published at the time of the analyses reported here, three of the nine loci recommended by Primmer et al. (2005) were tested (LOX1, Pca7 and ZL04), but none were found to be useful in either *Turdus* species.

It should be noted, however, that many of the DNA samples used in this study were of low concentration and/or poor quality, due to the type of tissue available (mostly contour feathers; details of extraction methodology are described in the following chapter). This explains why, for some primers, although amplifications were successful, the analysed fragments were of insufficient quality to allow unambiguous genotyping. When good quality DNA samples were available (extracted from muscle tissue), these usually produced results that were both clearer and stronger than those from feather extractions, making the former much easier to score. Therefore, although many of the previously-characterised primers proved unsuitable for this particular study, the problem was often attributable to

the quality of the DNA samples, rather than with the primers. It is expected that many of the rejected (previously-characterised) primers (such as Ase19, LOX1 and MJG1) will be useful for future studies of *Turdus* species where good quality DNA samples are available.

Conclusion

Screening of previously-characterised primers is potentially very useful, especially where DNA samples of the target species are of good quality/quantity. Indeed, beginning with this approach, rather than attempting to isolate novel loci, may minimise costs (in terms of both time and expense). Consultation of studies that have tested cross-species amplification of passerine microsatellite loci, as well as the BIRDMARKER database (http://www.shef.ac.uk/misc/groups/molecol/deborah-dawson-birdmarkers.html), should provide a useful starting point. Where DNA samples are likely to be of low quality/concentration (and this is unavoidable), isolation of novel loci may be a more useful approach, but considerable care should be taken during the DNA extraction procedure (e.g. the use of purification kits, which remove contaminants, and maximise the quantity of DNA obtained, is recommended).

CHAPTER 3: GENETIC POPULATION STRUCTURE OF *TURDUS MERULA* AND *T. ILIACUS* ANALYSED USING MICROSATELLITE MARKERS

Introduction

According to Balloux & Lugon-Moulin (2002), populations of most, if not all, species show some levels of genetic structuring. Even the European Eel *Anguilla anguilla*, once considered the classic example of a single panmictic population because all individuals are thought to migrate to the Sargasso Sea for reproduction, has been shown to be geographically structured, through the use of microsatellite analysis (Wirth & Bernatchez, 2001). Historical processes, geographical barriers, and life history traits (e.g. mating systems) may all influence the genetic structure of populations. In addition, as species' geographical distributions may be more extended than an individual's capacity to disperse, populations in close proximity may be genetically more similar to one another than to more distant populations (Balloux & Lugon-Moulin, 2002).

Migratory behaviour and gene flow

One factor that might limit the use of genetic markers for avian studies is that, since birds are highly mobile, there may be widespread dispersal and weak genetic differentiation between populations (Webster et al., 2002). A second caveat to using genetic markers for avian species is that populations inhabiting recently glaciated areas (such as the northern temperate zone) may be less genetically variable than populations inhabiting regions where their separation has been established for a longer period of time. This pattern is common to many different taxa (Merilä et al., 1997). These factors may explain why several phylogeographical analyses of migratory birds, particularly those utilising mtDNA (e.g. Bensch et al., 1999; Mila et al., 2000; Lovette et al., 2004) have revealed differentiation between large geographical regions, but little differentiation within regions (Webster et al., 2002). However, few studies to date have explored the utility of microsatellites for investigating migratory connectivity (but see Clegg et al., 2003).

Nonetheless, populations of many avian species differ in their migratory behaviours, e.g. propensity to migrate (i.e. migratory versus sedentary populations), and/or migratory routes (Davis et al., 2006), and this may be reflected in the genetic structure of populations. For example, it could be predicted that migratory habit enhances gene flow, so that population genetic structure will be weaker (indicating relatively high gene flow) in migratory populations than in those that are sedentary (Arguedas & Parker, 2000). Since Redwings (of the nominate race *iliacus*) are all long-distance migrants, tend to be nomadic, and are not thought to display strong site fidelity (Snow, 1986b; Reid, 1993), it might be expected that there is more mixing between breeding populations in this species than in the partially migrant Blackbird. Thus, levels of genetic population structure are likely to vary between the two species, reflecting their different migratory strategies.

A variety of genetic markers, particularly mtDNA, have already been used for tracing the origins of migratory birds. The different types of genetic markers, and their application to bird migration studies are summarised in Chapter 1. Since microsatellites have proven utility for population differentiation studies, on both a practical and theoretical level, and do not suffer from some important limitations of other genetic markers, this approach was used to investigate genetic structure in breeding populations of Blackbirds and Redwings.

Aims

The aim of the analyses described in this chapter was to use microsatellite markers to determine whether genetic population structure exists in the breeding ranges of Blackbirds and Redwings, and whether this information is useful for assigning wintering individuals to their source (i.e. breeding) population.

The following hypotheses were tested:

- Genetic population structure does exist within the breeding ranges of both Blackbirds and Redwings, but is strongest in Blackbirds.

- Microsatellite markers will allow discrimination between the two Redwing races *Turdus iliacus iliacus* and *T. i. coburni.*

Methods

Sampling

All feathers for this part of the study were collected with the assistance of bird ringers licensed under the European Union for Bird Ringing (EURING) scheme, who volunteered to participate in this project. Birds were mainly caught in mist-nets (in a few cases whoosh nets were used), and all feathers were collected without deliberate pulling or plucking; fortunately these species (particularly Blackbirds) are inclined to shed several contour feathers during routine ringing and processing (the reason for this is unclear, but one may speculate that this could be a defence mechanism).

Feathers were sampled from twelve breeding populations of Blackbirds, and nine breeding populations of Redwings (eight of the subspecies *iliacus*, and a single *coburni* population). Sampling locations are illustrated in Figure 3.1, and numbers listed in Table 3.1(a) (Blackbirds) and Table 3.1(b) (Redwings). Redwing samples from two of the sites in southern Finland (Espoo and Hanko) were combined, due to extremely small sample sizes (two and three respectively). Although this is clearly not an ideal strategy, it was felt that the two sites were located close enough to be considered as a single population, and that this was preferable to either considering the two sites separately, or excluding all five samples. This pooled 'population' is hereafter referred to as 'southern Finland'. Note that three-letter International Organization for Standardization (ISO) country codes (provided in Table 3.1) are used in subsequent Tables in the current and following chapters.

Feathers from non-breeding birds were collected from 15 sites in the UK and Ireland; sampling locations are provided in Figure 3.2, and numbers listed in Table 3.2. Note that the non-breeding Redwings (sampled at Bardsey, UK), were all natural casualties (the birds died by flying into a lighthouse), and thus whole

carcasses were collected, and stored at –4°C. Feathers requiring long periods of storage were also kept at –4°C; those requiring only short storage periods were kept in paper envelopes at room temperature, until required for analysis.



Figure 3.1 Map of the sample sites where feathers from twelve populations of breeding Blackbirds and nine populations of breeding Redwings were collected for comparative genetic analyses. Sites where Blackbirds were sampled are represented by blue triangles; Redwings by red diamonds; both species by white circles. The map was created using Google Earth.

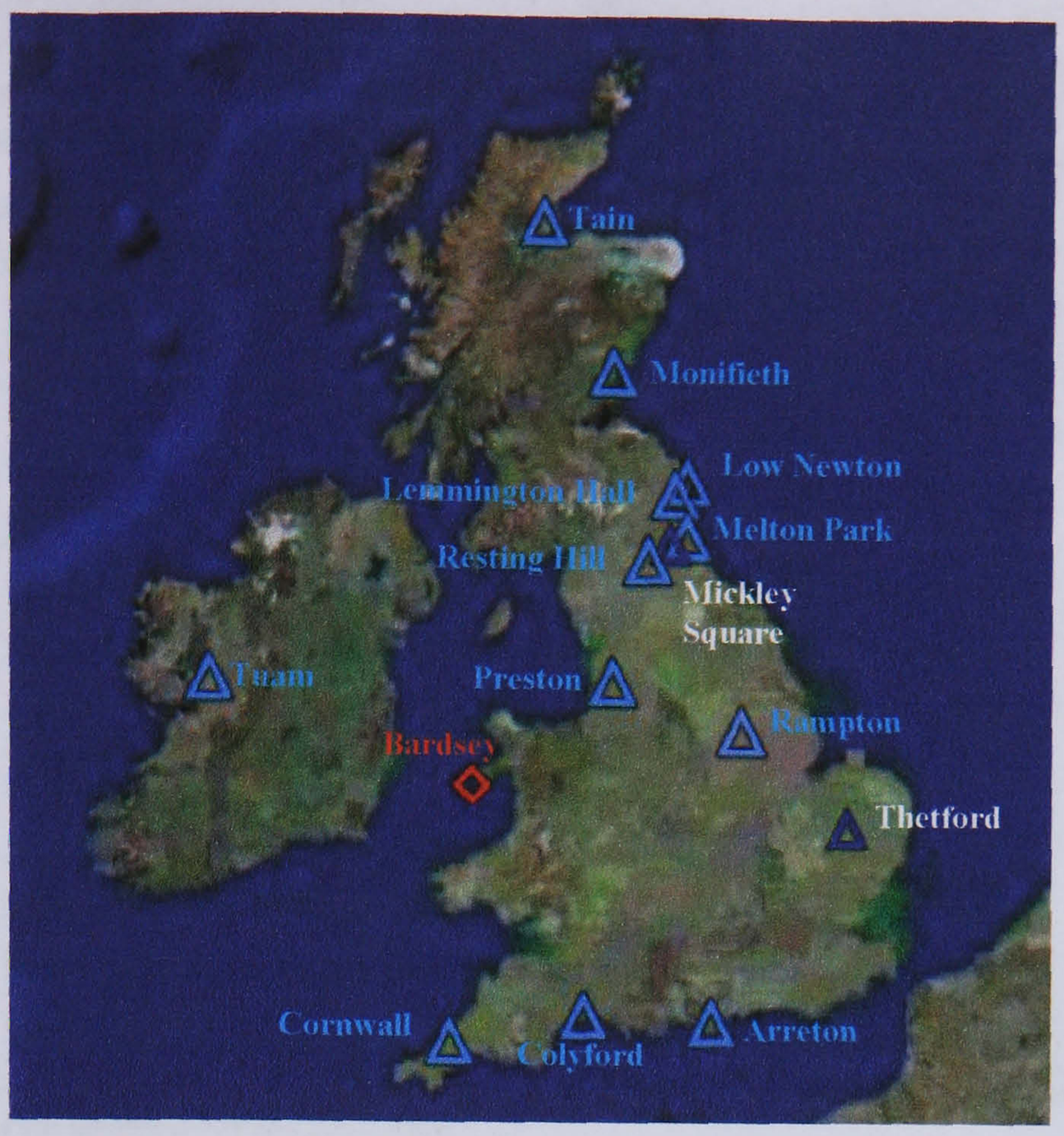


Figure 3.2 Map of the sample sites where feathers from UK and Irish-wintering Blackbirds and Redwings were collected for genetic analysis. Sites where Blackbirds were sampled are represented by blue triangles, with the darker shade (and white text) indicating the two sites where both breeding and overwintering Blackbirds were sampled; the Redwing sampling site is represented by a red diamond. The map was created using Google Earth.

Table 3.1(a) Sampling location (including ***three-letter International Organization for Standardization (ISO) country codes****) and size of twelve breeding populations of Blackbirds used to investigate genetic population structure. Samples were collected between May and September 2005.*

Name of main sample collector(s)	**Sample site**	**ISO country code**	***n***	**Latitude**	**Longitude**
Kjetil Aadne Solbakken	Titran, Norway	NOR	16	63.40N	08.19E
Alf Tore Mjos	Eidfjord, Norway	NOR	16	60.25N	07.08E
Aleksi Lehikoinen & Vilppu Välimäki	Hanko, Finland	FIN	11	59.49N	22.54E
David Leat	Skye, UK	GBR	5	57.15N	06.20W
Lars Hansen	Nyborg, Denmark	DNK	11	55.20N	10.48E
David Leat	Northumberland, UK	GBR	16	54.56N	01.54W
Andrey Mukhin	Kaliningrad, Russia	RUS	12	55.00N	20.34E
Jeff & Allison Kew	Norfolk, UK	GBR	14	52.24N	00.43E
Sigfried Schlegel	Annaberg-Buchholz, Germany	DEU	13	50.56N	13.00E
Jules Diederich	Schifflange, Luxembourg	LUX	12	49.30N	06.01E
Wolfgang Fiedler	Radolfzell, Germany	DEU	15	47.45N	9.00E
Gerhard Loewe	Ozzano, Italy	ITA	5	44.42N	10.08E

Table 3.1(b) Sampling location (including three-letter International Organization for Standardization (ISO) country codes), race, and size of breeding populations of Redwings used to investigate genetic population structure. Samples were collected between June and September 2005, with the exception of the Icelandic birds, which were collected in September 2004.

Name of main sample collector(s)	**Race**	**Sample site**	**ISO country code**	***n***	**Latitude**	**Longitude**
Morten Helberg	*iliacus*	Troms, Norway	NOR	16	68.45N	19.40E
Veikko Isomursu	*iliacus*	Rovaniemi, Finland	FIN	9	66.31N	25.45E
Tómas G. Gunnarsson	*coburni*	Selfoss, Iceland	ISL	19	64.07 N	20.29W
Kjetil Aadne Solbakken	*iliacus*	Titran, Norway	NOR	14	63.40N	08.19E
Thomas Holmberg	*iliacus*	Handol, Sweden	SWE	13	63.16N	12.27E
Petri Suorsa	*iliacus*	Konnevesi, Finland	FIN	6	62.37N	26.26E
Alf Tore Mjøs	*iliacus*	Eidfjord, Norway	NOR	10	60.25N	07.08E
Vilppu Välimäki	*iliacus*	Espoo, Finland*	FIN	2	60.11N	24.48E
Aleksi Lehikoinen	*iliacus*	Hanko, Finland*	FIN	3	59.49N	22.54E

*Note that samples from these two sites were combined, due to the small sample sizes involved. The pooled sites are henceforth referred to as the southern Finland population.

Table 3.2(a) Sampling location and size of non-breeding Blackbirds used to investigate genetic population structure. Samples were collected between October 2004 and March 2005. Sample sites are listed in order of descending latitude.

Name of main sample collector(s)	**Sample site**	***n***	**Latitude**	**Longitude**
Bob Swann	Tain, Ross & Cromarty, UK	12	57.48N	04.03W
Alan Barclay & Bruce Lynch	Monifieth, Angus, UK	8	56.29N	13.00W
Chris Redfern	Low Newton, Northumberland, UK	12	55.30N	01.37W
Philip & Hugh Hanmer	Lemmington Hall, Northumberland	12	55.23N	01.48W
Dave Sowter	Preston, Lancashire, UK	14	55.20N	02.41W
Bob Gajdus	Melton Park, Tyne & Wear, UK	24	55.01N	01.37W
David Leat	Mickley Square, Northumberland, UK	10	54.57N	01.53W
Martin Hughes	Resting Hill, Northumberland, UK	15	54.48N	02.13W
Chris Benson	Tuam, Galway, Ireland	23	53.30N	08.51W
Mike Archer	Rampton, Nottinghamshire, UK	8	53.17N	00.47W
Jeff & Allison Kew	Thetford, Norfolk, UK	14	52.24N	00.43E
Greg Conway	Colyford, Devon, UK	4	50.43N	03.03W
Anthony Roberts	Arreton, Isle of Wight, UK	8	50.40N	01.14W
Greg Conway	Cornwall*, UK	6	50.21N	04.56W

Note that birds from Cornwall were sampled from three sites, all within close proximity. Therefore longitude and itude provided in the table are means of the three sites.

Table 3.2(b) Sampling location and size of non-breeding Redwings used to investigate genetic population structure. Note that all samples were collected at the same site; only collection date varies.

Name of main sample collector	Sample site	Latitude	Longitude	*n*	Collection date
Ian Fisher	Bardsey, North Wales, UK	52.45N	04.46W	81	Oct 1995
				26	Mar 1998
				5	Oct 1998
				27	Oct 1999

DNA extraction

Where carcasses were available (i.e. non-breeding Redwings), DNA was extracted from muscle tissue, following the NaAc protocol described in Chapter 2). Where only feathers were available, DNA was extracted from the entire feather calamus, including the blood clot located in the superior umbilicus (Figure 3.3). Initial extractions followed the NaAc protocol (used for carcasses). However, DNA extracted from feathers in this way was found to produce poor results, due to low DNA concentration and/or presence of DNA inhibitors. Therefore the majority of feather extractions were carried out using a DNeasy® Tissue Kit (Qiagen), following the manufacturer's instructions. This purification kit effectively removes contaminants and enzyme inhibitors, and PCR amplification of the resulting samples was consistently better than standard NaAc extractions.

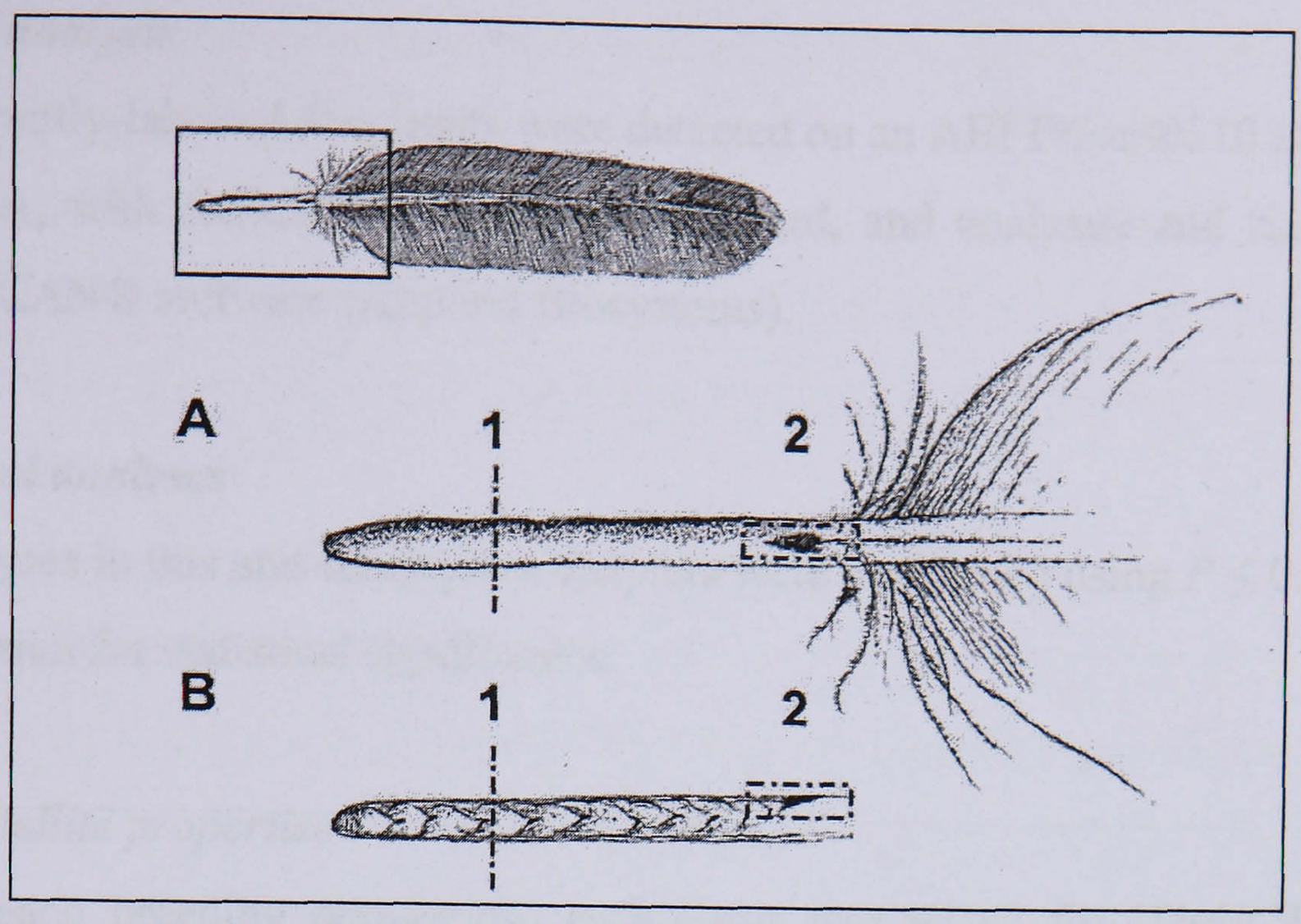


Figure 3.3 DNA sampling from feathers: (A) detail of a posterior view of the base of the feather, and (B) longitudinal cross-section through the feather calamus. Two different sampling areas for DNA are shown: (1) basal tip of the calamus and (2) blood clot from the superior umbilicus. The entire calamus (encompassing sample sites (1) and (2)) was used for DNA extraction in this study. From Horváth et al. (2005).

Microsatellites

Two microsatellite loci isolated from Redwings, and one isolated from Blackbirds, were used in conjunction with seven microsatellite loci (five per species) characterised for other species, as described in the previous chapter, and summarised in Table 3.3. For all samples, loci were amplified individually via PCR, as described in the previous chapter.

Table 3.3. Microsatellite loci used to amplify Turdus merula *and* T. iliacus *DNA samples for comparison of genetic structure of breeding populations.*

Locus	**Source species**	**Reference**	**Species in which locus was amplified**
RW4 RW9	*Turdus iliacus iliacus*	Novel loci	*T. iliacus* and *T. merula*
BB3	*Turdus merula*	Novel locus	*T. iliacus* and *T. merula*
Ase40	*Acrocephalus sechellensis*		*T. iliacus* and *T. merula*
PAT MP 2-43	*Poecile atricapillus*	Otter et al. (1998)	*T. iliacus* and *T. merula*
Cuμ28 Cuμ32	*Catharus ustulatus*	Gibbs et al. (1999)	*T. merula* only
TGU06 TGU07	*Taeniopygia guttata*	Slate et al. (2007)	*T. iliacus* only *T. iliacus* and *T. merula*
Mcyμ4	*Malurus cyaneus*	Double et al. (1997)	*T. iliacus* only

Genetic analysis

Fluorescently-labelled fragments were detected on an ABI Prism®310 automated sequencer, with ROX500 internal size standard, and analysed and sized using GENESCAN® software (Applied Biosystems).

Statistical analyses

All analyses in this and subsequent chapters were performed using $P \leq 0.05$ as the criterion for statistical significance.

Microsatellite properties

Within each breeding population, each locus was tested for Hardy-Weinberg equilibrium (to test whether the observed genotypes are the product of a random union of gametes), and all pairs of loci were tested for linkage disequilibrium (disequilibrium of Hardy-Weinberg due to loci being located close to each other on the same chromosome, and thus inherited together). Wintering birds, treated as a single population of each species, were also tested for Hardy-Weinberg equilibrium. These analyses were performed (separately for each species) using Arlequin software (version 2.000; Schneider et al., 2000).

Factorial correspondence analysis

For breeding birds, the software GENETIX (Belkhir et al., 1996-2004) was used to carry out a factorial correspondence analysis (FCA); a multivariate analysis method (sometimes referred to as Principal Coordinates Analysis, PCO), analogous to a principal components analysis (PCA) of the rows and columns of a table. However, whereas PCA is used for tables consisting of continuous data, a correspondence analysis can be applied to contingency tables (i.e. cross-tabulations), in which individuals are described by their allele frequencies (samples x alleles). The contingency table is used to measure the relatedness between any two samples in the k-dimensional space (k = number of alleles). The resulting factorial axes can be ordered according to their eigenvalues (the eigenvalue is a measure of the variance between samples), with the first axis corresponding to the largest eigenvalue and so on. The ordination of populations along the major factorial axes can be used to visualise the genetic similarity between populations in a three-dimensional scatterplot (e.g. She et al., 1987; Lu

et al., 2001; D'Amato, 2006). In addition to investigating the extent of population differentiation and genetic relationships among samples, FCA is useful for detecting individuals that are potential outliers (possibly due to genotyping errors). A single Redwing (from Rovaniemi, Finland) and a single Blackbird (from Titran, Norway) were identified as clear outliers and were omitted from subsequent analyses.

Population genetic structuring

The most commonly reported method for investigating population genetic structuring is to use fixation indices to estimate patterns of gene flow among populations. Two sets of such statistics are typically used for microsatellite data. The oldest (and most widely used) are the *F*-statistics. Originally devised to describe the effect of inbreeding within samples (Wright, 1921, 1922), the inbreeding coefficient (*F*) was expanded to account for hierarchical levels of population subdivision (Wright, 1951). F_{ST} is a measure of allele frequency divergence among subpopulations. Since its original inception by Wright, F_{ST} has been redefined by Nei (1977) to account for loci with multiple alleles:

$$F_{ST} = (H_T - H_S) / H_T,$$

where H_T is the expected Hardy-Weinberg heterozygosity of the total population (i.e. heterozygosity *between* subpopulations), and H_S is the average expected heterozygosity derived from each individual (i.e. mean heterozygosity *within* subpopulations).

More recently, Slatkin (1995) devised R_{ST}, which is an F_{ST} analogue, based explicitly on the stepwise mutation model (SMM), which some microsatellites appear to follow. R_{ST} can be defined as:

$$R_{ST} = (S - SW) / S,$$

where S is the average squared difference in allele size between all pairs of alleles, and SW the average sum of squares of the differences in allele size within each subpopulation. Thus, R_{ST} can be calculated from the variances of allele

sizes, whereas F_{ST} is typically derived from the variances of allele *frequencies* (Balloux & Lugon-Moulin, 2002). Both F_{ST} and R_{ST} range from 0 to 1, where 0 indicates no population divergence and 1 indicates complete differentiation among populations. For each species, Arlequin software was used to perform global and locus-by-locus Analyses of Molecular Variances (AMOVAs; 1000 permutations), in order to compare the genetic variance (measured as F_{ST}) attributable to between (versus within) breeding populations. Subsequently, both F_{ST} and R_{ST} were estimated for each pair of breeding populations. Permutation testing (100 replicates) was used to assess whether pairwise F_{ST} and R_{ST} differed significantly from zero. Since it is possible that different parts of the UK and Ireland may receive migrants from different breeding sites, AMOVAs were also performed for wintering birds grouped by collection site (for Blackbirds) or period (for Redwings).

Neighbour-joining trees

For breeding birds, allele frequency data (population-pairwise F_{ST} values) calculated using Arlequin, were entered into PHYLIP (Phylogeny Inference Package, version 3.66; Felsenstein, 2005), and used to construct radial neighbour-joining trees using the program TREEVIEW (version 1.6.6; Page, 1996). Neighbour-joining is a clustering method used for the creation of phylogenetic trees, based on a matrix of pairwise evolutionary distances relating to the set of taxa being studied. The method was first introduced by Saitou & Nei (1987). At each step of the algorithm, the neighbour-joining method selects as neighbours that pair of current taxa, which most decreases the total tree length (the principle of minimum evolution). Unlike the UPGMA (Unweighted Pair Group Method with Arithmetic mean) algorithm for phylogenetic tree reconstruction, neighbour-joining does not assume that all lineages evolved at the same rate and produces an unrooted tree (Gascuel & Steel, 2006).

Cluster analysis

A fundamental prerequisite of traditional methods of measuring population genetic structure (such as F_{ST}) is the definition of populations, which is often based on geographical origin of samples. However, geographical proximity of populations does not always reflect the genetic structure of populations. An

alternative approach is to use Bayesian clustering method, such as the software STRUCTURE, developed by Pritchard et al. (2000), which uses a model-based method to detect clusters of individuals on the basis of their genotypes (Pritchard et al., 2000; Evanno et al., 2005; Pérez Garrido, 2006). The model defines *K* clusters (where *K* may be unknown), each of which is characterised by a set of allele frequencies at each locus. Individuals are assigned probabilistically to one (or more, if their genotypes indicate that they are admixed) cluster(s). STRUCTURE assumes that within populations, loci are at both Hardy-Weinberg and linkage equilibrium, but does not assume a particular mutation process (Pritchard et al., 2000).

In order to test the strength of breeding population genetic structure, STRUCTURE (version 2) was used without prior population designation, to determine whether distinct clusters of populations could be discriminated. The admixture model was selected, as was the option of correlated allele frequencies, because Falush et al. (2003) found that this configuration performed best in cases of subtle population substructure. Preliminary investigations indicated that a burn-in length of 40,000 iterations, followed by 100,000 MCMC (Markov chain Monte Carlo) repetitions was sufficient. Breeding data were analysed under these settings five times for each value of *K*, varying *K* from 1 to 15 in Blackbirds, and from 1 to 10 in Redwings (since there were more Blackbird than Redwing breeding populations)

Assignment Tests

Assignment tests are another method of addressing classification problems, and attempt to assign individuals of unknown origin to their most likely source population, based on likelihoods that the genotype of the individual to be assigned occurs in each of two or more reference populations (e.g. Rannala & Mountain, 1997; Manel et al., 2005). For each individual, probabilities of occurrence in the reference populations can also be calculated using MCMC (Markov Chain Monte Carlo) simulations based on the allele frequencies of the reference populations. By repeating the simulations many times, the expected distribution of genotypes in each reference population can be obtained, which in

turn is used to generate a distribution of genotype likelihood values (Manel et al., 2005).

Breeding Blackbirds were assigned (a) to two groups: UK and non-UK; (b) three groups: UK, Fennoscandia and Continental, and the software GENECLASS2 (Piry et al., 2004) used to determine what percentage of breeding birds could be correctly assigned to their source population. Similarly, Redwings were grouped into separate races (*iliacus* and *coburni*) in order to determine whether sufficient separation occurs between the two to allow assignment of birds of unknown race. GENECLASS2 offers criteria for calculating -log values of likelihoods, and simulation algorithms for calculating likelihood probabilities. Both approaches were tested.

Note that, for Redwings, since assignment tests performed well with the breeding data (see Results section), both methods (-log likelihood, and likelihood probability) were used to predict race of non-breeding Redwings. Assignment tests for breeding Blackbirds generally did not perform well, and therefore assignment of non-breeding Blackbirds was not attempted. However, all genetic variables (both FCA coordinates and assignment test results) are included in assignment models in Chapter 6 (in which different techniques are combined).

Results

Breeding Birds

Microsatellite properties

For Blackbirds, a total of 94 alleles were detected at the eight microsatellite loci across the twelve breeding populations. The number of alleles per locus ranged from four to 21. For Redwings, a total of 79 alleles were detected at the eight microsatellite loci across the eight breeding populations. The number of alleles per locus also ranged from four to 21. Allele frequencies of each population (of both species) are provided in Appendix 2. Other properties of the microsatellite loci used in the analyses (including allele size range) are provided in Chapter 2. Summary statistics for microsatellite loci, including the number of individuals

(n), the number of alleles (A) and proportions of observed (H_O) and expected (H_E) Hardy-Weinberg heterozygosities per population and per locus are provided in Table 3.4.

At locus RW9 in Blackbirds, significant deviation from Hardy-Weinberg expectations in seven of the twelve populations indicates a heterozygote deficiency at this locus. This suggests the presence of one or more null (non-amplifying) alleles at locus RW9. In order to ensure that the inclusion of this locus did not bias the results, all subsequent analyses were carried out excluding locus RW9.

Linkage disequilibrium tests indicated that in most populations (of both species), one or more loci did not segregate independently (Table 3.5). However, in both species, the number of exceptions was relatively small (18/252 pairwise tests or 7.1% in Blackbirds; 15/224 or 6.7% in Redwings), and spread across most of the populations. For the purposes of analysis, all loci are thus considered to segregate independently.

Table 3.4 Summary statistics for (a) Blackbird and (b) Redwing microsatellite loci including: the number of individuals (n), the number of alleles (A) and proportions of observed (H_O) and expected (H_E) Hardy-Weinberg heterozygosities per population and per locus. Significant departures from Hardy-Weinberg expectations ($P<0.05$) are depicted in bold red type, with superscripts [d] and [e] indicating heterozygote deficiency or excess respectively. Note that these P values do not account for multiple comparisons.

(a)		Cuμ28	Cuμ32	BB3	PAT43	Ase40	RW4	RW9	TGU07
Kal*, RUS	***n***	11	12	12	12	11	12	12	12
	A	8	5	3	10	12	4	7	4
	H_E	0.88	0.74	0.42	0.90	0.91	0.57	0.87	0.61
	H_O	0.91	0.75	0.33	0.67	0.82	0.75	**0.67** [d]	0.67
Nyborg, DNK	***n***	11	8	11	10	10	10	10	11
	A	6	4	3	11	6	4	6	7
	H_E	0.75	0.66	0.39	0.94	0.82	0.69	0.84	0.75
	H_O	**0.91** [e]	0.75	0.45	1.00	0.80	0.90	**0.60** [d]	0.73
North*, GBR	***n***	16	14	16	16	15	15	15	15
	A	10	4	3	15	16	5	7	9
	H_E	0.73	0.71	0.41	0.93	0.93	0.65	0.85	0.80
	H_O	**0.88** [e]	**0.43** [d]	0.25	1.00	**0.73** [d]	0.53	**0.60** [d]	0.73
Hanko, FIN	***n***	11	10	11	11	11	11	11	11
	A	11	5	4	14	9	6	7	5
	H_E	0.93	0.77	0.58	0.95	0.89	0.59	0.86	0.70
	H_O	1.00	0.40	0.45	1.00	0.91	0.64	**0.55** [d]	0.91
Buch*, DEU	***n***	12	12	13	13	12	13	10	13
	A	11	4	3	11	10	5	5	8
	H_E	0.91	0.71	0.50	0.91	0.91	0.67	0.86	0.73
	H_O	**0.92** [e]	0.83	0.31	0.92	0.75	0.77	**0.20** [d]	0.62
Radolf*, DEU	***n***	14	14	15	15	14	15	13	14
	A	11	6	4	13	11	6	8	8
	H_E	0.92	0.78	0.40	0.93	0.87	0.69	0.93	0.75
	H_O	0.71	0.71	0.40	0.87	0.71	0.73	**0.31** [d]	0.57
Ozzano, ITA	***n***	5	4	5	5	5	5	4	5
	A	8	3	2	10	7	3	6	4
	H_E	0.98	0.75	0.60	1.00	0.91	0.73	0.96	0.82
	H_O	1.00	0.75	0.60	1.00	1.00	0.80	0.75	0.60
Norf*, GBR	***n***	14	14	14	14	14	14	14	14
	A	9	4	3	13	12	6	6	8
	H_E	0.85	0.77	0.27	0.93	0.93	0.69	0.85	0.76
	H_O	1.00	0.86	0.21	1.00	0.93	**0.57** [d]	0.57	0.79
Schiff*, LUX	***n***	11	12	12	12	12	11	12	12
	A	10	4	3	15	12	4	6	9
	H_E	0.90	0.76	0.37	0.96	0.91	0.52	0.79	0.89
	H_O	0.91	0.83	0.33	0.92	0.92	0.45	**0.33** [d]	0.83
Eidfjord, NOR	***n***	16	16	16	16	16	16	15	16
	A	10	5	4	15	12	4	7	7
	H_E	0.87	0.75	0.55	0.94	0.90	0.55	0.80	0.74
	H_O	0.75	0.94	0.50	1.00	0.94	0.56	0.73	0.81
Titran, NOR	***n***	14	15	15	14	15	15	13	15
	A	9	5	4	14	16	7	4	7
	H_E	0.80	0.71	0.48	0.94	0.96	0.75	0.75	0.83
	H_O	0.79	0.80	0.40	**0.79** [d]	0.93	0.80	0.54	0.73
Skye, GBR	***n***	5	3	5	5	5	5	4	4
	A	3	4	2	4	7	4	4	4
	H_E	0.71	0.93	0.38	0.76	0.91	0.71	0.82	0.82
	H_O	0.60	0.67	0.20	0.80	1.00	1.00	1.00	1.00

(b)		Ase40	Mcyμ4	BB3	PAT43	RW4	RW9	TGU06	TGU07
Hanko,	***n***	5	5	5	5	5	5	5	5
FIN	**A**	3	4	3	7	6	5	6	4
	H_E	0.64	0.53	0.73	0.91	0.89	0.84	0.87	0.84
	H_O	1.00	0.40	0.60	1.00	1.00	0.80	1.00	0.40
Konn*,	***n***	6	6	6	6	6	6	6	6
FIN	**A**	2	2	4	7	5	3	3	5
	H_E	0.48	0.53	0.83	0.88	0.79	0.68	0.62	0.79
	H_O	0.67	0.50	0.83	1.00	0.50	0.50	0.67	1.00
Rov*,	***n***	8	8	8	8	7	8	8	8
FIN	**A**	4	3	4	7	9	3	5	5
	H_E	0.80	0.61	0.84	0.86	0.95	0.61	0.73	0.85
	H_O	0.50	0.63	0.63	0.88	**0.71** [d]	0.63	0.50	0.75
Selfoss,	***n***	14	19	19	18	17	18	19	19
ISL	**A**	7	3	3	11	11	3	5	6
	H_E	0.85	0.15	0.37	0.88	0.90	0.61	0.74	0.76
	H_O	0.64	0.11	0.26	0.78	1.00	0.72	0.74	0.63
Eidfjord,	***n***	9	10	10	10	9	10	5	9
NOR	**A**	6	4	4	7	6	4	4	6
	H_E	0.77	0.71	0.76	0.83	0.80	0.44	0.71	0.81
	H_O	0.67	0.50	0.50	0.70	**0.89** [e]	0.50	0.40	0.89
Titran,	***n***	14	14	14	13	11	11	12	14
NOR	**A**	6	3	5	10	11	3	5	6
	H_E	0.68	0.20	0.67	0.85	0.94	0.62	0.76	0.80
	H_O	0.57	0.21	0.71	0.69	0.82	0.45	0.83	0.86
Troms,	***n***	16	16	15	16	16	16	15	15
NOR	**A**	9	4	4	9	10	6	7	7
	H_E	0.80	0.46	0.78	0.82	0.82	0.58	0.83	0.87
	H_O	0.81	0.50	0.67	0.75	**0.88** [e]	0.69	0.87	0.73
Handol,	***n***	9	13	13	13	12	13	7	12
SWE	**A**	5	3	4	9	11	5	4	6
	H_E	0.82	0.22	0.76	0.79	0.89	0.47	0.57	0.76
	H_O	0.67	0.15	0.46	0.92	1.00	**0.23** [d]	0.71	0.83

* Abbreviations: Buch: Bucholz; Kal: Kaliningrad; Konn: Konnevesi; Norf: Norfolk; North: Northumberland; Schiff: Schifflange; Radolf: Radolfzell: Rov: Rovaniemi

Table 3.5(a) Matrix of linkage disequilibrium tests between each pairwise combination of seven microsatellite loci, within each of twelve breeding Blackbird populations sampled from across Europe. Numbers refer to populations for which the test was significant. Populations: (1) Nyborg, DNK; (2) Northumberland, GBR; (3) Hanko, FIN; (4) Bucholz, DEU; (5) Radolfzell, DEU; (6) Norfolk, GBR; (7) Schifflange, LUX; (8) Titran, NOR.

	Cuμ28	Cuμ32	BB3	PAT43	Ase40	RW4
Cuμ32	3					
BB3	-	-				
PAT43	2, 8	3, 5	-			
Ase40	3, 4, 5	-	4, 6, 7	1		
RW4	-	-	-	3	-	
TGU07	-	2, 4	6	1, 4	-	-

Table 3.5(b) Matrix of linkage disequilibrium tests between each pairwise combination of eight microsatellite loci, within each of eight breeding Redwing populations sampled from across Europe. Numbers refer to populations for which the test was significant. Populations: (1) Rovaniemi, FIN; (2) Selfoss, ISL; (3) Eidfjord, NOR; (4) Titran, NOR; (5) Handol, SWE.

	Ase40	**Mcyμ4**	**BB3**	**PAT43**	**RW4**	**RW9**	**TGU06**
Mcyμ4	2						
BB3	3	4					
PAT43	-	-	5				
RW4	5	-	-	1, 4			
RW9	-	-	-	4	5		
TGU06	-	2	-	3, 4	2	-	
TGU07	-	2	-	3	-	-	

Factorial correspondence analysis

For Blackbirds, the first three principal factors of the factorial correspondence analysis explained a similar amount of the total variation: 3.31%, 3.09% and 3.03% respectively. Similarly, for Redwings, the first three principal factors explained consistently small amounts of the total variation: 4.60%, 4.26% and 3.93% respectively. This indicates that there is very little genetic structure between populations of either species, and examination of the 3D graphical presentation shows that there are no distinct clusters in the multivariate space, even between the two Redwing races *iliacus* and *coburni* (Figure 3.4).

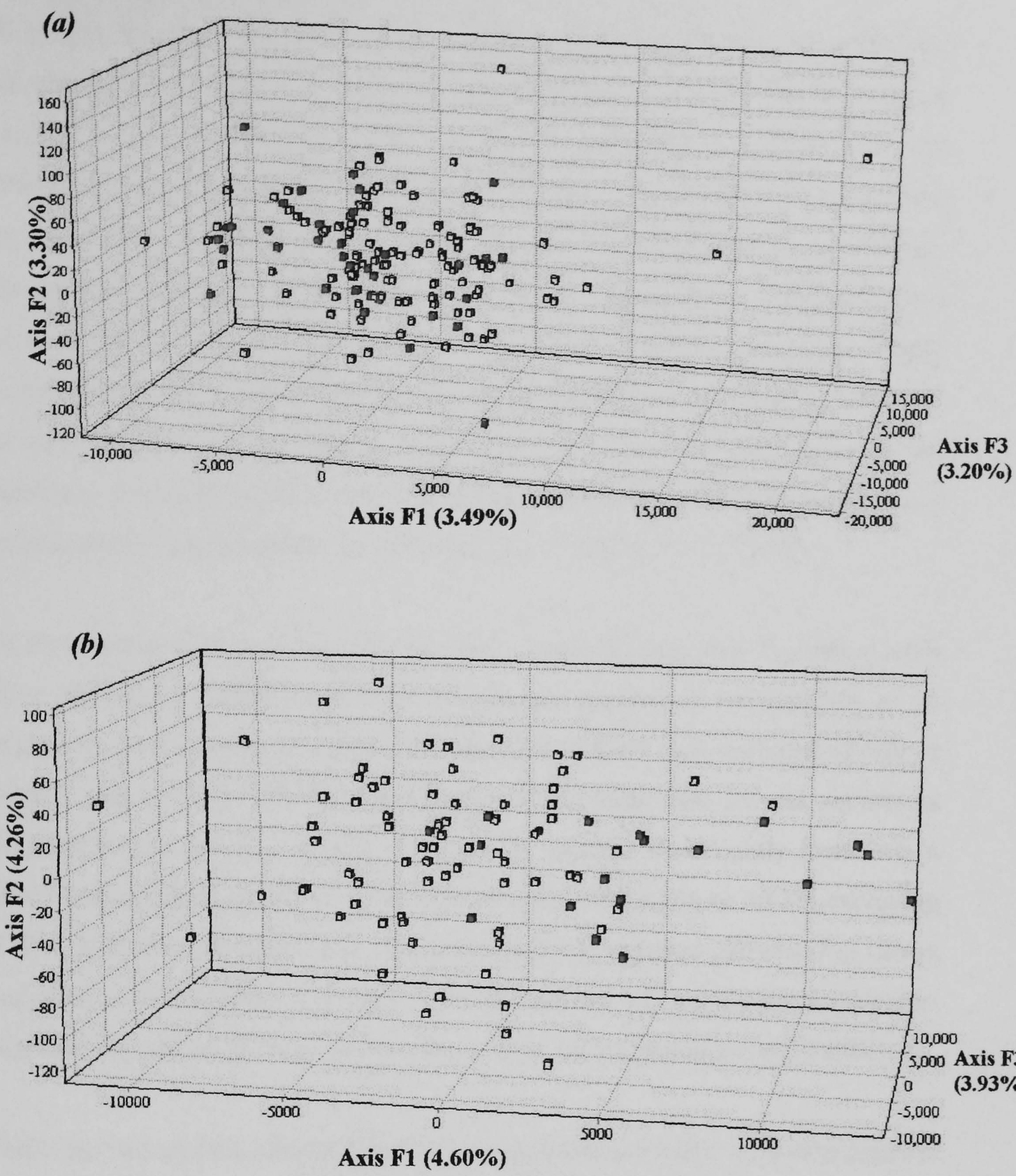


Figure 3.4 Factorial correspondence analysis based on the allele frequencies from (a) seven microsatellite loci genotyped in breeding populations of Blackbirds; (b) eight microsatellite loci genotyped in breeding populations of Redwings. In each case, the first three factors are plotted. In (a) the three UK-breeding populations are shaded (red is Skye; pink is Northumberland; blue is Norfolk). In (b) Redwings of the nominate race iliacus *are white; those of the Icelandic race* coburni *are shaded red.*

Population genetic structuring

For both species, low but significant differentiation between populations was demonstrated by global and locus-by-locus AMOVAs, which compare variation within and between populations. Results are summarised in Table 3.6. For Blackbirds, loci Cuμ28 and PAT43 appear to be the most informative, followed by Ase40. This is not surprising, given that these three loci had the most alleles. In contrast, the most informative loci in Redwings appear to be a combination of those with the fewest alleles: Mcyμ4 and BB3 (only four and five alleles respectively), along with the most polymorphic locus for this species: RW4 (21 alleles). However, when the Icelandic race (*coburni*) was removed from the analysis, global AMOVA did not detect any significant differentiation between populations of the nominate race (*iliacus*) ($F_{ST} = 0.00129$; $P = 0.358$).

In Blackbirds, there were several significant population-pairwise F_{ST} values, with little difference between results from pairwise population comparisons of all eight loci, and pairwise comparisons when locus RW9 was excluded (28/66 or 42.4%, and 26/66 or 39.4% respectively). There were also several significant population-pairwise R_{ST} values, although the number was slightly lower when RW9 was excluded (25/66 or 37.9% for all eight loci; 20/66 or 30.3% excluding RW9). In Redwings, there were fewer significant population-pairwise F_{ST} values (relative to Blackbirds) and only a small number of significant population-pairwise R_{ST} values (8/28 or 28.6%, and 4/28 or 14.3% respectively) (Table 3.7).

There are two striking results from these comparisons. Firstly, in Blackbirds, the only population that has high (i.e. significant) population-pairwise values of *both* F_{ST} and R_{ST} is Skye, UK, although Titran, Norway also has several significant values of both F_{ST} and R_{ST}. Secondly, all pairwise comparisons between the Icelandic Redwings and other populations produced significant F_{ST} values, but this was not reflected in R_{ST} values. Indeed, there does not appear to be any significant relationship between F_{ST} and R_{ST} values (Figure 3.5).

Table 3.6 Summary of genetic variance attributable to between (versus within) breeding population differences for (a) Blackbirds (12 populations) and (b) Redwings (8 populations), analysed via locus-by-locus and global (i.e. total across loci) AMOVAs. Significant values (P<0.05) are depicted in bold red type.

(a) Blackbirds

Locus	df	F_{ST}	P	% var.
Cuμ28	11	**0.03749**	**<0.001**	3.75
Cuμ32	11	0.00360	0.332	0.36
BB3	11	-0.00367	0.652	-0.37
PAT43	11	**0.02400**	**<0.001**	2.40
Ase40	11	**0.01713**	**0.016**	1.71
RW4	11	0.00521	0.135	0.52
TGU07	11	-0.00370	0.654	-0.37
Global	291	**0.01244**	**<0.001**	1.24

(b) Redwings

Locus	df	F_{ST}	P	% var.
Ase40	7	0.01666	0.208	1.66
Mcyμ4	7	**0.09488**	**0.002**	9.49
BB3	7	**0.08749**	**<0.001**	8.75
PAT43	7	0.01440	0.132	1.44
RW4	7	**0.01739**	**0.045**	1.74
RW9	7	0.01161	0.190	1.16
TGU06	7	0.02232	0.062	2.23
TGU07	7	0.00771	0.274	0.77
Global	181	**0.02313**	**<0.001**	2.40

Table 3.7(a). Matrix of F_{ST} (bottom) and R_{ST} statistics (top) in twelve populations of breeding Blackbirds sampled from across Europe. Values departing significantly from zero are depicted in bold red type.

	Kal*, RUS	Nyborg, DNK	MS*, GBR	Hanko, FIN	Buch*, DEU	Radolf*, DEU	Ozzano, ITA	Norfolk, GBR	Schiff*, LUX	Eidf*, NOR	Titran, NOR	Skye, GBR
Kal*, RUS		-0.02004	**0.13289**	0.01139	-0.01460	0.03013	**0.11645**	**0.07397**	0.03440	0.03561	**0.15690**	**0.20762**
Nyborg, DNK	0.00567		0.05124	0.02470	-0.03396	0.01071	0.09277	0.00406	-0.00055	0.01195	**0.06748**	**0.25866**
North*, GBR	**0.02113**	**0.02104**		**0.11286**	0.05112	0.04801	0.00287	-0.01601	0.07713	0.05135	-0.02949	**0.35748**
Hanko, FIN	-0.00174	**0.03252**	**0.02199**		-0.01217	**0.03730**	0.12171	0.07408	-0.01335	-0.01901	**0.14134**	**0.19793**
Buch*, DEU	0.01469	**0.02702**	**0.02000**	-0.00116		-0.01394	0.04715	0.00164	-0.00223	-0.01120	**0.07352**	**0.21936**
Radolf*, DEU	0.01384	-0.0005	0.01568	0.00524	0.00358		0.01156	-0.00651	0.05703	0.02764	**0.06395**	**0.34706**
Ozzano, ITA	-0.02415	0.00459	0.00521	-0.00848	0.00181	-0.00139		-0.00060	0.11901	0.06351	0.00478	**0.47769**
Norfolk, UK	0.00700	0.00357	-0.00400	0.00783	0.00761	-0.00072	-0.01170		0.05081	0.03515	0.00241	**0.30801**
Schiff*, LUX	**0.03549**	**0.03067**	**0.02318**	0.01222	**0.02529**	0.00686	0.00705	0.01130		-0.01280	**0.09503**	0.08417
Eidf*, NOR	0.00791	**0.01358**	**0.02392**	-0.01056	**0.01586**	0.00087	-0.01155	0.01061	0.00005		**0.07558**	**0.21411**
Titran, NOR	**0.01560**	0.01558	**0.02305**	0.01234	**0.02003**	-0.00231	-0.01225	**0.01419**	**0.02215**	0.00998		**0.36073**
Skye, GBR	**0.04070**	**0.05274**	0.03261	**0.03877**	-0.00096	**0.03602**	**0.06457**	0.01754	**0.03102**	**0.04144**	0.03733	

***Abbreviations: Kal: Kaliningrad; MS: Mickley Square, Northumberland; Buch: Bucholz; Radolf: Radolfzell; Schiff: Schifflange; Eidf: Eidfjord**

Table 3.7(b). Matrix of F_{ST} (bottom) and R_{ST} statistics (top) in eight populations of breeding Redwings sampled from across Europe. Values departing significantly from zero are depicted in bold red type.

	Southern FIN	**Konnevesi, FIN**	**Rovaniemi, FIN**	**Selfoss, ISL**	**Eidfjord, NOR**	**TITRAN, NOR**	**TROMS, NOR**	**Handol, SWE**
Southern FIN		-0.02656	-0.06629	0.08151	0.03621	-0.01837	**0.07557**	0.01599
Konnevesi, FIN	-0.00170		-0.03915	-0.03972	-0.09431	0.02663	-0.03503	-0.08296
Rovaniemi, FIN	0.00264	0.01447		0.05468	0.02137	-0.02388	**0.08391**	0.01422
Selfoss, ISL	**0.06689**	**0.08175**	**0.08522**		-0.04833	0.11643	-0.00049	-0.02373
Eidfjord, NOR	-0.01257	0.00089	-0.0192	**0.06723**		0.08803	-0.00223	-0.01476
TITRAN, NOR	0.00176	0.02008	0.0139	**0.03191**	0.02481		**0.17832**	**0.10044**
TROMS, NOR	-0.01888	0.01161	-0.00018	**0.05293**	-0.02877	0.00091		-0.04321
Handol, SWE	0.01632	**0.03857**	-0.00661	**0.05707**	0.02698	0.00524	-0.02348	

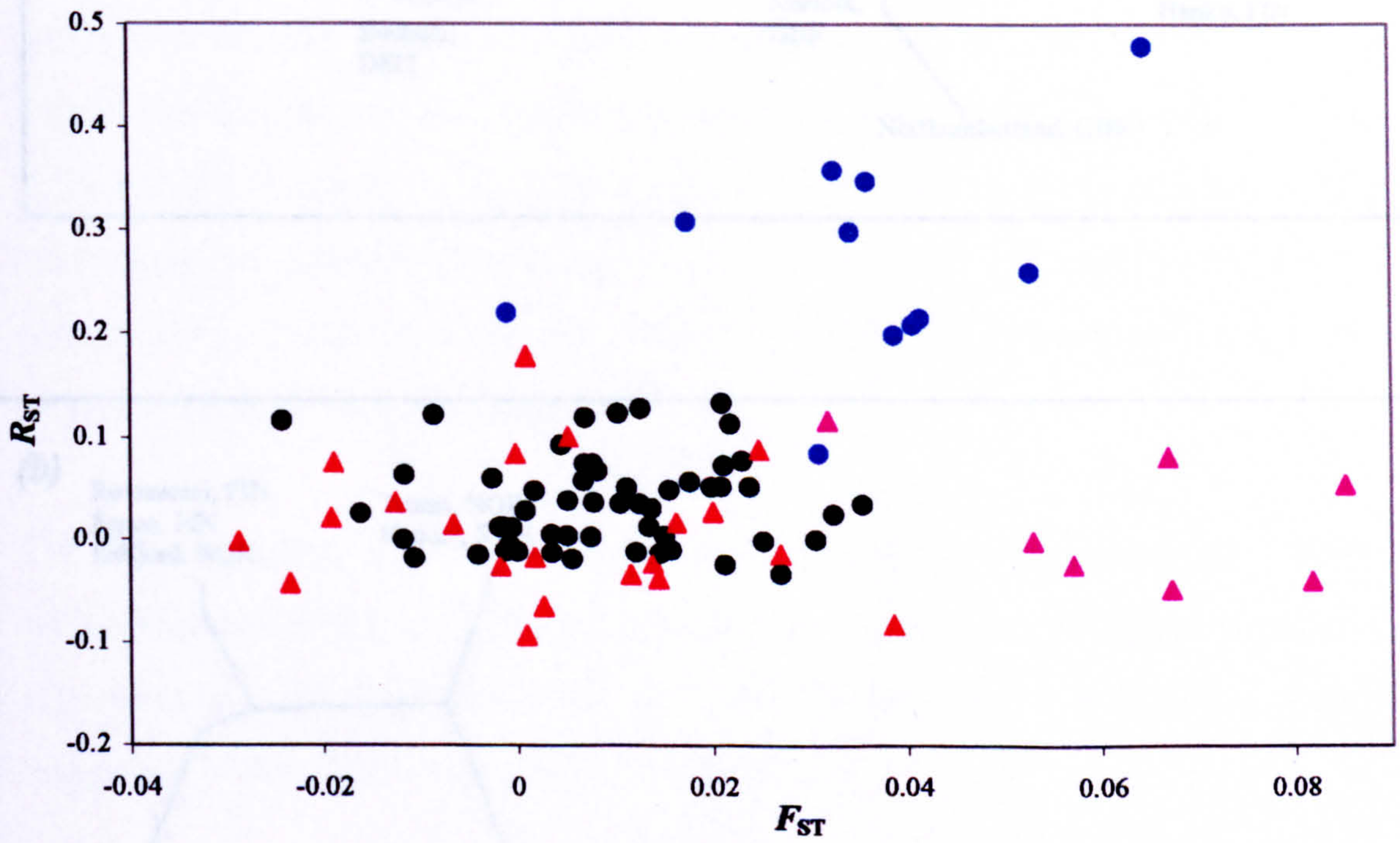


Figure 3.5 Comparison of population-pairwise F_{ST} and R_{ST} values for populations of breeding Redwings (represented by triangles) and Blackbirds (represented by circles), sampled from across Europe. In general, there does not appear to be any significant association between the two sets of values. The pink triangles (▲) represent comparisons between a population of the Icelandic Redwing subspecies coburni *with seven populations of the continental race iliacus. The resulting F_{ST} values were significant, but the R_{ST} values were not. The blue circles (●) represent comparisons between a population of Blackbirds sampled on Skye with eleven other breeding populations sampled across Europe. These were the only pairwise comparisons (for either species) to result in consistently high values of both F_{ST} and R_{ST}. Eight microsatellite loci were used for the Redwing population comparisons, and seven for the Blackbird population comparisons.*

Neighbour-joining trees

Neighbour-joining trees revealed a complex pattern of genetic relationships between Blackbird populations, and a very simple structure in Redwings (Figure 3.6). However, there were no distinct clusters of populations that could easily be explained in terms of geographic proximity.

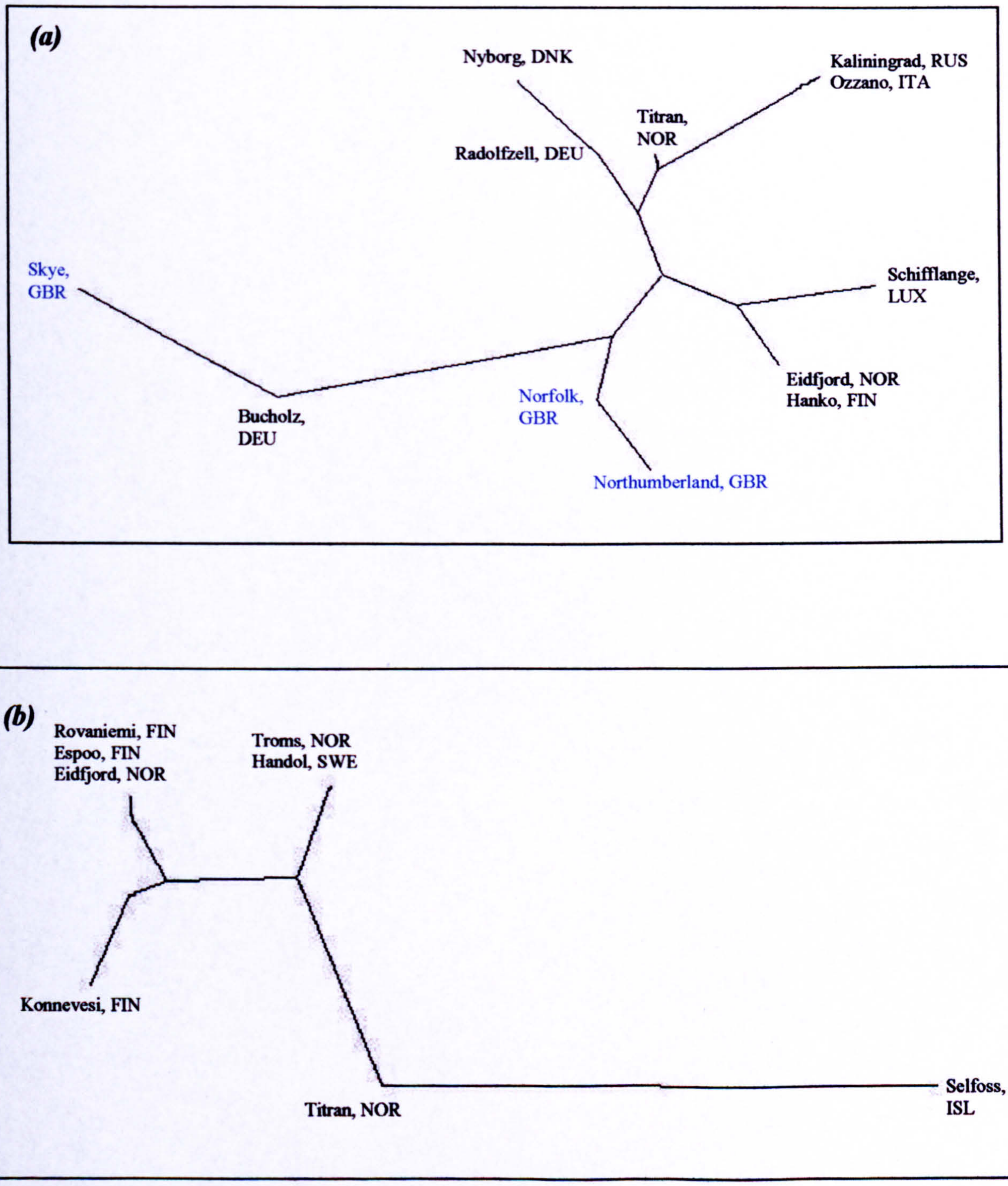


Figure 3.6 Radial (unrooted) neighbour-joining tree for breeding populations of (a) Blackbirds (12 populations), and (b) Redwings (8 populations), constructed from population-pairwise F_{ST} matrices. Names of Blackbird populations breeding in the UK are given in blue text.

Cluster analysis

For both species, the estimated logarithm of probability of the data [ln Pr (X / K)] was maximum for K = 1 and the posterior probability [P (K / X)] of having only one population was 1. This suggests that, in both species, the breeding population is completely panmictic, and lacks any clear genetic structure.

Assignment tests

Both methods of breeding population assignment (-log values of likelihoods, and probabilities) produced similar results. For Blackbirds assigned to two groups, the –log likelihood method correctly assigned 70.3% of individuals (102/145), while the likelihood probability method performed slightly better, correctly assigning 75.2% of individuals (109/145) (Table 3.8).

For Blackbirds assigned to three groups, the –log likelihood method correctly assigned 49.0% of individuals (71/145); the likelihood probability method again performed slightly better, correctly assigning 54.5% of individuals (79/145). Using the former method, correct assignment was fairly consistent between different breeding groups. In contrast, the latter method correctly assigned a high percentage of Continent-breeding birds (77.9%), but percentage of correct assignments of birds from the other two groups (UK and Fennoscandia) was little or no better than random (Table 3.9).

For Redwings, the –log likelihood calculations method correctly assigned 87.9% of individuals (80/91 individuals), whereas the method including the simulation algorithm (to calculate likelihood probabilities) was more accurate, correctly assigning 94.5% (86/91 individuals) (Table 3.10).

Table 3.8 Results of assignment tests for two breeding populations of Blackbirds: UK, and non-UK, using (a) –log likelihood calculations following the method of Baudouin & Lebrun (2001); (b) a simulation algorithm following the method of Cornuet et al. (1999) to calculate likelihood probabilities. Correct assignments are displayed in bold red type.

(a) assignments based on –log likelihood calculations

source	**assigned population**	
population	UK	non-UK
UK (n = 35)	**19 (54.3%)**	16 (45.7%)
non-UK (n = 110)	27 (24.5%)	**83 (75.5%)**

(b) assignments based on likelihood probabilities

source	**assigned population**	
population	UK	non-UK
UK (n = 35)	**12 (34.3%)**	23 (65.7%)
non-UK (n = 110)	13 (11.8%)	**97 (88.2%)**

Table 3.9 Results of assignment tests for three breeding populations of Blackbirds: UK, Fennoscandia and Continental, using (a) –log likelihood calculations following the method of Baudouin & Lebrun (2001); (b) a simulation algorithm following the method of Paetkau et al. (2004) to calculate likelihood probabilities. Correct assignments are displayed in bold red type.

(a) assignments based on –log likelihood calculations

source	**assigned population**		
population	UK	Continent	Fennoscandia
UK (*n* = 35)	**17 (48.6%)**	12 (34.3%)	6 (17.1%)
Continent (*n* = 68)	17 (25.0%)	**33 (48.5%)**	18 (26.5%)
Fennoscandia (*n* = 42)	5 (11.9%)	16 (38.1)	**21 (50.0%)**

(b) assignments based on likelihood probabilities

source	**assigned population**		
population	UK	Continent	Fennoscandia
UK (*n* = 35)	**13 (37.1%)**	19 (54.3%)	3 (8.6%)
Continent (*n* = 68)	9 (13.2%)	**53 (77.9%)**	6 (8.8%)
Fennoscandia (*n* = 42)	4 (9.5%)	25 (59.5)	**13 (31.0%)**

Table 3.10 Results of assignment tests for two races of Redwings: **iliacus** *and* **coburni**, *using (a) – log likelihood calculations (b) a simulation algorithm to calculate likelihood probabilities; both methods followed that of Rannala & Mountain (1997).*

(a) assignments based on –log likelihood calculations

identified race	**assigned race**	
	iliacus	*coburni*
iliacus (n = 72)	**67 (93.1%)**	5 (6.9%)
coburni (n = 19)	6 (31.6%)	**13 (68.4%)**

(b) assignments based on likelihood probabilities

identified race	**assigned race**	
	iliacus	*coburni*
iliacus (n = 72)	**72 (100%)**	0
coburni (n = 19)	5 (26.3%)	**14 (73.7%)**

Winter Birds

Microsatellite properties

For non-breeding Blackbirds, a total of 92 alleles were detected at the eight microsatellite loci. The number of alleles per locus ranged from four to 21. For non-breeding Redwings, a total of 104 alleles were detected at the eight microsatellite loci across the eight breeding populations. The number of alleles per locus ranged from five to 29. For Blackbirds, these numbers are similar to those found in breeding populations, but for Redwings, the number of alleles per locus was generally higher than those found in breeding populations. Summary statistics for microsatellite loci, including the number of individuals (n), the number of alleles (A) and proportions of observed (H_O) and expected (H_E) Hardy-Weinberg heterozygosities per locus are provided in Table 3.11.

In non-breeding Blackbirds, loci Cuμ28 and TGU07 displayed significant deviations from Hardy-Weinberg expectations, indicating heterozygote deficiencies at these loci. However, this may be due to the pooling of individuals from many different breeding populations, rather than the presence of null alleles at these loci. The results from breeding birds support this. Conversely, although locus RW9 appears to be in Hardy-Weinberg equilibrium for non-breeding Blackbirds (in contrast to the results for breeding Blackbirds), a substantial number of wintering individuals could not be scored for one or both alleles at this locus (51/170, or 30%). Therefore, as with breeding birds, the AMOVA between sites was carried out excluding locus RW9.

In non-breeding Redwings, exact tests for Hardy-Weinberg equilibrium revealed a significant deficit of heterozygotes at locus BB3. This is surprising, because this locus was found to be in equilibrium for breeding populations, and, with the exception of one individual, all wintering birds were scoreable for both alleles at this locus. It is possible that this deviation from Hardy-Weinberg equilibrium is due to differences between the two Redwing races, since the winter Redwings analysed in this part of the study are not thought to include any Icelandic birds. It is also possible that genotyping errors occurred. Whatever the explanation, in order to ensure that the inclusion of this locus did not bias the results, the AMOVA between different sampling periods was carried out excluding locus BB3.

Global AMOVAs did not detect any significant differentiation between sample sites for either species (F_{ST} = 0.00210; P = 0.100 for Blackbirds; F_{ST} = 0.00121; P = 0.397 for Redwings). However, the locus-by-locus AMOVAs revealed a significant differentiation between sample sites of Blackbirds at locus RW4 (F_{ST} = 0.03133; P = 0.005).

Table 3.11 Summary statistics for (a) Blackbird and (b) Redwing microsatellite loci including: the number of individuals (n), the number of alleles (A) and proportions of observed (H_O) and expected (H_E) Hardy-Weinberg heterozygosities per population and per locus. Significant departures from Hardy-Weinberg expectations ($P<0.05$) are depicted in bold red type, with superscript d indicating heterozygote deficit.

(a)	Cuμ28	Cuμ32	BB3	PAT43	Ase40	RW4	RW9	TGU07
n	157	161	169	148	158	163	119	160
A	15	6	4	20	21	8	9	9
H_E	0.90	0.75	0.37	0.93	0.90	0.61	0.84	0.75
H_O	**0.88 [d]**	0.73	0.37	0.88	0.90	0.58	0.78	**0.73 [d]**

(b)	Ase40	Mcyμ4	BB3	PAT43	RW4	RW9	TGU06	TGU07
n	139	131	138	139	137	138	135	138
A	19	5	6	20	29	6	10	9
H_E	0.78	0.37	0.75	0.87	0.94	0.42	0.76	0.81
H_O	0.76	0.37	**0.58 [d]**	0.87	0.94	0.44	0.72	0.79

Assignment tests

For non-breeding Redwings, the –log likelihood method assigned 82.5% of individuals (113/137 individuals) to the nominate race *iliacus*; the remaining birds (24 individuals) were assigned to the Icelandic race *coburni*. In contrast, the likelihood probability method assigned 96.4% of individuals (132/137 individuals) to the nominate race *iliacus*. Of the remaining birds, three were assigned to the Icelandic race *coburni*, and two could not be assigned to either race (probability of belonging to each race was 0).

Discussion

Breeding Birds

Blackbirds

In general, low but significant levels of genetic differentiation were found in breeding populations of Blackbirds, as indicated by the global AMOVA and population-pairwise F_{ST} values. Significant population-pairwise R_{ST} values were far fewer, and in general, there appeared to be little association between F_{ST} and R_{ST}, with the possible exception of birds breeding on the Isle of Skye (Figure 3.5). For this population, most pairwise comparisons with other breeding Blackbird populations differed significantly in both F_{ST} and R_{ST} values (Table 3.7(a)). Indeed, the neighbour-joining tree constructed from population-pairwise F_{ST} comparisons suggests that the Skye population is genetically distinct (although it appears to be most closely related to the two other British-breeding populations, Norfolk and Northumberland, along with Annaberg-Buchholz in West Germany (Figure 3.6(a)).

Blackbirds have been gradually extending their breeding range northwards and vestwards during the past century. For example, the species began colonising the utlying northern and western islands of Britain during the first half of the 20th :ntury (Venables & Venables, 1952), and within the last two decades, a eeding population of around 60 pairs have become established on the small, 'shore island of Heligoland, German Bight (Sacher et al., 2006). Although such onisations have occurred very recently (in the evolutionary timescale), the

founder effect and genetic drift can lead to relatively rapid genetic differentiation. The founder effect was defined by Mayr (1942) as the effect of establishing a new population by a small number of individuals, carrying only a small fraction of the parental population's total genetic variation. In addition, a founder population may be very small, and show random genetic drift, and an increase in inbreeding due to small population size. Random genetic drift is the statistical effect that results from the influence that chance has on the survival of alleles, causing an allele to become more common or more rare over successive generations. Ultimately, random genetic drift may lead to an allele becoming fixed or removed from the population's gene pool (e.g. Hedrick, 2005; Allendorf & Luikart, 2007).

A number of subspecies endemic to Scottish Islands have been described, including the Hebridean Song Thrush *Turdus philomelos hebridensis*, the Shetland Starling *Sturnus vulgaris zetlandicus*, and several subspecies of Wren: the Shetland Wren *Troglodytes troglodytes zetlandicus*, Fair Isle Wren *T. t. fridariensis*, Hebridean Wren *T. t. hebridensis*, and St Kilda Wren *T. t. hirtensis* (McGowan et al., 2003). According to McGowan et al. (2003), although these races were first described between 50 and 100 years ago, many have been poorly studied, and little is known about their status, distribution or movements. These races are identified as distinct due to their morphological characteristics; whilst it is likely that, in many cases, these morphological distinctions will be coupled with genetic differences between races (as appears to be the case with Redwings), to date, no genetic studies have been conducted. It is therefore intriguing (and quite feasible) that Blackbirds sampled on Skye appear to be genetically distinctive from those breeding on the mainland, and this possibility certainly warrants further investigation.

The only other Blackbird population that appeared to have substantial levels of differentiation based on R_{ST} as well as F_{ST} is Titran, Norway, which is located on the western tip of the offshore island of Frøya. Although the neighbour-joining tree indicates that the Titran population appears to be less genetically distinctive than that of Skye (Figure 3.6(a)), the Titran population does appear to be genetically distinct from the other two Fennoscandian Blackbird populations of

Eidfjord and Hanko. However, it seems unlikely, that the apparent genetic distinctiveness of Titran birds is a result of the same phenomena (the founder effect and random genetic drift) as Skye birds, because Titran is a regular stopover site for migrants, and each autumn, 100-200 Blackbirds (and a similar number of Redwings) are ringed there (Kjetil Aa. Solbakken *pers. comm.*), although information about recruitment into the local (i.e. Titran) breeding population is not available. It is possible that the genetic distinctiveness of the Titran population is instead due to historical events, e.g. migratory Blackbirds from different populations following separate migratory routes, allowing genetic differences to evolve.

Redwings

The most striking result for Redwings was the distinction, inferred by population-pairwise F_{ST} comparisons, between the Icelandic race *coburni* and the Continental race *iliacus*. The indication that these two races are more different genetically than are different populations of the same race is not unexpected. Surprisingly, however, this distinction was not reflected in population-pairwise R_{ST} comparisons. When the Icelandic population was excluded, no significant genetic variation was detected between populations of the Continental race. This is likely to be at least partly due to the very small sample sizes used in the study, but may also reflect the fact that Redwings are highly nomadic, and may effectively form a single panmictic population (at least in Fennoscandia). It would be interesting to obtain samples from breeding populations further east, to test whether genetic structuring exists between these two (broad) regions.

Comparison between species

Although a direct comparison of levels of genetic population structure between species is not possible (since a different suite of microsatellite loci were used, and those that were shared have different levels of polymorphism between the two species), when the Redwing analysis was confined to the nominate race (i.e. excluding the Icelandic population), Blackbird populations appeared to have greater levels of genetic structuring. This may be a reflection of the different migratory and dispersal strategies employed by the two species. Redwings are long-distance migrants and do not appear to exhibit strong natal fidelity (Snow,

1986b; Reid, 1993), whereas Blackbirds are partial migrants, and over much of its range, the species is largely sedentary (Chamberlain & Main, 2002). Migratory habit in birds is related to site fidelity, with migratory species tending to disperse further (Paradis et al., 1998). Both sedentariness and high site fidelity tend to result in low levels of gene flow, and thus increased differentiation between populations (Korfanta et al., 2005).

Other studies have found differences in levels of genetic structuring between migratory and sedentary sister taxa. For example, a study by Arguedas & Parker, (2000) of population structure in migratory and sedentary House Wrens (*Troglodytes aedon* and *T. musculus* respectively) found evidence to suggest that migratory behaviour enhances gene flow, with significantly higher levels of gene flow in migratory birds compared to sedentary birds. Similarly, in a subspecies of the Burrowing Owl *Athene cunicularis*, Korfanta et al. (2005) found that genetic differentiation was at least twice as great in resident Florida and California populations as in migratory western populations.

Comparison of F_{ST} *versus* R_{ST}

R_{ST} is thought to reflect more accurately the mutation pattern of microsatellites (Slatkin, 1995), and under ideal conditions, i.e. large population sizes and many loci, generally appears to perform better than F_{ST} (Gaggiotti et al., 1999; Balloux & Lugon-Moulin, 2002). This is expected, because under any mutation model with some 'memory' in allele size, the effect of mutation becomes more important than that of migration (defined here as movement of individuals between breeding groups, resulting in genetic exchange). However, when the number of loci or samples is relatively small, or when degrees of differentiation are expected to be low (as may often be the case when investigating intraspecific genetic variation), F_{ST} performs better than R_{ST} (Balloux et al., 2000; Balloux & Lugon-Moulin, 2002). Since all of these considerations (i.e. sample size, loci number, and low level of differentiation) apply to this study, it is not surprising that F_{ST} values appear to be more sensitive in detecting differentiation between populations than do R_{ST} values.

Population structure

For both species, the factorial correspondence analyses revealed that there were no distinct clusters of populations, even at a broadscale (Figure 3.4), which is consistent with the indication (provided by the global AMOVAs and population-pairwise F_{ST} comparisons) that levels of population genetic structuring are very weak. However, although attempts to characterise populations based on genetic structure alone appears to reveal little about source populations of birds, the results of the assignment tests suggest that this approach may have potential, if combined with other methods (such as stable isotope analyses).

Clustering analyses

Results of the cluster analyses suggested that breeding populations of both species were entirely panmictic. A similar result was obtained in a study of genetic structure of a Roe Deer (*Capreolus capreolus*) population (Coulon et al., 2006), yet using an alternative Bayesian model (GENELAND; Guillot et al., 2005), defined two genetic units, which showed weak but significant differentiation (as indicated by F_{ST} values and an AMOVA). This suggests that the program GENELAND may be more sensitive than STRUCTURE in detecting weak genetic structure. However, the number of loci used in this study was relatively small; perhaps the identification (or isolation) of additional loci would be beneficial for any future studies. Note that GENELAND was not used in the present study, because it was developed for landscape genetics (an amalgamation of population genetics and landscape ecology; Manel et al. 2003), with the aim of providing information on how landscape and environmental features influence gene flow, population structure and local adaptation (Guillot et al., 2005), an issue that is not under consideration in the present study.

Assignment tests

In contrast to the complete absence of genetic population structure indicated by the clustering analyses, the likelihood probability assignment test was able to separate the two Redwing races (which cannot always be done through observation, or even in the hand, Chris Benson, *pers. comm.*) with a high degree of accuracy. However, further sampling (particularly of additional Icelandic populations) would be useful, in order to test the applicability of these markers to

a larger population size. When Blackbirds were assigned to two groups (UK and non-UK), both assignment methods were poor at assigning UK-breeding birds to their breeding origin, but much better at assigning non-UK birds to their correct group. When Blackbirds were assigned to three groups (UK, Continent and Fennoscandia), the –log likelihood method correctly assigned approximately half of the birds to their correct group, and predictive performance was consistent across groups. In contrast, the likelihood probability method assigned a relatively high proportion of Continent-breeding Blackbirds to their correct group (~78%), but assignment of Blackbirds breeding in the UK and Fennoscandia was no better than random. Clearly genetic markers used in isolation (i.e. without additional types of data) are not useful for determining breeding origins of this species.

Wintering birds

No genetic variation between sample sites (Blackbirds) or periods (Redwings) was revealed for birds overwintering (or passing through) the UK and Ireland. This suggests that different sites (or the same site during different periods in the case of Redwings) are receiving migrants from a mixture of breeding origins. However, without systematic sampling of regions, it is difficult to determine whether geographical patterns (between wintering sites) occur at all. Given that migrant birds arrive first on the east coast, it seems likely that sites do differ in the origins of migrants that they receive in the winter, particularly Blackbirds (whereas Redwings appear to exhibit nomadic movements). It is also likely that sites will differ in the proportions of migrants versus resident Blackbirds during the winter. Unfortunately, elucidating these potential differences is hampered by the lack of strong genetic structure in either species.

Both assignment methods predicted that a small number of non-breeding Redwings were of the Icelandic race, which is intriguing. Although the total number of recoveries is rather small ($n \sim 140$), Icelandic-ringed Redwings have never been recovered anywhere in England or Wales (Milwright, 2002a). However, according to Witherby et al. (1938), the Icelandic race has been identified in southern parts of Britain. Note, however, that the likelihood probability method was more accurate than the –log likelihood method at assigning breeding Redwings to their correct race, and the former method

assigned only three birds to the Icelandic race (versus 24 assigned as *coburni* by the –log likelihood method). It is of course possible that the birds identified as Icelandic may have been misassigned; some populations of the nominate race may be genetically more similar to Icelandic birds than those sampled in the present study. Alternatively, genotyping errors may have led to erroneous classifications.

Limitations

Sample sizes

A number of studies have cited the need for large population sizes and/or numbers of loci, in order to produce meaningful results (e.g. Ruzzante, 1998; Rosenberg et al., 2001). However, this was simply not feasible for the present study. Indeed, the fact that promising results have been obtained from such small samples, using a non-invasive (and therefore highly practical) sampling method, is encouraging.

Low quality/quantity DNA

Non-invasive sampling techniques, as used in this study, are known to produce low quantity and/or quality DNA, which promotes genotyping errors. Allelic dropouts occur when only one of the two alleles present at a heterozygous locus is amplified. Equally problematic are false alleles: allele-like artefacts generated by PCR (Pompanon et al., 2005). A number of approaches can be adopted in order to limit genotyping errors. The most conservative method would be to repeat each DNA amplification, independently for each locus, several times (the multiple-tubes approach; Taberlet et al., 1996). However, this is obviously more time-consuming and costly than a single-tube approach (Taberlet et al., 1999). In the present study, repeat amplifications were performed whenever there was any doubt in the allelic size-scoring (e.g. when amplification was weak, or when peaks were not clearly separated), and those individuals that could not be genotyped at more than two (of the original set of eight) loci were not included in the genetic analyses.

Linkage disequilibrium

Significant results were obtained for nine of the twelve Blackbird populations, and five of the eight Redwing populations Although this indicates that loci may not be segregating independently, the numbers of significant results were relatively small, and spread widely across populations, with no consistent patterns. In comparison, Soha et al. (2004) reported seven exceptions to 66 tests of linkage disequilibrium (10.6%) in their genetic analysis of White-crowned Sparrows (*Zonotrichia leucophrys pugetensis*) populations, which they felt was insufficient to indicate that loci were not segregating independently. According to Pompanon et al. (2005), genotyping errors could markedly affect linkage estimations by masking the true segregation of alleles. Thus, it seems unlikely that loci in this study were linked.

Conclusion

In general, Blackbird populations appear to be more genetically structured than Redwings, which may be related to differences in migratory patterns, such as tendency to migrate and breeding site fidelity. Icelandic Redwings (*T. i. coburni*) appear to be strongly differentiated from the Continental race (*T. i. iliacus*), whereas little or no differences were detected between populations of the same race (*iliacus*). For both species, F_{ST} performed better than R_{ST} in detecting genetic structure. The cluster analysis indicated absence of genetic structure in both species, although genetic assignment tests performed well when used to separate the two races of Redwings. Performance of assignment tests in assigning Blackbirds was weak, although there were several limitations to the present study (in terms of sample size, and quality/quantity of DNA), suggesting that the use of additional microsatellite loci might lead to improvements.

CHAPTER 4: IDENTIFYING BREEDING ORIGINS OF TWO *TURDUS* SPECIES OVERWINTERING IN THE UK AND IRELAND USING STABLE ISOTOPE RATIOS

Introduction

Use of stable isotopes to elucidate patterns of migration

The intrinsic isotopic profile or 'signature' of an individual's tissues is controlled mainly by local dietary inputs (including water), which in turn are affected by a number of complex, inter-related environmental factors, such as geological and soil composition, climate and altitude (Bowen et al., 2005). A useful feature of stable isotope ratios for ecological studies is that different types of tissue integrate local diet over different temporal scales. For example, whilst blood and muscle have rapid turnover times of days to weeks, bone collagen averages the individual's lifetime of dietary inputs (Hobson & Wassenaar, 1997). Thus, not only can stable isotope ratios provide information on very recent dietary inputs, since different tissues provide dietary information that is integrated over different time periods, it is possible to reconstruct dietary *histories* of individuals. This has facilitated numerous studies aiming to trace the origin(s) of migratory animals, because the sample site can be linked with the site in which a particular tissue was formed prior to migration (Kelly & Finch, 1998).

Stable isotope ratios are being used to link breeding and non-breeding grounds of migratory birds in a growing number of studies (e.g. Chamberlain et al., 1997; Hobson & Wassenaar, 1997, 2001; Cherel et al., 2000; Hobson et al., 2001; Meehan et al., 2003; Atkinson et al., 2005), most of which utilise feathers. Feathers are an appropriate tissue to use in this context because, once fully-grown, vascular connections to them atrophy and the feathers become inert. Consequently, the isotopic ratios of feathers provide a fixed record of the ratios in the environment where the feathers were grown. Since the primary moult for most temperate passerines occurs after breeding and usually precedes autumn migration (Kelly & Finch, 1998), feathers subsequently collected at wintering sites (i.e. grown on the breeding grounds) provide a means of locating the

breeding origins of these birds. Many of these stable isotope studies support the results of ringing data (e.g. Mehl et al., 2004; Royle & Rubenstein, 2004; Atkinson et al., 2005; Bearhop et al., 2005; Gunnarsson et al., 2005; Mazerolle et al., 2005; Yohannes et al., 2005; Bensch et al., 2006; Neto et al., 2006), thus demonstrating the value of augmenting conventional tracking methods with newer techniques.

Useful isotopic patterns in the environment

Table 4.1 provides a brief summary of the various stable isotope patterns in the terrestrial environment and the biological/biogeochemical processes influencing these patterns. Environmental isotope abundance can also be influenced by anthropogenic factors. For example, the use of fertilisers may affect isotopic ratios of nitrogen. In addition, marine environments are more enriched in $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$ and $\delta^{34}S$ (i.e. have higher values) than terrestrial environments, and thus may be useful for birds that move seasonally between marine and freshwater sites (Rubenstein & Hobson, 2004; Hobson, 2005a). Several isotopes have been utilised in studies attempting to elucidate the origins of migratory birds, including $\delta^{13}C$, $\delta^{15}N$ (the analysis of which is coupled with that of $\delta^{13}C$), $\delta^{18}O$ (e.g. Hobson et al., 2004a), $\delta^{34}S$ (e.g. Hebert & Wassenaar, 2005a) and $\delta^{87}Sr$ (e.g. Chamberlain, 1997); so far $\delta^{13}C$, and in particular $\delta^{2}H$, have generally proved to be the most informative (Appendix 1 provides additional examples of studies).

Table 4.1 Summary of biological/biogeochemical processes influencing stable isotope abundance and natural isotope patterns in the terrestrial environment. After Rubenstein & Hobson (2004).

Element **Stable isotope ratios** **Notation**	**Biological/biogeochemical processes influencing isotope abundance**	**Natural pattern in terrestrial environment**
Carbon ^{13}C: ^{12}C $\delta^{13}C$	Varies between C_3, C_4 and CAM plant species, due to photosynthetic processes Minor stepwise trophic enrichment of $\delta^{13}C$.	Decreases with increasing latitude Increases with increasing altitude Mesic habitats comparatively more depleted (i.e. values are lower) than xeric habitats in C_3-based systems Marine habitats comparatively more depleted than terrestrial habitats
Nitrogen ^{15}N: ^{14}N $\delta^{15}N$	Varies in plants according to how they fix N Systematic enrichment with trophic level	Xeric habitats comparatively more depleted than mesic habitats Tissues of terrestrial carnivorous vertebrates enriched relative to terrestrial herbivorous vertebrates
Hydrogen (deuterium) ^{2}H: ^{1}H $\delta^{2}H$	Varies in meteoric waters with precipitation patterns, temperature, elevation and relative humidity	Decreases with increasing latitude Decreases with increasing altitude Highest in summer and lowest in winter above 30° latitude Marine habitats comparatively more enriched than terrestrial habitats Decreases moving inland Older soils comparatively more enriched than younger soils
Oxygen ^{18}O: ^{16}O $\delta^{18}O$	As for hydrogen	As for hydrogen
Sulphur ^{34}S: ^{32}S $\delta^{34}S$	Varies with distribution of light and heavy sulphides in bedrock, atmospheric deposition and quality of plant growing conditions.	Estuarine and marsh habitats comparatively more enriched than marine habitats Marine habitats comparatively more enriched than terrestrial habitats
Strontium ^{87}Sr: ^{86}Sr $\delta^{87}Sr$	Varies in plants with type of geological substrate and atmospheric deposition	Older soils comparatively more enriched than younger soils Calcium-rich soils comparatively more enriched than calcium-poor soils
Lead $^{208, 207}Pb$: ^{206}Pb $\delta^{206, 207, 208}Pb$	Varies in plants with type of geological substrate	- Older soils comparatively more enriched than younger soils

Carbon

The majority of the global vegetation coverage consists of C_3 plants (e.g. Ehleringer, 1991). However, higher temperatures generally favour the growth of C_4 plants in arid or semiarid regions (Still et al., 2003), an example of which is the Gramineae (such as maize or sugar-cane). CAM plants, such as Cactaceae, occur mainly in arid regions. However, within many species ranges, there is no useful spatial resolution of the distribution of C_3 versus C_4 biomes, and therefore this information is quite limited for inferring migratory origins. In addition to having lower $\delta^{13}C$ than both C_4 and CAM plants, C_3 plants show variation in $\delta^{13}C$ based on mechanisms associated with water-use efficiency. The overall result is that C_3 plants generally become more enriched in $\delta^{13}C$ (resulting in higher $\delta^{13}C$ values) in more xeric habitats than in cooler or more mesic environments. An additional phenomenon is that marine biomes are more enriched in $\delta^{13}C$ than terrestrial or freshwater biomes. These patterns are more useful for inferring habitat use, rather than for tracking migrants *per se* (Hobson, 2005a). However, combining ratios of different stable isotopes, such as $\delta^{13}C$ and $\delta^{2}H$, will give information at multiple scales, and may therefore be a more useful approach than using a single isotope in isolation.

Nitrogen

Agricultural practices can alter $\delta^{15}N$ values in both upland and wetland systems (Hobson, 2005a). According to Hobson (2005a), nitrogen isotopes in the soil may be variable within and between sites, but two processes may result in agricultural soils having higher $\delta^{15}N$ values than temperate forest soils. These are (i) the presence of animal-based fertilisers, and (ii) the greater evaporation of isotopically lighter nitrogenous compounds such as ammonia from agricultural soils (as a result of tillage, and the relatively low acidity of agricultural soils) (Nadelhoffer & Fry, 1994; Hobson, 2005a). Some studies have found useful differences in feather $\delta^{15}N$ values between birds originating from different habitat types (e.g. Hobson, 1999b; Hebert & Wassenaar, 2001). However, Alexander et al. (1996) found high variability in $\delta^{15}N$ values of tissues of several shorebird species feeding in an agricultural matrix, and it remains unclear how scale-dependent these habitat differences in $\delta^{15}N$ values are (Hobson, 2005a).

Hydrogen

In North America, δ^2H in precipitation (δ^2H_p) has a continental-scale gradient, from enriched (higher) values in the southeast to relatively depleted (lower) values in the northwest (Hobson & Wassenaar, 1997; Hobson, 2005a) (Figure 4.1(a)). This phenomenon has evidently been well exploited, since much of the existing research into the use of stable isotopes for determining origins of migratory birds has been undertaken in the Americas, focusing on Neotropical (North - South America) migrants breeding in North America. In contrast, there appears to be a relative paucity of studies considering migratory movements of species spending part of their annual cycle in Europe, particularly those that breed there. European-based studies are emerging (e.g. Chamberlain et al., 2000; Evans et al., 2003; Neto et al., 2006; Newton et al., 2006), and recently, Hobson et al. (2004b) created δ^2H_p maps of Europe (Figure 4.1(b)). It was found that δ^2H in feathers (δ^2H_f) was correlated with collection site δ^2H_p (both mean annual and growing-season values, $r^2 = 0.66$ or 0.65 respectively), and that there was a general decrease in δ^2H_p along a southwest to northeast gradient. However, some of these recent studies suggest that there may be less potential for determining the origins of individuals within the European continent than within North America (Hobson et al., 2004a; Bowen et al. 2005).

Although distinct and predictable geographical variation in δ^2H_p occurs in the Americas, and most of Europe (though probably to a lesser extent), this is not the case in many different latitudes in Africa. Due to an east-west moisture gradient across sub-Saharan Africa, $\delta^{13}C_f$ and $\delta^{15}N_f$ have proved to be more informative than δ^2H_f for determining the origins of Palearctic (Eurasia - Africa) migrants (Pain et al., 2004; Rubenstein & Hobson, 2004). However, there appears to be a general consensus that further investigations are required, to determine just how useful patterns of stable isotope ratios are for determining origins of Palearctic migrants.

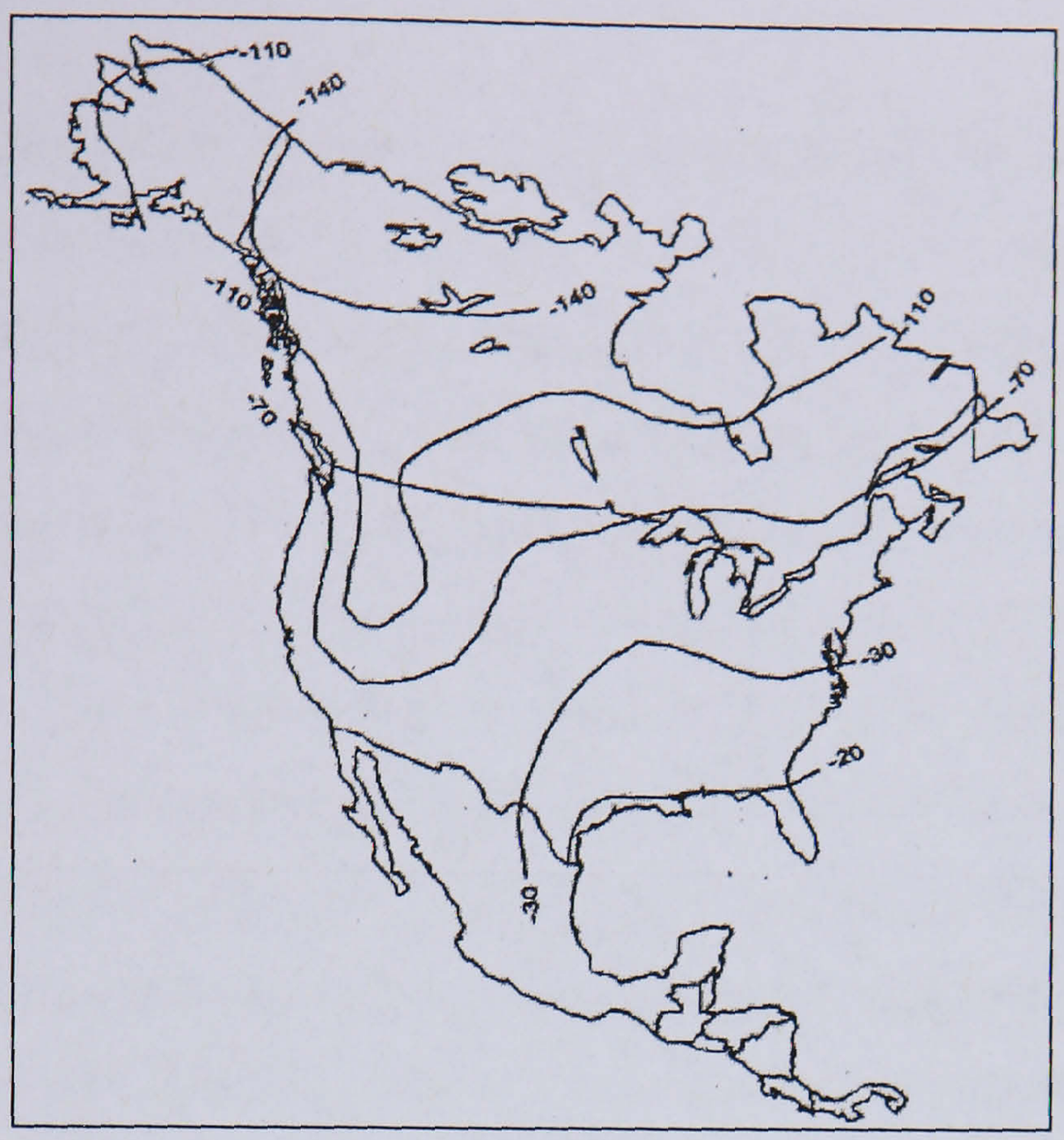


Figure 4.1(a) Contours of average growing season δ^2H in precipitation for North America (altitude-corrected and precipitation amount-weighted), based on 1969-1994 data from the global network of isotopes in precipitation (GNIP) database. This database is administered by the International Atomic Energy Association (IAEA) and World Meteorological Organization (WMO). From Hobson & Wassenaar (1997).

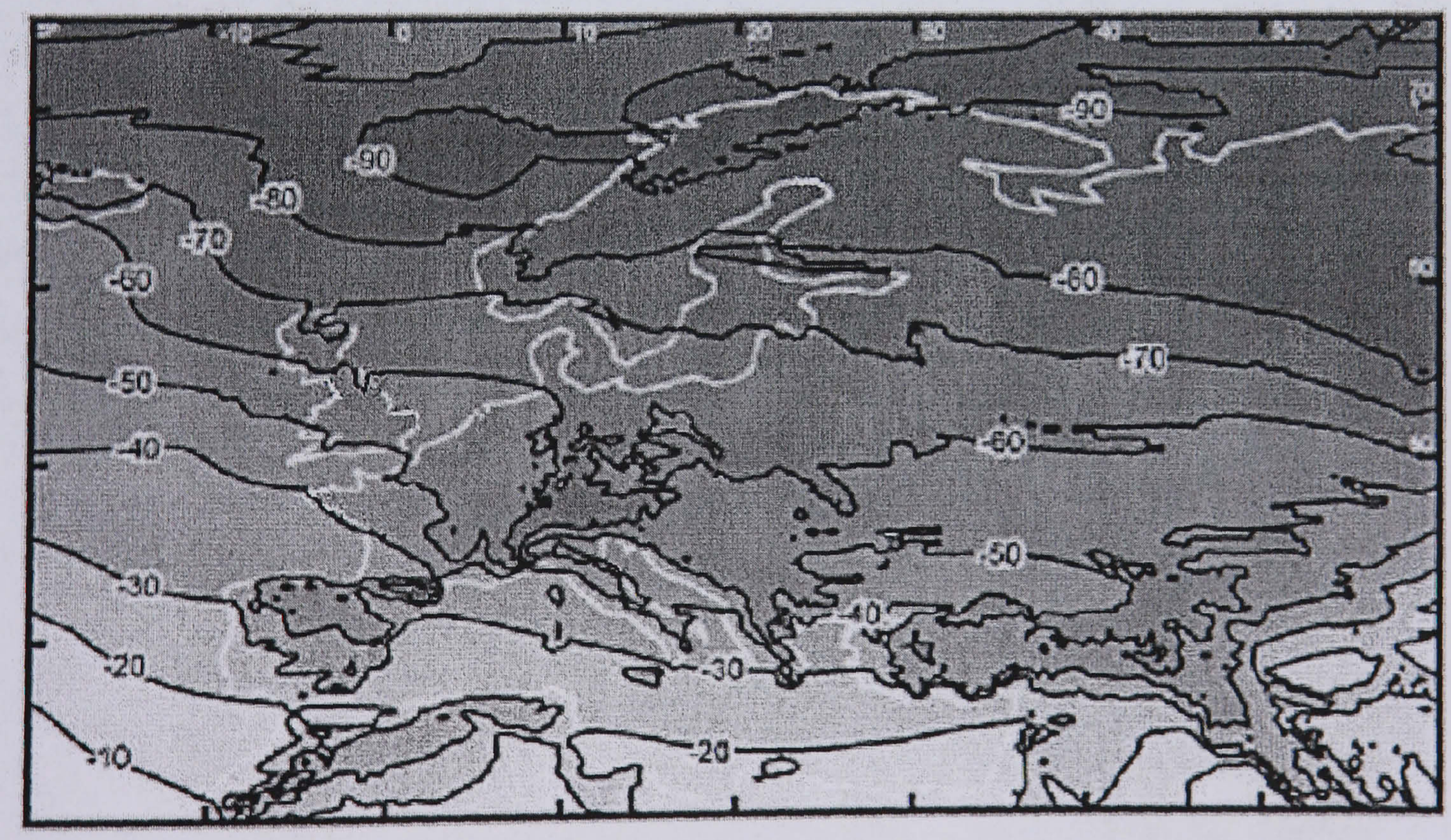


Figure 4.1(b) Contour map of weighted, altitude-corrected δ^2H patterns in precipitation in Europe, based on 1960-2000 data from the GNIP database. From Hobson et al. (2004a).

Caveats

In addition to potential difficulties in interpreting geographical patterns of stable isotope ratios, it is important to be aware of assumptions relating to the life history of migratory species, as these may influence isotopic data (Rubenstein & Hobson, 2004). For example, although it is often assumed that all individuals in a sampling area have similar tissue isotope ratios, this is not necessarily the case. Juvenile birds, for instance, may consume a different diet or feed at a different trophic level to adults (Rubenstein & Hobson, 2004). In addition, nutritional status may have a confounding influence on stable isotope values of tissues. For example, Cherel et al. (2005) demonstrated that a period of fasting (25 days) in the King Penguin *Aptenodytes patagonicus* resulted in a depletion of $\delta^{15}N$ values in blood and feathers. Similarly, water stress can have important effects on the nitrogen isotopic ratio of tissues (Kelly, 2000).

There may also be temporal variation in stable isotope studies. This is illustrated by a study of the effects of altitude and year on the isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of male Black-throated Blue Warblers (*Dendroica caerulescens*), breeding along a steep altitudinal gradient in the Appalachian Mountains (Graves et al., 2002). The study found age-specific and altitudinal effects in $\delta^{13}C$ values, and age-specific and temporal effects in $\delta^{15}N$ values. The cause of the temporal fluctuation was unknown, but the authors suggested drought-stress as one possible explanation, because a number of studies have documented an inverse relationship between $\delta^{15}N$ values in vertebrate tissues and rainfall (e.g. Ambrose, 1986; Heaton, 1986; Cormie, 1994).

Geographical and seasonal climatic variation, e.g. in bedrock, altitude or rainfall, can also affect isotope studies, as can anthropogenic factors (Rubenstein & Hobson, 2004). Most notably, land use practices can alter $\delta^{15}N$ values in both upland and wetland systems. For example, this can result in agricultural soils becoming more $\delta^{15}N$-enriched than temperate forest soils (Hobson, 2005a). Testing for differences related to biometric data and social grouping can help to identify any dependent variables, and the use of multiple stable isotopes should help to mitigate anthropogenic and other effects.

Indeed, a number of recent studies suggest that the use of stable isotope ratios does not provide a universal panacea for identifying patterns of movements (e.g. Fox et al., 2007). In particular, concerns over the general applicability are δ^2H values are beginning to arise (Meehan et al., 2003; Smith & Dufty, 2005; Wunder, et al. 2005; Newton et al., 2006; Rocque et al., 2006; Langin et al., 2007). For example, Rocque et al. (2006) analysed $\delta^{13}C_f$, $\delta^{15}N_f$ and δ^2H_f values in two generations of feathers collected from three bird species: American and Pacific Golden Plovers *Pluvialis dominica* and *P. vulva*, and Northern Wheatears *Oenanthe oenanthe*, that breed sympatrically in Alaska, and overwinter on three different continents: South America, the Pacific islands and Asia, and Africa, respectively. Since these species grow feathers on their Alaskan breeding grounds, as well as replacing some feathers (on specific body areas) on their wintering grounds, it was expected that these two generations of feathers would differ markedly in their isotopic signatures. Rocque et al. (2006) predicted that feathers grown in Alaska would be depleted in δ^2H, with low variation (e.g. between –130 and –110‰), because birds were sampled in a small geographical area (of Alaska), with minimal topographic relief. In contrast, it was hypothesised that feathers grown on the wintering grounds would be comparatively enriched in δ^2H (between –60 and –10‰), and would be characterised by relatively high variability in δ^2H. Contrary to expectations it was found that Alaskan-grown Plover feathers displayed high variation, especially those of American Plovers, which ranged from –175 to –62‰; δ^2H values of winter-grown American Plovers feathers were not consistent with predicted values, and (in direct contrast to feathers grown in Alaska) variation among these feathers was much lower than expected.

Consequently, Rocque et al. (2006) were unable to confidently assign Plover feathers to origin of growth on the continental scale. Moreover, based on δ^2H_p maps of North America, they were only able to assign Alaskan-breeding Plovers to within several thousand kilometres of their actual breeding origins. Equally worrying, no differences in any of the three isotopes were detected between Northern Wheatear feathers grown on breeding grounds in Alaska, and those grown on African winter quarters. Rocque et al. (2006) conclude that the use of isotope ratios to trace migratory movements may not be applicable for all

migration systems or bird species, especially on relatively small spatial scales. It certainly appears that the pattern of geographic variation in δ^2H_f is more complex than initially envisaged.

Aims

The general aim of this part of the study was to determine the breeding origins of Blackbirds and Redwings overwintering in Britain & Ireland, using stable isotopes ($\delta^{13}C$, $\delta^{15}N$ and δ^2H) in contour feathers. More specifically, the following issues were addressed: do stable isotopes of feathers sampled at different breeding sites vary in predictable patterns across Europe? Is there sufficient variation between different sites to assign wintering individuals of both species back to their breeding origins? If this is not possible, can we distinguish between UK-resident Blackbirds (those that bred there), and migrants (those that bred elsewhere in Europe)? Similarly, is it possible to discriminate between the two Redwing races *iliacus* and *coburni*? How much intra-specific variation exists within breeding populations? Do age- or sex-specific differences in stable isotope ratios occur within these populations? Where both species were sampled at a particular breeding site, do isotope ratios differ significantly between the two species? Are there differences in stable isotope signatures of wintering birds (of either species) between different regions of the UK (i.e. what is the extent of migratory connectivity)? These questions led to the following hypotheses:

- δ^2H_p varies with both longitude and latitude, and is the most useful stable isotope for discriminating between breeding populations of both Blackbirds and Redwings.

- There is little variation in stable isotope ratio within breeding populations, and no difference between the two sexes, different age groups, or between the two different species.

- Distinctive breeding population 'signatures' (i.e. a combination of $\delta^{13}C_f$, $\delta^{15}N_f$ and δ^2H_f) will allow discrimination between wintering Blackbirds from

breeding populations in the UK, Fennoscandia, and continental Europe, and between wintering Redwings from Iceland, Fennoscandia and Russia.

- There are differences in stable isotope signatures of wintering Blackbirds between different regions of the UK and Ireland (relatively high migratory connectivity), but not between Redwings overwintering/stopping over in different regions of the UK (low migratory connectivity).

Methods

Sampling

Sampling of feathers is described in the preceding chapter. Where possible (i.e. where sufficient material existed), feathers selected for stable isotope analysis were those from which DNA was extracted for the genetic component of this project (Chapter 3).

Ageing and sexing

Birds were designated the following age codes, based on the EURING (European Union for Bird Ringing) system of age codes, in which odd numbers are applied to birds whose year of hatching is known accurately, whereas even numbers indicate a lack of precise knowledge. Age code 1 indicates a pullus (nestling); 3 indicates a bird that hatched during the current year (of sampling); 4 indicates a bird that hatched before the current year, but exact year unknown; 5 indicates a bird that definitely hatched during the previous year; 6 is an adult bird that hatched before the previous year (but exact hatching year unknown). The EURING system is based on calendar years, so that at 00:01 hours on 1st January, each bird moves into the next age category (e.g. age 3 becomes age 5) (Redfern & Clark, 2001). Due to a relatively low proportion of breeding birds with EURING age codes 4, 5 and 6, these birds were all pooled and recoded as 4.

Redwings are sexually monomorphic; where carcasses were available (all birds from Bardsey and the Farne Islands) birds were sexed via dissection. Where only feathers were available, birds were sexed via molecular methods (as described in

the previous chapter). However, this was not always possible (either due to insufficient quality or quantity of DNA), and a relatively high proportion of Redwings (~20%), remained unsexed. Although Blackbirds are sexually dimorphic, it is often difficult to sex juveniles before they have completed post-juvenile moult; such individuals were also sexed via molecular methods. Birds of unknown sex or age codes were included in General Linear Models (described below), but not in subsequent post hoc analyses of age and sex.

(i) Breeding Populations

Feathers were sampled from twelve breeding populations of Blackbirds, and nine breeding populations of Redwings (eight of the subspecies *iliacus*, and a single *coburni* population). Sampling locations, elevation, dates of collection (month and year) and sex/age composition of samples are listed in Table 4.2. Note that both Redwings and Blackbirds were sampled at three of the breeding sites: Hanko, Finland; Titran, Norway and Eidfjord, Norway. Although, for all three sites, both species were caught at the same location, different elevations are given for the two species at the last site. This is because Blackbirds at this site breed at low altitudes, while most Redwings are found at higher altitudes; often breeding above 1000 metres (Alf Tjore Mjøs, *pers. comm.*), and altitude is known to affect stable isotope ratios.

Where possible, feathers of nestlings (EURING code 1) and fledglings (EURING code 3) were selected, in order to be certain that the feathers were grown on the collection site. Juveniles of both species undergo a post-juvenile moult in late summer (usually August and September), replacing all their body plumage apart from the retrices (tail feathers) and remiges (primaries, secondaries and tertials). Adult birds (those in their second autumn and older) generally undergo a complete moult between July (Blackbirds) or August (Redwings), and early October (Snow, 1969; Simms, 1978). (See Chapter 5 for further discussion of moult period of British- and Irish-breeding Blackbirds.) Sampling contour feathers from birds hatched before the current (sampling) year (i.e. those aged as 4, 5 or 6) can thus be problematic, because it is difficult to ascertain whether feathers collected early in the breeding season were newly grown (i.e. following moult), and therefore representative of isotopic ratios at the sample site, or

whether they were old, pre-moult feathers grown during the previous breeding season (and possibly a different location). On the other hand, if birds aged as 4+ are sampled later in the year, they may have dispersed into the area from a different breeding location, and thus any feathers sampled may not have been grown at the sample site. According to Cramp & Simmons (1988b), autumn movements of Blackbirds begin in late September, with main passage occurring in October and early November. Redwings generally leave Fennoscandia in a broad front, between late September and mid-November, although departure from Siberia begins as early as the end of August.

Despite these caveats, where juvenile feather samples were in limited supply, feathers from adult birds sampled in August and September were used (as well as four adult birds from late July, which were either undergoing, or had completed their moult), with a cut-off date of 25th September. However, four Blackbirds sampled outside this period were included in the stable isotope analyses, and are described in the Discussion.

(ii) Non-breeding Populations

Outside the breeding season, Blackbirds were sampled at eleven sites; Redwings were sampled at three different sites, and on repeated occasions at one of these sites (Bardsey, North Wales, UK). Details of birds collected and sampling locations are provided in Table 4.3.

Note that not all samples produced useable ratios for all three isotopes, either due to the failure of a particular analysis, or because a value was classed as an outlier (the latter are considered in the Discussion). Thus, different numbers of samples are provided where necessary (with explanatory notes below the affected Tables).

Table 4.2(a) Sampling location, elevation (metres above sea level), collection date, and sex/size compositions of twelve breeding populations of Blackbirds used to investigate the relationship between feather $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values and breeding origin. Populations are listed in order of descending latitude.

Sample site	Latitude	Longitude	*n*	Elevation (masl)	Sex composition	Age composition (EURING age codes)	Collection Date
Titran, NOR	63.40N	08.19E	11	0	♀: 9 ♂: 2	code 3: 4 code 6: 3** unknown: 4	Sep 2005
Eidfjord, NOR	60.25N	07.08E	9-12*	25	♀: 5 ♂: 7	code 3: 10 code 6: 2**	Sep 2005
Hanko, FIN	59.49N	22.54E	10	8	♀: 6 ♂: 4	code 3: 7 code 4: 3	Aug-Sep 2005
Skye, GBR	57.15N	06.20W	5	10	♀: 4 ♂: 1	code 3: 2 code 4: 1 code 6: 2**	Aug 2005
Nyborg, DNK	55.20N	10.48E	10	10	♀: 4 ♂: 6	code 3: 5 code 6: 5**	Jun-Sep 2005
Kaliningrad, RUS	55.00N	20.34E	12	9	♀: 1 ♂: 10 unknown: 1	code 3: 10 code 4: 1 unknown: 1	Jul-Aug 2005
Mickley Square, Northumberland, GBR	54.56N	01.54W	9	149	♀: 4 ♂: 5	code 3: 6 code 4: 2 code 6: 1**	Jul-Aug 2005
Norfolk, GBR	52.24N	00.43E	10	27	♀: 5 ♂: 4 unknown: 1	code 3: 10	May-Jul 2005
Bucholz, DEU	50.56N	13.00E	4-5*†	673	♀: 2 ♂: 3	code 3: 5	Jun-Jul 2005
Schifflange, LUX	49.30N	06.01E	10-12*	331	♀: 7 ♂: 4 unknown: 1	code 3: 11 code 5: 1**	Jul-Oct† 2005
Radolfzell, Germany	47.45N	9.00E	3-4*	393	♀: 3 ♂: 1	code 3: 4	May-Jun 2005
Ozzano, ITA	44.42N	10.08E	2-3‡	249	♀: 2 ♂: 1	code 3: 2 code 4: 1	Jul-Sep 2005

Notes: *lower number refers to samples analysed for $\delta^{15}N$ only; †lower number refers to samples analysed for $\delta^{13}C$ only; ‡lower number refers to samples analysed for $\delta^{2}H$ only
**recoded as age group 4 for analysis purposes

Table 4.2(b). Race, sampling location, elevation (metres above sea level), collection date, and sex/size compositions of nine breeding populations of Redwings used to investigate the relationship between feather $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values and breeding origin. Populations are listed in order of descending latitude.

Race	Sample site	Latitude	Longitude	Elevation (masl)	*n*	Sex composition	Age composition (EURING age codes)	Collection Date
iliacus	Troms, NOR	68.45N	19.40E	0	14	♀: 4 ♂: 8 unknown: 2	code 3: 10 code 4: 4	Sep 2005
iliacus	Rovaniemi, FIN	66.31N	25.45E	91	4	♀: 3 ♂: 1	code 1: 4	June 2005
coburni	Selfoss, ISL	64.07 N	20.29W	1	19-22**	♀: 9 ♂: 8 unknown: 5	code 3: 16 code 4: 6	Sep 2004
iliacus	Titran, NOR	63.40N	08.19E	0	7-8*‡	♀: 3 ♂: 5	code 3: 4 code 4: 3 unknown: 1	Aug-Sep 2005
iliacus	Handol, SWE	63.16N	12.27E	538	12-13*†	♀: 1 ♂: 4 unknown: 9	code 3: 12 code 4: 2	Jul-Aug 2005
iliacus	Konnevesi, FIN	62.37N	26.26E	98	6	♀: 4 unknown: 2	code 1: 5 code 3: 1	Jul 2005
iliacus	Hanko, FIN	59.49N	22.54E	8	3	♀: 2 ♂: 1	code 3: 2 code 4: 1	Aug 2005
iliacus	Eidfjord, NOR	60.25N	07.08E	1000	9-10*	♀: 1 ♂: 9	code 3: 6 code 4: 1 code 6: 2*** unknown: 1	Sep 2005
iliacus	Espoo, FIN	60.11N	24.48E	27	2	♂: 2	code 1: 2	Aug 2005

Notes: *lower number refers to $\delta^{15}N$; †lower number refers to samples analysed for $\delta^{13}C$; ‡lower number refers to $\delta^{2}H$;
**For Selfoss, 19 samples were analysed for $\delta^{2}H$; 21 for $\delta^{15}N$ and 22 for $\delta^{13}C$.
***recoded as age group 4 for analysis purposes

Table 4.3(a) Sampling location, collection date, and sex/size compositions of Blackbirds used to investigate the relationship between feather $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values between UK and Irish overwintering sites and breeding origin. Sample sites are listed in order of descending latitude.

Sample site	**Latitude**	**Longitude**	***n***	**Sex composition**	***Age composition*** *(EURING age codes)*	**Collection date**
Tain, Ross & Cromarty, UK	57.48N	04.03W	14	♀: 1 ♂: 13	code 3: 10 code 4: 4	Dec 2004
Monifieth, Angus, UK	56.29N	13.00W	12-14‡	♀: 6 ♂: 8	code 4: 1 code 5: 5 code 6: 8	Dec 2004- Feb 2005
Low Newton, Northumberland, UK	55.30N	01.37W	12	♀: 7 ♂: 5	code 3: 8 code 4: 4	Oct 2004
Preston, Lancashire, UK	55.20N	02.41W	15	♀: 7 ♂: 8	code 4: 3 code 5: 12	Jan 2005
Mickley Square, Northumberland, UK	54.57N	01.54W	12	♀: 7 ♂: 5	code 3: 4 code 4: 3 code 5: 3 code 6: 2	Oct 2004- Jan 2005
Tuam, Galway, Ireland	53.30N	08.51W	15	♀: 7 ♂: 8	code 3: 4 code 4: 5 code 5: 2 code 6: 4	Dec 2004- Jan 2005
Rampton, Nottinghamshire, UK	53.17N	00.47W	14	♀: 7 ♂: 7	code 3: 1 code 4: 1 code 5: 4 code 6: 8	Dec 2004- Feb 2005
Thetford, Norfolk, UK	52.24N	00.43E	15	♀: 6 ♂: 9	code 3: 4 code 4: 6 code 5: 2 code 6: 3	Nov 2004- Mar 2005
Colyford, Devon, UK	50.43N	03.03W	5	♀: 2 ♂: 3	code 3: 1 code 4: 2 code 6: 2	Dec 2004- Jan 2005
Arreton, Isle of Wight, UK	50.40N	01.14W	13	♀: 3 ♂: 10	code 5: 9 code 6: 4	Jan-Feb 2005
Cornwall*, UK	50.21N	04.56W	10-12†	♀: 7 ♂: 5	code 3: 1 code 4: 8 code 5: 1 code 6: 2	Dec 2004- Jan 2005

*Birds from Cornwall were sampled from three sites, all within close proximity; longitude and latitude provided in the table are means of the three sites. ‡Lower number refers to $\delta^{2}H$; †lower number refers to samples analysed for $\delta^{13}C$

Table 4.3(b) Sampling location, collection date, and sex/size compositions of Redwings used to investigate the relationship between feather $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values and breeding origin. Sample sites are listed in order of descending latitude.

Sample site	Latitude	Longitude	*n*	Sex composition	Age composition (EURING age codes)	Collection date
Farne Islands, Northumberland, UK	55.37N	01.36W	13	♀: 4 ♂: 7 unknown: 2	code 3: 11 code 4: 2	Oct 2004
Tuam, Galway, Ireland	53.30N	08.51W	24	♀: 2 ♂: 2 unknown: 20	code 3: 3 code 4: 3 code 5: 6 code 6: 12	Dec 2004-Mar 2005
Bardsey, North Wales, UK	52.45N	04.46W	80-82*	♀: 46 ♂: 35 unknown: 1	code 3: 53 code 4: 28 unknown: 1	Oct 1995
Bardsey, North Wales, UK	52.45N	04.46W	7	♀: 6 ♂: 1	code 3: 4 code 4: 3	Oct 1998
Bardsey, North Wales, UK	52.45N	04.46W	27	♀: 22 ♂: 5	code 3: 10 code 4: 17	Oct 1999
Bardsey, North Wales, UK	52.45N	04.46W	26	♀: 20 ♂: 6	code 3: 7 code 4: 19	Mar 1998
Woking, Surrey, UK	51.17N	00.31W	15	♀: 5 ♂: 5 unknown: 5	code 3: 1 code 4: 1 code 5: 7 code 6: 6	Dec 2004-Mar 2005

Notes: *80 samples were analysed for $\delta^{2}H$; 82 for $\delta^{15}N$ and $\delta^{13}C$

Stable isotope analysis

Contour feathers were washed in 0.25M NaOH followed by 2-3 washes in deionised water, and dried overnight at 50 °C. The dried feathers were cut into sections (excluding the basal calamus) to allow accurate weighing of 0.6-0.8 mg into individual tin capsules for simultaneous carbon and nitrogen isotope analysis and silver capsules for hydrogen isotope analysis. All isotope ratios were measured via continuous-flow stable isotope mass spectrometry (CF-IRMS), using a Costech ECS 4010 elemental analyser interfaced with a ThermoElectron Delta XP Plus isotope ratio mass spectrometer. Analyses were carried out at the East Kilbride node of the NERC Life Sciences Mass Spectrometry Facility (Scottish Universities Environmental Research Centre).

Stable isotope ratios are expressed as the normalised ratio of the sample to a primary international standard, in parts per thousand (per mil, ‰):

$$\delta X = [(R_{sample} / R_{standard}) - 1] * 1000$$

where R_{sample} and $R_{standard}$ are the ratios of heavy to light isotopes for the sample and the standard respectively. $R_{standard}$ for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ is Pee Dee Belemnite (V-PDB), atmospheric nitrogen (AIR), and Vienna Standard Mean Ocean Water (VSMOW) respectively (Gannes et al., 1998; Darling & Talbot, 2003).

For carbon and nitrogen isotope measurements, repeat analyses of internal gelatin and two alanine standards of different isotope ratio routinely incorporated into each carbon/nitrogen sample run, showed that $\delta^{15}N$ and $\delta^{13}C$ can be measured with an accuracy and precision of ± 0.2‰ and 0.1‰ respectively. These internal standards are prepared every six weeks and checked against the secondary international standards USGS 24 graphite, IAEA CH6-sucrose, IAEA-CH7-polythene, and IAEA-N1, N2 and USGS-25 (all ammonium sulphate). Repeat analyses of keratin standards incorporated into each hydrogen sample run showed that $\delta^{2}H$ can be measured with an accuracy and precision of ± 2 ‰.

Analyses of $\delta^{2}H$ are affected by uncontrolled isotopic exchange between labile hydrogen in feathers and ambient water vapour in the laboratory (Wassenaar & Hobson, 2000a). However, Wassenaar & Hobson (2003) demonstrated that routine use of calibrated keratin working standards and a "comparative equilibration" technique can be used to correct for this effect. As recommended by Wassenaar & Hobson (2003), the keratin standards CFS (chicken feather) and BWB-II (Bowhead whale baleen) and comparative equilibration were used to correct samples for exchangeable hydrogen.

Statistical analysis

All data in this and subsequent chapters were analysed using SPSS (version 14.0) statistical software. All Analyses of Variance (ANOVAs) were performed using the default Type III sum-of-squares method.

(i) Breeding Populations

Stable isotope ratios of each individual breeding population were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Outliers, identified via quantile-quantile (q-q) plots, were excluded from subsequent

analyses (possible reasons for these outlying values are considered in the Discussion). For each species, Levene's test was used to check for homogeneity of variances between population isotope ratios.

Despite some heterogeneity in isotope ratios between breeding populations, a General Linear Model (GLM) Univariate ANOVA was used for each species to investigate whether mean stable isotope ratios of feathers ($\delta^{15}N_f$, $\delta^{13}C_f$ and $\delta^{2}H_f$) differed between birds of different sexes, age groups, and breeding populations, because this type of analysis is relatively robust to such natural variability (Glass et al., 1972). As a precaution, for those isotope ratios that exhibited unequal variances between populations, a one-way ANOVA was used to determine Welch's statistic, as this is preferable to the F statistic when the assumption of equal variances does not hold (Welch, 1937). Since some individuals had data missing for individual isotopes, a separate GLM was used for each isotope (i.e. to minimise exclusion of individuals with missing values). The T3 post hoc test was used for pairwise comparisons (Tamhane, 1979); modified by Dunnett (1980). This test was selected because it is robust to both heterogeneous variance, and unequal sample sizes (Day & Quinn, 1989).

Mean $\delta^{2}H_p$ values of the sample sites for the months April to September (defined as the 'Growing Season') were obtained from the Online Isotopes in Precipitation Calculator (OIPC, version 2.1; available at http://www.waterisotopes.org/), which uses data derived primarily from the Global Network for Isotopes in Precipitation (GNIP), administered by the International Atomic Energy Association (IAEA) and World Meteorological Organization (WMO). Values from this restricted period were selected (rather than using values for the entire year) to more accurately reflect $\delta^{2}H_p$ values during the period when feathers were grown (since there may be seasonal fluctuations $\delta^{2}H_p$, e.g. Smith & Dufty, 2005). Estimates of $\delta^{2}H_p$ are calculated according to an algorithm developed by Bowen & Wilkinson (2002) and refined by Bowen & Revenaugh (2003) and Bowen et al. (2005). Note that the calculations take elevation into account. Regression analyses were carried out to determine whether there was (i) an association between $\delta^{2}H_p$ and latitude; (ii) between mean $\delta^{2}H_f$ and $\delta^{2}H_p$ of each site; (iii) an association between $\delta^{2}H_p$ and

latitude. Where both species were sampled at the same site and did not differ in mean δ^2H_f (as was the case for both Hanko and Titran), data were pooled. For Eidfjord, Norway, there was a significant inter-specific difference in mean δ^2H_f, and also in δ^2H_p, because although sampled at the same site, the two species breed at different altitudes, which influenced δ^2H_p values; species-specific mean δ^2H_f and site $\delta 2H_p$ values were thus included for this site.

For each species, Pearson's correlations were used to determine (i) whether there was an association between either population mean $\delta^{13}C_f$ or $\delta^{15}N_f$ values, with latitude; (ii) between population mean δ^2H_f, $\delta^{13}C_f$, or $\delta^{15}N_f$ values, with longitude. In addition, for each isotope, one-way ANOVA linear contrasts were used to investigate relationships between mean isotope ratio of breeding populations of each species and latitude or longitude.

(ii) Non-breeding Populations

Two Blackbird samples with unusually enriched $\delta^{13}C$ values (more than seven times the standard deviation of the mean of the wintering Blackbird dataset) were excluded from analyses. Possible reasons for these outlying values are considered in the Discussion. Since it is possible that different parts of the UK and Ireland may receive migrants from different breeding sites, $\delta^{15}N_f$, $\delta^{13}C_f$ and δ^2H_f values of the different winter sites were compared via GLM Multivariate ANOVA. This is particularly important for Blackbirds because populations in different regions may contain different proportions of residents versus migrants (as well as migrants from different origins). For Blackbirds, sex and age groups were also included as factors in the GLM, since data for all of these variables was complete for most individuals. However, since information about sex could not be obtained for a number of Redwings, in this species differences in isotope ratios between the sexes were tested independently. Posthoc testing (Dunnett's T3 test) was used to determine where statistical differences between individual populations occurred. Levene's test was used to check for homogeneity of variances in isotope ratios between sample sites.

(iii) Species Comparisons

For each of the three breeding sites where both *Turdus merula* and *T. iliacus* were sampled (Hanko, Finland; Eidfjord, Norway; Titran, Norway), a Multivariate GLM was used to determine whether mean stable isotope ratios differed between the two species.

(iv) Discriminant analysis

To determine the utility of stable isotopes for distinguishing between UK and non-UK breeding populations of Blackbirds, quadratic discriminant function analysis was used. Breeding Blackbirds were classified into (a) two groups: UK and non-UK; (b) three groups: UK, Fennoscandia and Continent. Different combinations of isotopes were tested, and the most effective combination (in terms of % of accurate assignments) used to assign wintering birds to their most likely source population, with the winter data calibrated against breeding data. Discriminant analyses were not used for Redwings, because stable isotope ratios were not normally distributed (a strict assumption of any discriminant function analysis, e.g. Tan et al., 2006).

Results

All isotope values are reported in parts per thousand (‰).

Breeding Populations

Homogeneity of variances

For breeding Blackbird populations, Levene's test was significant for all three isotopes: $\delta^{15}N_f$ (Levene statistic = 6.228; $P < 0.001$), $\delta^{13}C_f$ (Levene statistic = 2.311; $P = 0.015$) and $\delta^{2}H_f$ (Levene statistic = 4.566; $P < 0.001$). For Redwings, Levene's test was significant for both $\delta^{15}N_f$ (Levene statistic = 4.472; $P < 0.001$) and $\delta^{2}H_f$ (Levene statistic = 5.826; $P < 0.001$). With the exception of Blackbird $\delta^{13}C_f$, these values remained significant even if populations with small sample sizes ($n < 6$) were excluded. This indicates that variance in stable isotope ratios was not equal between breeding sites, and therefore Welch's statistic is reported (in addition to F statistics).

Blackbirds

For Blackbirds, both the *F* statistics and Welch statistics revealed that there were significant differences in mean $\delta^{15}N_f$, $\delta^{13}C_f$ and $\delta^{2}H_f$ values between breeding populations. There was also a significant difference in mean $\delta^{2}H_f$ values between age groups, and significant interactions between the factors age and sex, as well as between age sex and population. There was a significant difference in $\delta^{13}C_f$ values between age groups, but no significant interactions between factors. There was no significant difference in any of the three isotopes between the two sexes. These results are summarised in Table 4.4(a); pairwise population comparisons (Dunnett's T3 post hoc test) of mean $\delta^{2}H_f$ between the four possible combinations of age and sex categories (adult male, adult female, hatch-year male, and hatch-year female) are provided in Table 4.4(b). Mean isotope ratios for each breeding population are provided in Table 4.5(a), and pairwise population comparisons (Dunnett's T3 post hoc test) in Table 4.5(b).

Table 4.4(a) Results of General Linear Model (GLM) Univariate Analyses used to test whether mean stable isotope ratios of $\delta^{15}N_f$, $\delta^{13}C_f$ and $\delta^{2}H_f$ differed between twelve breeding populations of Blackbirds. Factors used in the model were: sex, age group (either hatched in the sample year, or hatched prior to the sample year) and population. Significant results are depicted in bold red type. Since the variance of each isotope was not equal between populations, the Welch statistic is also provided. This was significant for all three isotopes ($P < 0.001$).

Factor	Dependent Variable	*n*	Welch Statistic	df	*F*	*P*
population	$\delta^{15}N_f$	97	**30.049**	11	**3.741**	**0.001**
	$\delta^{13}C_f$	101	**8.017**	11	**4.559**	**<0.001**
	$\delta^{2}H_f$	99	**27.990**	11	**4.114**	**<0.001**
sex	$\delta^{15}N_f$			2	0.302	0.741
	$\delta^{13}C_f$			2	0.383	0.684
	$\delta^{2}H_f$			2	1.577	0.215
age	$\delta^{15}N_f$			2	0.583	0.562
	$\delta^{13}C_f$			2	**6.821**	**0.002**
	$\delta^{2}H_f$			2	**5.677**	**0.006**
population * sex	$\delta^{15}N_f$			12	0.434	0.943
	$\delta^{13}C_f$			12	1.257	0.269
	$\delta^{2}H_f$			12	0.917	0.536
population * age	$\delta^{15}N_f$			8	0.469	0.873
	$\delta^{13}C_f$			8	1.001	0.446
	$\delta^{2}H_f$			6	1.815	0.112
sex * age	$\delta^{15}N_f$			2	0.185	0.831
	$\delta^{13}C_f$			2	0.802	0.453
	$\delta^{2}H_f$			2	**3.581**	**0.034**
population * sex * age	$\delta^{15}N_f$			3	0.363	0.780
	$\delta^{13}C_f$			3	0.022	0.995
	$\delta^{2}H_f$			3	**2.788**	**0.049**

Table 4.4(b) Pairwise comparisons (Dunnett's T3 post hoc test) of mean δ^2H_f values of Blackbird feathers between birds of different age and sex categories: birds coded as 3 are those that hatched during the year of sampling (i.e. fledglings and nestlings); those coded as 4 hatched before the year of sampling. Significant values are depicted in bold red type.

	Comparisons			
Age code and sex	4 male (n = 8)	4 female (n = 12)	3 male (n = 36)	3 female (n = 36)
4 male	-	0.828	**<0.001**	0.051
4 female			**0.025**	0.732
3 male				0.092

Table 4.5(a) Mean $\delta^{15}N$, $\delta^{13}C$, and δ^2H values of Blackbird feathers collected from twelve different European breeding populations, listed in order of ascending $\delta^{13}C$ values.

	$\delta^{15}N_f$‰			$\delta^{13}C_f$‰			δ^2H_f‰		
Population	***n***	**mean**	**SD**	***n***	**mean**	**SD**	***n***	**mean**	**SD**
Northumberland, GBR	9	8.1	0.7	9	-24.8	0.4	9	-85.2	7.9
Norfolk, GBR	10	5.5	1.2	10	-24.6	0.6	10	-80.9	7.5
Skye, GBR	5	7.8	1.2	5	-24.6	0.3	5	-79.1	12.1
Kaliningrad, RUS	12	7.1	2.8	11	-24.4	0.9	10	-94.8	3.8
Hanko, FIN	10	6.0	1.5	10	-24.1	0.3	10	-86.3	6.4
Schifflange, LUX	10	7.5	0.8	12	-23.9	0.8	12	-74.0	12.2
Annaberg-Bucholz, DEU	5	7.9	1.4	4	-23.9	0.0	4	-95.7	2.2
Nyborg, DNK	10	7.4	1.4	10	-23.9	0.5	10	-79.3	13.8
Radolfzell, DEU	3	5.9	1.1	4	-23.7	0.4	4	-92.5	3.3
Eidfjord, NOR	9	4.8	0.1	12	-23.5	0.5	12	-72.1	6.8
Titran, NOR	11	9.3	2.4	11	-23.4	0.8	11	-70.6	7.8
Ozzano, ITA	3	5.8	0.6	3	-23.2	0.7	2	-77.1	1.0

Table 4.5(b) Pairwise comparisons (Dunnett's T3 post hoc test) of population mean $\delta^{15}N$, $\delta^{13}C$, and $\delta^{2}H$ values of Blackbird feathers collected from twelve different European breeding populations. For clarity, only significant values are displayed. Populations are listed in order of descending latitude.

		Population comparisons (*P*)										
Population	**Isotope**	Eidf*, NOR	Hanko, FIN	Skye, UK	Nyb*, DNK	Kal*, RUS	MS*, UK	Thet*, UK	Buch*, DEU	Schiff*, LUX	Radolf*, DEU	Ozz*, ITA
Titran, NOR	$\delta^{15}N_f$	**0.004**	-	-	-	-	-	**0.015**	-	-	-	**0.048**
	$\delta^{13}C_f$	-	-	0.035	-	-	0.008	0.045	-	-	-	-
	$\delta^{2}H_f$	-	0.004	-	-	<0.001	0.036	-	<0.001	-	<0.001	-
Eidfjord, NOR			-	-	0.008	-	<0.001	-	-	<0.001	-	-
			-	0.004	-	-	<0.001	0.015	-	-	-	-
			0.004	-	-	<0.001	-	-	<0.001	-	<0.001	-
Hanko, FIN				-	-	-	-	-	-	-	-	-
				-	-	-	0.028	-	-	-	-	-
				-	-	-	-	-	-	-	-	-
Skye, UK					-	-	-	-	-	-	-	-
					-	-	-	-	-	-	-	-
					-	-	-	-	-	-	-	-
Nyborg, DNK						-	-	-	-	-	-	-
						-	0.019	-	-	-	-	-
						-	-	-	-	-	-	-
Kal*, RUS							-	-	-	-	-	-
							-	-	-	-	-	-
							-	0.007	-	0.004	-	<0.001
MS*, UK								0.002	-	-	-	-
								-	0.003	-	-	-
								-	-	-	-	-
Thetford, UK									-	0.031	-	-
									-	-	-	-
									0.005	-	-	-
Bucholz, DEU										-	-	-
										-	-	-
										0.003	-	0.003
Schiff*, LUX											-	-
											-	-
											0.015	-
Radolf*, DEU												-
												-
												0.019

*Abbreviations: Eidf: Eidfjord; Nyb: Nyborg; Kal: Kaliningrad; MS: Mickley Square; Thet: Thetford; Buch: Bucholz; Schiff: Schifflange; Radolf: Radolfzell; Ozz: Ozzano

Redwings

For Redwings, F statistics revealed significant differences in all three isotopes ($\delta^{15}N_f$, $\delta^{13}C_f$ and $\delta^{2}H_f$) between breeding populations. The Welch statistic was also significant for $\delta^{15}N$ and $\delta^{2}H$ ($P < 0.001$). There was also a significant difference in $\delta^{15}N_f$ between age groups, with mean $\delta^{15}N_f$ values being highest in adult birds, and a significant interaction between the factors age and sex. There was a significant difference in $\delta^{2}H_f$ between age groups, but no significant interactions between factors. There were no significant differences in any of the three isotopes between the two sexes. These results are summarised in Table 4.6(a); pairwise population comparisons (Dunnett's T3 post hoc test) of mean $\delta^{2}H_f$ between age categories are provided in Table 4.6(b). Mean isotope ratios for each breeding population are provided in Table 4.7(a), and pairwise population comparisons (Dunnett's T3 post hoc test) in Table 4.7(b).

Table 4.6(a) Results of General Linear Model (GLM) Univariate Analyses used to test whether mean stable isotope ratios of $\delta^{15}N_f$, $\delta^{13}C_f$ and $\delta^{2}H_f$ differed between nine breeding populations of **Redwings**. *Factors used in the model were: sex, age group, and population. Significant results are depicted in bold red type. Since the variance of $\delta^{15}N$, and $\delta^{2}H$ was not equal between populations, the Welch statistic is also provided. This was significant for both isotopes (P < 0.001).*

Factor	Dependent Variable	*n*	Welch Statistic	df	*F*	*P*
population	$\delta^{15}N_f$	79	**13.843**	8	**5.005**	**<0.001**
	$\delta^{13}C_f$	81	-	8	**3.397**	**0.004**
	$\delta^{2}H_f$	77	**72.866**	8	**17.847**	**<0.001**
sex	$\delta^{15}N_f$			2	0.708	0.498
	$\delta^{13}C_f$			2	1.656	0.201
	$\delta^{2}H_f$			2	0.587	0.560
age	$\delta^{15}N_f$			3	**3.126**	**0.034**
	$\delta^{13}C_f$			3	0.249	0.861
	$\delta^{2}H_f$			3	**3.305**	**0.028**
population * sex	$\delta^{15}N_f$			6	0.508	0.800
	$\delta^{13}C_f$			7	0.486	0.840
	$\delta^{2}H_f$			7	0.742	0.638
population * age	$\delta^{15}N_f$			5	**4.797**	**0.001**
	$\delta^{13}C_f$			6	1.005	0.443
	$\delta^{2}H_f$			5	0.525	0.756
sex * age	$\delta^{15}N_f$			2	1.030	0.365
	$\delta^{13}C_f$			2	0.165	0.848
	$\delta^{2}H_f$			2	0.217	0.806
population * sex * age	$\delta^{15}N_f$			1	0.045	0.832
	$\delta^{13}C_f$			1	0.510	0.478
	$\delta^{2}H_f$*			-	-	-

*Note: for $\delta^{2}H_f$ possible interaction between **population * sex * age could not be tested due to insufficient samples.**

Table 4.6(b) Pairwise comparisons (Dunnett's T3 post hoc test) of mean $\delta^{2}H$ values of Redwing feathers between birds of different age categories (samples were collected from nine different European breeding populations). Age code 1 indicates a pullus (nestling); birds coded as 3 are those that hatched during the year of sampling (i.e. fledglings and nestlings); those coded as 4 hatched before the year of sampling. Significant values are depicted in red type.

Age group comparisons (*P*)

Age code	1 (*n* = 11)	3 (*n* = 48)	4 (*n* = 17)
1	-	**0.010**	**0.001**
3			0.272

Table 4.7(a) Mean $\delta^{15}N$, $\delta^{13}C$, and $\delta^{2}H$ values of Redwing feathers collected from nine different European breeding populations, listed in order of ascending $\delta^{2}H$.

Population	$\delta^{15}N_f$‰			$\delta^{13}C_f$‰			$\delta^{2}H_f$‰		
	n	mean	SD	*n*	mean	SD	*n*	mean	SD
Rovaniemi, FIN	4	5.5	0.8	4	-25.0	0.5	4	-108.5	1.8
Konnevesi, FIN	6	4.6	1.9	6	-23.6	0.6	6	-99.4	4.7
Handol, SWE	12	3.8	0.5	13	-23.6	0.3	12	-97.2	3.3
Troms, NOR	14	5.4	1.6	14	-23.9	0.5	14	-92.1	4.9
Eidfjord, NOR	9	4.7	0.6	10	-23.2	0.7	10	-89.0	9.5
Selfoss, ISL	21	4.9	1.3	22	-24.0	0.6	19	-86.8	4.3
Espoo, FIN	2	6.1	0.2	2	-23.1	0.4	2	-85.5	2.8
Hanko, FIN	3	4.1	0.3	3	-23.9	0.4	3	-83.9	2.5
Titran, NOR	8	8.7	3.4	7	-23.7	0.4	7	-66.3	3.7

Table 4.7(b) Pairwise comparisons (Dunnett's T3 post hoc test) of population mean $\delta^{15}N$, $\delta^{13}C$, and $\delta^{2}H$ values of Redwing feathers collected from nine different European breeding populations. For clarity, only significant values (depicted in bold type) are displayed. Populations are listed in order of descending latitude.

		Population comparisons (*P*)							
Population	**Isotope**	Rovaniemi, FIN	Selfoss, ISL	Titran, NOR	Handol, SWE	Konnevesi, FIN	Eidfjord, NOR	Espoo, FIN	Hanko, FIN
Troms, NOR	$\delta^{15}N_f$	-	-	-	**0.050**	-	-	-	-
	$\delta^{13}C_f$	-	-	-	-	-	-	-	-
	$\delta^{2}H_f$	**<0.001**	-	**<0.001**	-	-	-	-	-
Rovaniemi, FIN			-	-	-	-	-	-	-
			-	-	-	-	**0.032**	-	-
			<0.001	**<0.001**	**<0.001**	-	**0.002**	-	**0.003**
Selfoss, ISL				-	**0.036**	-	-	-	-
				-	-	-	-	-	-
				<0.001	**<0.001**	**0.010**	-	-	-
Titran, NOR					-	-	**0.041**	-	-
					-	-	-	-	-
					<0.001	**<0.001**	**<0.001**	-	**0.003**
Handol, SWE						-	-	**0.025**	-
						-	-	-	-
						-	-	-	**0.018**
Konnevesi, FIN							-	-	-
							-	-	-
							-	-	**0.008**
Eidfjord, NOR								-	-
								-	-
								-	-
Espoo, FIN									**0.047**
									-
									-

Geographical trends

There was a strong correlation between collection site δ^2H_p, estimated for the months April to September (the period defined as the 'Growing Season' in the GNIP database), and latitude ($r_{1, 17} = 0.991$; $P < 0.001$) (Figure Fig 4.2(a)). One of the Norwegian sites (Titran) appeared to have outlying mean values of δ^2H_f for both Redwings and Blackbirds, and a second Norwegian site (Eidfjord) had an outlying mean δ^2H_f value for Blackbirds. Excluding these, δ^2H_f was correlated with both collection site δ^2H_p ($r_{1, 16} = 0.640$; $P = 0.006$), and latitude ($r_{1, 16} = 0.525$; $P = 0.031$) (Figure 4.2(b)). However, when each species was considered separately, there was no significant relationship between mean site δ^2H_f values and either δ^2H_p or latitude for either species.

There were no significant correlations between either $\delta^{13}C_f$ or $\delta^{15}N_f$ and latitude in either species, nor were there any significant associations between longitude and any of the three isotope ratios. However, for Blackbirds, there was a significant linear $\delta^{13}C$ trend for longitude ($t_{100} = -3.454$; df = 29.518; $P = 0.002$) but not latitude, and for Redwings, there was a significant linear $\delta^{13}C_f$ trend for latitude (t_{81} -4.536; df = 9.807; $P = 0.001$), but not longitude. There were no significant linear trends in δ^2H_f (excluding Titran birds and Eidfjord Blackbirds) or $\delta^{15}N_f$ values for either species.

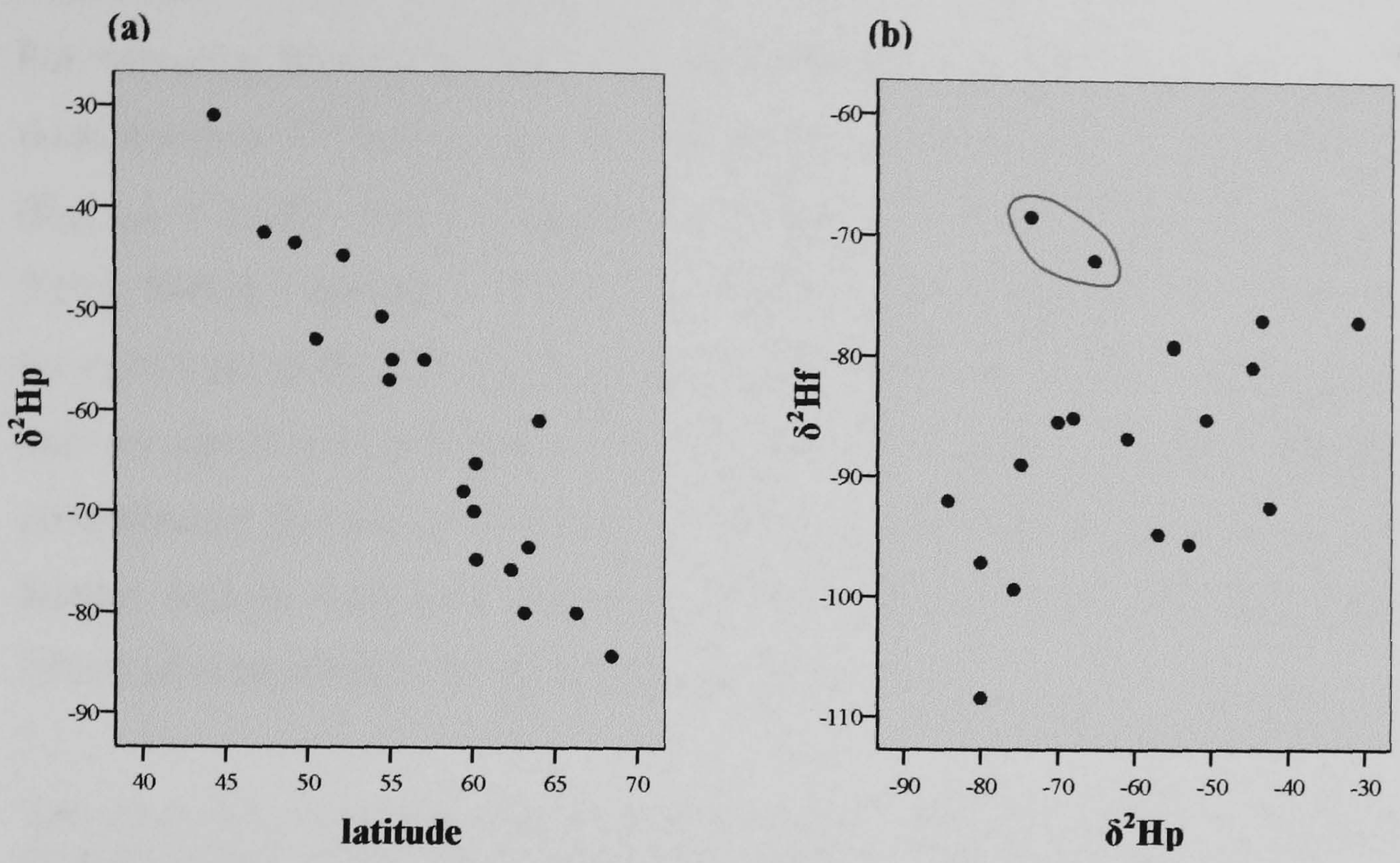


Figure 4.2(a) Scatterplot of mean δ^2H_p for the months April-September, of different European sites versus their latitude. δ^2H_p means were calculated using the Online Isotopes in Precipitation Calculator (OIPC, version 2.1; available at http://www.waterisotopes.org/), which uses data from the GNIP database. There was a strong correlation between collection site δ^2H_p and latitude ($r_{1, 17} = 0.991$; $P < 0.001$).

(b) Scatterplot of mean δ^2H_f of Blackbirds and Redwings, versus mean δ^2H_p of the European breeding sites at which the birds were sampled. One of the Norwegian sites (Titran) appeared to have outlying mean values of δ^2H_f for both Redwings and Blackbirds, and a second Norwegian site (Eidfjord) had an outlying mean δ^2H_f value for Blackbirds. These values, circled in blue, were excluded from the regression analysis. δ^2H_f was correlated with collection site δ^2H_p ($r_{1, 16} = 0.640$; $P = 0.006$).

Wintering Populations

Homogeneity of variances

For Blackbird sample sites, Levene's test was significant for all three isotopes: $\delta^{15}N_f$ (Levene statistic = 2.610; $P = 0.006$), $\delta^{13}C_f$ (Levene statistic = 3.339; $P = 0.001$) and δ^2H_f (Levene statistic = 3.028; $P < 0.002$). For Redwings, Levene's test was significant for $\delta^{13}C_f$ only (Levene statistic = 2.213; $P < 0.044$). This indicates that, in some cases, variance in stable isotope ratios was not equal between non-breeding sites, and in such cases, Welch's statistic is reported (in addition to F statistics).

Blackbirds

For wintering Blackbirds, there was a significant difference in the means of all three isotopes: $\delta^{15}N_f$ ($F_{10,\ 131} = 3.243$; **Welch's statistic = 6.622**; $P \leq 0.01$), $\delta^{13}C_f$ ($F_{10,\ 129} = 2.182$; **Welch's statistic = 12.689**; $P \leq 0.027$), and $\delta^{2}H_f$ ($F_{10,\ 129} = 2.623$; **Welch's statistic = 7.7232**; $P \leq 0.008$), between sample sites. There were no significant differences in any of the three isotopes between sex or age groups, and no significant interactions between any main effects. **Pairwise population** comparisons (Dunnett's T3 post hoc test) are illustrated in Table 4.8. Mean feather isotope values of breeding Blackbird feathers and individual isotope values of wintering birds are illustrated in Figure 4.3.

Table 4.8 Pairwise comparisons (Dunnett's T3 post hoc test) of sample site mean $\delta^{15}N$, $\delta^{13}C$ and $\delta^{2}H$ of Blackbird feathers collected from eleven different British and Irish wintering sites. For clarity, only significant values are displayed. Populations are listed in order of descending latitude.

		Population									
Site	**Isotope**	Monifieth	Low Newton	Preston	Mickley Square	Tuam	Rampton	Thetford	Devon	Arreton	Cornwall
Tain	$\delta^{15}N_f$	-	-	-	-	-	-	**0.001**	-	-	-
	$\delta^{13}C_f$	-	-	-	-	**0.012**	-	-	-	-	-
	$\delta^{2}H_f$	-	-	-	-	-	-	-	-	-	-
Monifieth			-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	**0.011**	-	-
Low Newton				-	-	-	-	-	-	-	-
				-	-	-	-	-	-	-	-
				-	-	-	-	-	**0.011**	-	-
Preston					-	-	-	-	**0.050**	-	-
					-	-	-	-	-	-	-
					-	-	**0.010**	**0.025**	-	**0.039**	-
Mickley Square						-	-	-	-	-	-
						-	-	-	-	-	-
						-	-	-	-	-	-
Tuam							-	-	**0.023**	-	-
							<0.001	**0.003**	**<0.001**	-	**0.009**
							-	-	-	-	-
Rampton								**0.006**	-	-	-
								-	-	-	-
								-	**0.003**	-	-
Thetford									**0.006**	-	-
									-	-	-
									0.001	-	-
Devon										-	-
										-	-
										0.001	**0.009**
Arreton											-
											-
											-

Redwings

For the nine wintering Redwing groups, there was a significant difference in δ^2H_f values between groups ($F_{6,\ 186} = 2.931$; $P = 0.009$), but no significant difference in either $\delta^{15}N_f$ or $\delta^{13}C_f$ values. There were no significant differences in any of the three isotope ratios between either sex or age group, although there was a significant difference in $\delta^{13}C_f$ values when the factors age and sample site were combined ($F_{8,\ 186} = 2.158$; $P = 0.033$). Post hoc testing (Dunnett's T3) did not reveal any significant pairwise group differences in δ^2H_f values.

Species comparisons

There were three breeding sites at which both species were sampled: two in Norway: Titran and Eidfjord, and a single site in Finland: Hanko. There were no significant differences in any of the three isotopes ($\delta 15N_f$, $\delta^{13}C_f$ and δ^2H_f) between species sampled at Titran, or Hanko. For species sampled at Eidfjord, there was a highly significant difference in δ^2H_f ($F_{1,\ 20} \leq 25.974$; $p < 0.001$), but no significant difference in either $\delta^{15}N_f$ or $\delta^{13}C_f$ values (Figure 4.3). For comparison purposes, plots of mean isotope ratios of all breeding populations (i.e. both species) and individual winter birds are illustrated in Figure 4.4.

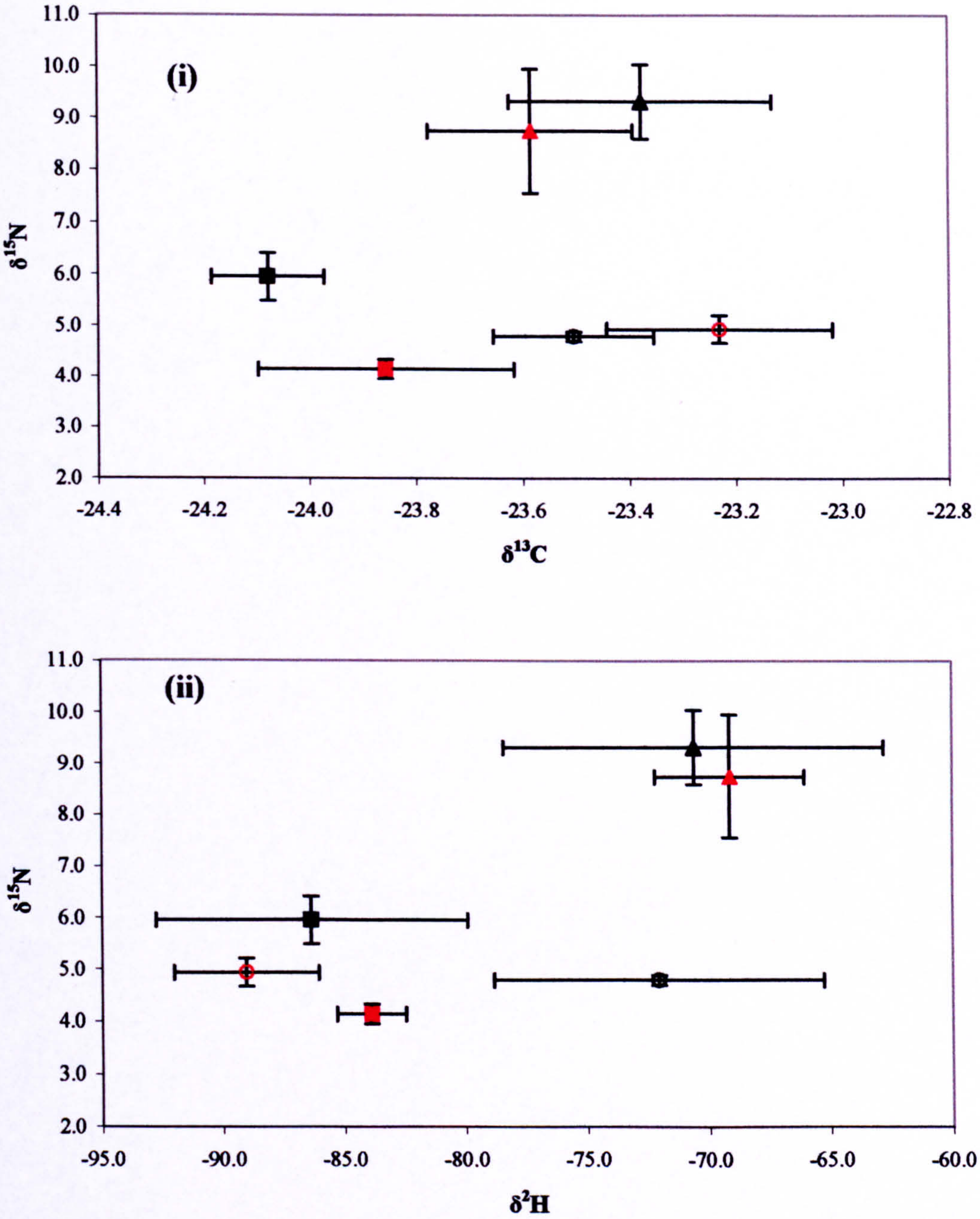
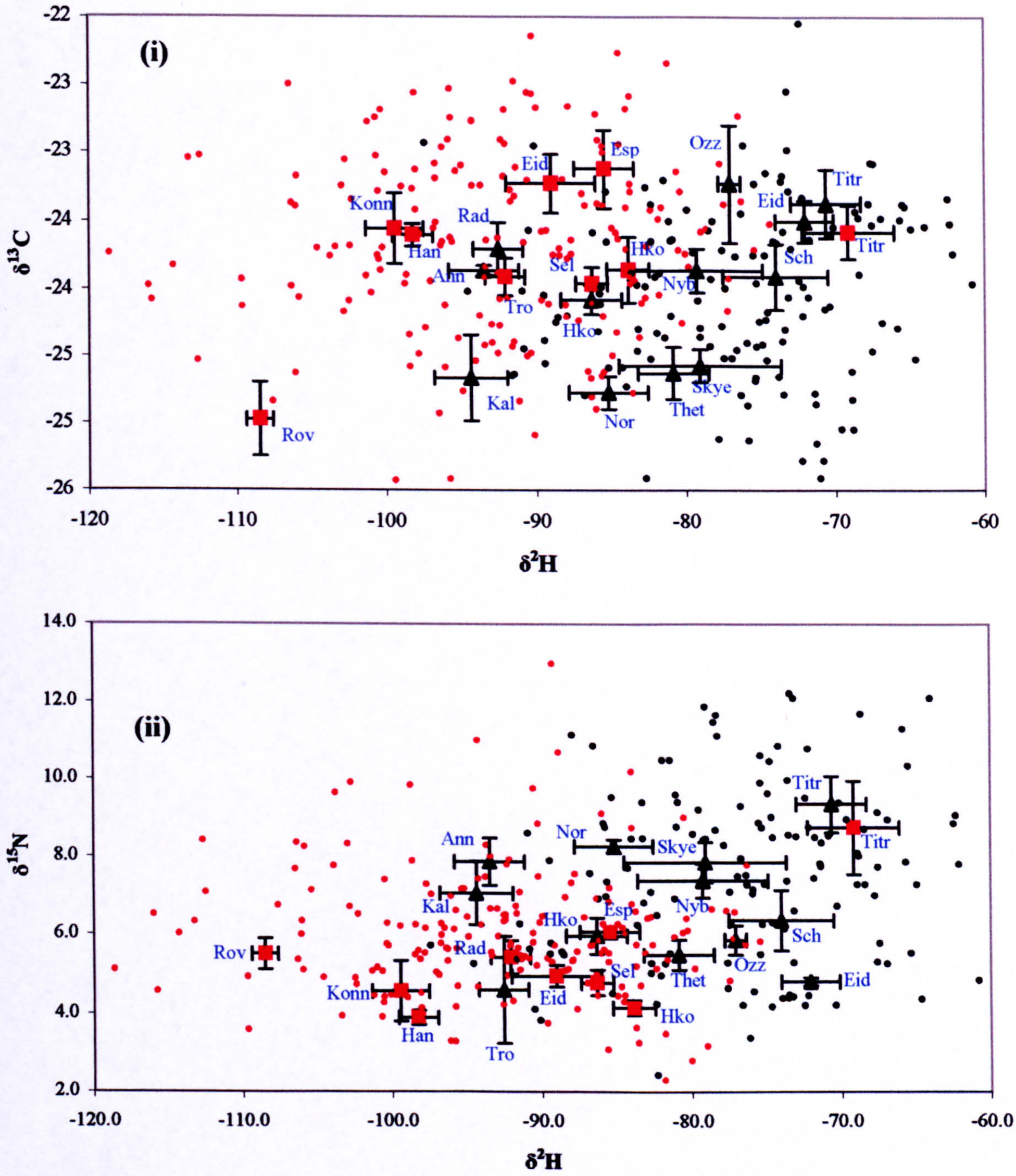


Figure 4.3 Species comparisons of mean feather isotope ratios of Blackbirds (points shaded black) and Redwings (points shaded red) collected from three different Scandinavian breeding sites: Hanko in Finland (represented by squares ■ and ■), Eidfjord (represented by open circles ○ and ○) and Titran (represented by triangles ▲ and ▲) in Norway. Values for (i) $\delta^{13}C_f$ and $\delta^{15}N$; (ii), $\delta^{2}H_f$ and $\delta^{15}N_f$ are reported in parts per thousand (‰). Each point represents the mean (± SE) of birds of each species sampled at that location.



Breeding populations:

Rov:	Rovaniemi, FIN	Han:	Handol, SWE	Nyb:	Nyborg, DNK
Kal:	Kaliningrad, RUS	Ann:	Annaberg-Bucholz, DEU	Sch:	Schifflange, LUX
Nor:	Northumberland, UK	Rad:	Radolfzell, DEU	Eid:	Espoo, FIN
Thet:	Thetford, UK	Tro:	Troms, NOR	Ozz:	Ozzano, ITA
Skye:	Skye, UK	Sel:	Selfoss, ISL	Titr:	Titran, NOR
Konn:	Konnevesi, FIN	Hko:	Hanko, FIN		

Figure 4.4 Mean feather isotope values of Redwings and Blackbirds collected from eighteen different European breeding sites: (i) $\delta^{13}C$ and $\delta^{2}H$; (ii) $\delta^{15}N$ and $\delta^{2}H$. Breeding Blackbirds are represented by black triangles (▲); breeding Redwings by red squares (■). Each of these points represents the species mean (± SE) of all birds sampled at that location. Superimposed on this are individual isotope values of feathers collected from UK and Ireland winter sites. Blackbirds are represented by black dots (•), Redwings by red dots (•).

Discriminant analyses

Of all the isotopes, $\delta^{13}C_f$ was found to have the most discriminatory power for assigning Blackbirds to UK and non-UK breeding groups. Using $\delta^{13}C_f$ values alone, 79.3% of samples (92/116 individuals) were correctly assigned to the UK or non-UK group. The discriminant function derived from $\delta^{13}C_f$ values was significant (canonical function 1: eigenvalue = 0.389; Wilks' Lambda = 0.720; χ^2 = 37.277; df = 1; $P < 0.001$). The additional inclusion of δ^2H_f values improved the classification performance by only 0.9% (correct assignment of a single additional individual) (Table 4.9(a)). $\delta^{15}N_f$ values could not be included because values were not normally distributed. A single discriminant function was derived from $\delta^{13}C_f$ and δ^2H_f values, and was also significant (canonical function 1: eigenvalue = 0.428; Wilks' Lambda = 0.700; χ^2 = 40.225; df = 2; $P < 0.001$). This latter function was used to assign winter Blackbirds to UK and non-UK breeding groups.

Using $\delta^{13}C_f$ and δ^2H_f (but excluding $\delta^{15}N_f$) values was also the most powerful combination for assigning breeding Blackbirds to three groups: UK, Continent and Fennoscandia. Overall, 71.2% of samples (83/116 individuals) were correctly assigned (Table 4.9(b)). Two significant discriminant functions were derived from $\delta^{13}C_f$ and δ^2H_f values (canonical function 1-2: eigenvalue = 0.459; Wilks' Lambda = 0.601; χ^2 = 57.225; df = 4; $P < 0.001$; canonical function 2: eigenvalue = 0.140; Wilks' Lambda = 0.877; χ^2 = 14.734; df = 1; $P < 0.001$).

43.0% of winter Blackbirds (58/135 individuals) were assigned to the UK breeding population (i.e. these birds were classified as UK and Ireland residents), and the remaining 57.0% (77 individuals) to the non-UK breeding group (i.e. these birds were classified as migrants), based on the quadratic discriminant derived from $\delta^{13}C_f$ and δ^2H_f values (Figure 4.5).

Table 4.9 Results of discriminant analyses for (a) two breeding populations of Blackbirds: UK and non-UK, based on $\delta^{15}N$, $\delta^{13}C$, and $\delta^{2}H$ in body feathers; (b) three breeding populations of Blackbirds: UK, Fennoscandia and Continental, based on $\delta^{13}C$ and $\delta^{2}H$ in body feathers. Correct assignments are displayed in bold red type.

(a) assignments based on 2 source populations

source population	**assigned population**	
	UK	non-UK
UK	**31 (93.9%)**	2 (6.1%)
non-UK	19 (22.9%)	**64 (77.1%)**

(b) assignments based on 3 source populations

source population	**assigned population**		
	UK	Continent	Fennoscandia
UK	**30 (90.9%)**	2 (6.1%)	1 (3.0%)
Continent	9 (19.6%)	**23 (50.0%)**	14 (30.4%)
Fennoscandia	5 (14.3%)	5 (14.3%)	**25 (71.4%)**

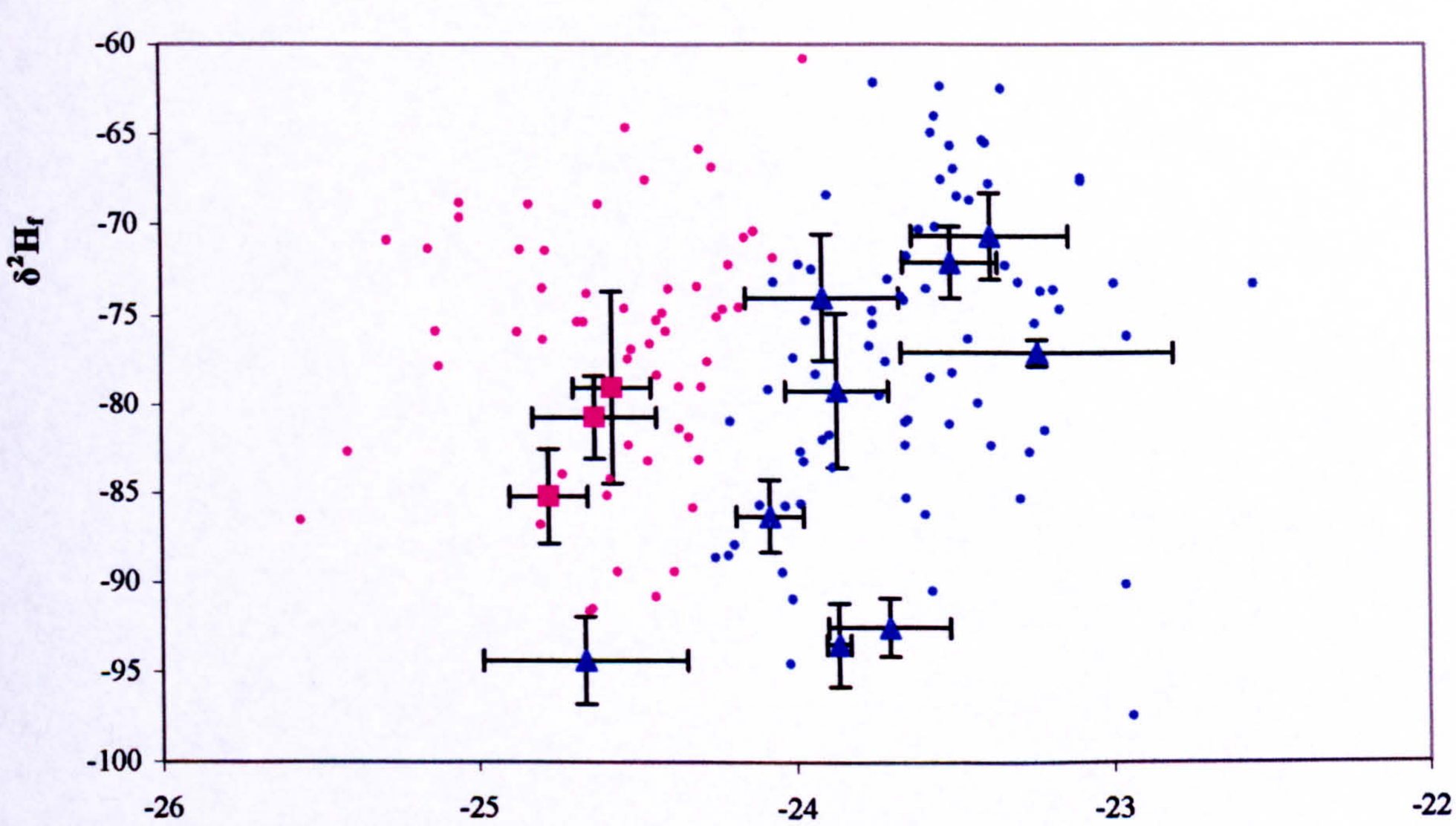


Figure 4.5 Mean $\delta^{13}C_f$ and $\delta^{2}H_f$ values of Blackbirds collected from UK (pink squares ■) and European breeding populations (blue triangles▲). Each point represents the mean (± SE) of birds of each species sampled at that location. Superimposed on this are individual isotope values of Blackbird feathers collected from eleven different UK and Ireland wintering sites. Those coloured pink (●) were assigned to a UK breeding origin; those coloured blue (●) to a UK breeding origin, using a quadratic discriminant function analysis based on $\delta^{13}C_f$ and $\delta^{2}H_f$

Discussion

Patterns of δ^2H_f

It was predicted that δ^2H_f would be the most useful isotope for discriminating between birds of different breeding origin, because a number of studies demonstrated that in North America, δ^2H_p varies with latitude across the continent, a phenomenon that a number of studies of bird migration have exploited, (e.g. Chamberlain et al., 1997; Hobson & Wassenaar, 1997, 2001; Hobson et al., 2001; Kelly et al., 2002; Atkinson et al., 2005). In addition, Hobson et al. (2004a) recently created δ^2H_p maps of Europe, and found that δ^2H_f was strongly correlated with collection site δ^2H_p. Indeed, there was a strong correlation between collection site δ^2H_p, estimated for the months April-September (to correspond with the time that the study species grow new contour feathers, and allow for isotopic equilibration between diet and feathers) and latitude (the r^2 value indicated that 88.2% of the variance in mean site δ^2H_p values could be explained by latitude, although note that altitude was factored into the δ^2H_p estimations). Excluding Titran Blackbirds and Redwings, and Eidfjord Blackbirds (for reasons discussed below), mean site δ^2H_f values were also correlated with both collection site mean δ^2H_p values and latitude, although the relationships were much weaker (r^2 values indicated that 27.5% of the variance could be explained by latitude alone; 40.9% of the variance in mean site δ^2H_f values could be explained by collection site mean δ^2H_p values).This may in part be due to the sample sizes involved; the GNIP database holds data collected (at monthly intervals) over a period of approximately 40 years, whereas the number of feathers analysed in the present study was comparatively small, and reflect a single snapshot in time. Thus, there is likely to be far less variance in the δ^2H_p values than in the δ^2H_f values.

The relatively weak correlations between δ^2H_f values and latitude/ site δ^2H_p values found in the present study contrasts somewhat to that of the study by Hobson et al. (2004a), which reported "strong correlations" between δ^2H of feathers from resident European birds and that of mean annual precipitation (both mean annual and growing-season values, $r^2 = 0.66$ or 0.65 respectively), despite the use of a similarly small number of samples from each site. However, Hobson

et al. (2004a) sampled a greater number of sites (n = 38) than the present study, covering a much larger range, notably including a number of sites from more northeasterly and southwesterly latitudes. Unsurprisingly, the resulting δ^2H_f range (from –131 to –38‰) was also considerably larger than that obtained here. The extended sample range of Hobson et al. (2004a) may thus account for the stronger correlation between δ^2H_f and collection site δ^2H_p compared with that found in the present study.

In a related study, Bowen et al. (2005) used precipitation isotope values (δ^2H_p and $\delta^{18}O_p$) to create global basemaps of isotope data. Using the same δ^2H_f data reported in the preceding Hobson et al. (2004a) study, Bowen et al. (2005) acknowledged that there may be less potential for determining the origins of individuals within the European continent than within North America; the range of δ^2H_p values was narrower (with a total range of ~100‰ from the Mediterranean coastal regions to northern Scandinavia), and the correlation between δ^2H_p and δ^2H_f weaker, in Europe than in North America (r^2 = 0.65 to 0.67, versus 0.85 to 0.86). Bowen et al. (2005) concede that, "Across large regions of central Europe δ^2H_p is almost invariant.... It is unlikely that the δ^2H and $\delta^{18}O$ tracer method could successfully resolve the origin of most materials within central Europe, but clearly potential does exist for the forensic assignment of samples within some regions, including Iberia, Western Europe, and Scandinavia."

One study that has used successfully used stable hydrogen isotope ratios to distinguish between different origins of a European migrant is that of Bearhop et al. (2005). They found that δ^2H values in the claw tips of several passerine species (both resident and migrant) grown in two European regions Britain & Ireland or Iberia were significantly different. This allowed Bearhop et al. (2005) to subsequently infer the winter origins of Blackcaps (*Sylvia atricapilla*) caught on breeding grounds in southern Germany and Austria. This important study also found that birds from the same wintering sites were mating assortatively on their breeding grounds. Blackcaps only began overwintering in Britain & Ireland in the past 50 years, and this novel migratory behaviour has been shown to have a genetic basis (Helbig, 1991; Berthold et al., 1992). Thus, assortative mating may

be the mechanism behind this rapid change in migratory behaviour (Bearhop et al., 2005).

Within-site variation of $\delta^2 H_f$ ratios

One of the problems encountered in the present study during analysis of δ^2H_f was the large within-site variation. Mean standard deviation of site δ^2H_f ranged from ± 1.0‰ to ± 13.8‰ in Blackbirds, and from ± 1.8‰ to ± 9.5‰ in Redwings. Closer inspection of the literature revealed that this level of variation is not uncommon. For example, despite the small number of individuals sampled at each site, considerable within-site variation in δ^2H_f was apparent in the study of Hobson et al. (2004a). For instance, the δ^2H_f of six individuals (including three or more species) sampled at Castelfidardo, Italy ranged from –54 to –70‰. This amount of variation was not unusual; even when a number of individuals of the same species were sampled at the same site, the variation was often between ± 8 and 12.5‰. Far more serious, was the finding reported by Wunder et al. (2005) of δ^2H of Mountain Plover (*Charadrius montanus*) chick feathers, collected in a single location in a single year, ranging from –7‰ to almost -60‰. Consequently, Wunder et al. (2005) questioned the concept of a characteristic range of δ^2H values for any single location. There are a number of reasons why such variation may occur, and the extent to which geospatial patterns of isotope ratios in avian tissues are influenced by age-specific, altitudinal, and temporal factors remains largely unexplored (Graves et al., 2002).

Temporal variation in feather isotopic ratios cannot be ruled out in the present study, because most samples (and all those from breeding sites) were collected during one season only. Even over a small timescale, temporal differences in feather isotopic ratios may occur. For example, Smith & Dufty (2005) reported a depletion in δ^2H_p in North America, between the months April - May, relative to June - July. According to Smith & Dufty (2005), isotopic equilibration between diet and feathers of an individual bird occurs gradually (circa 20-45 days; Bearhop et al., 2002), and thus the hydrogen isotopic composition of feathers may reflect that of precipitation prior to feather growth. This is another factor that should be considered when utilising δ^2H_f for bird migration studies; if juvenile feathers are grown before those of adults (patterns of moult typically

differ between adults and juveniles), juvenile and adult feathers grown at the same location may differ.

Both Meehan et al. (2003) and Smith & Dufty (2005) found adult birds (study species were Cooper's Hawks *Accipiter cooperii*, and Northern Goshawks *Accipiter gentiles*, respectively) had considerably higher δ^2H_f values than nestlings. The underlying causes of these age-related discrepancies remain largely unexplained, although Smith & Dufty (2005) speculate that they appear to be physiological rather than ecological. More recently, Langin et al. (2007) reported that juvenile tissues (feathers and blood) from five- to ten-day-old American Redstarts (*Setophaga ruticilla*) had more negative δ^2H values than those from adults. In contrast to Smith & Dufty (2005), Langin et al. (2007) suggested that these age-related differences in δ^2H values most likely reflected differences in diet. These findings have important implications; hatch-year birds may be selected for studies, because it is easier to be certain that sampled feathers were grown at or close to the collection site (e.g. Hobson & Wassenaar, 2001; Norris et al., 2005), but as Langin et al. (2007) concluded, feathers sampled from recently hatched juveniles may not provide a reliable estimate of expected local isotopic signatures for comparison with adult feathers of unknown origin.

Unexpected differences in δ^2H_f values between the sexes have also been reported by Newton et al. (2006), who sampled Bullfinches at a number of sites. This study found that δ^2H_f values were generally higher in males than females but, again, this difference could not be attributed to ecological differences (such as moult area or timing), suggesting the underlying reason was instead due to some physiological difference between the sexes (Newton et al., 2006). In addition, Langin et al. (2007) found that, within adult American Redstarts, primary feathers had significantly more negative δ^2H values than contour feathers.

Patterns of $\delta^{13}C_f$ and $\delta^{15}N_f$

For both species, there were significant differences in mean $\delta^{13}C_f$ and $\delta^{15}N_f$ values between sites. Significant linear trends in $\delta^{13}C_f$ (with longitude in Blackbirds, and latitude in Redwings) indicated that this isotope would be more

useful (for predicting species origins) than $\delta^{15}N_f$, which showed no linear trends for either species. This is not surprising, given that factors including land-use practices in the foraging area (e.g. use of agricultural fertilisers) and nutritional status of an individual are known to affect the $\delta^{15}N$ values of it's tissues (e.g. Hobson, 1999b; Hebert & Wassenaar, 2001; Cherel et al., 2005). Thus, local-scale variation is likely to confound any patterns that may exist at a broader geographical scale. Discriminant function analyses indicated that $\delta^{13}C_f$ had the highest discriminatory power of all the isotopes for differentiating between UK and non-UK breeding Blackbirds. This is an interesting and unexpected finding, because it suggests that $\delta^{13}C_f$ is potentially very useful for assigning Blackbirds to their breeding origin.

Unusual breeding site profiles

Titran and Eidfjord, the most northerly sites sampled for Blackbirds, had the highest (least negative) mean δ^2H_f values of all Blackbird breeding populations sampled, which is contrary to the expected latitudinal trend in δ^2H_f values (that predicts the most northerly sites will have the lowest, or most negative values). Blackbird feathers grown at these two sites also had the highest mean $\delta^{13}C_f$ value, with the exception of Ozzano, Italy. A possible explanation for these values would be a heavy marine influence on these sites; marine environments are more enriched (i.e. have less negative values) in $\delta^{13}C$, $\delta^{15}N$ and δ^2H than terrestrial environments (Rubenstein & Hobson, 2004). This is certainly likely to be the case for Titran, which is situated on the western tip of the island of Frøya. In addition, Redwings sampled at this site had the highest mean δ^2H_f values of all Redwing breeding populations sampled, despite the inclusion of a number of more southerly sample sites. For both species, the Titran site also produced the highest mean $\delta^{15}N_f$ value, which is further evidence of a probable marine influence on this site.

However, the unusual isotope values for the Eidfjord site are less easily explained by a strong marine influence. Although Eidfjord Blackbirds had one of the highest mean δ^2H_f values, Redwings from the same site did not have a similarly high mean δ^2H_f value. In fact, the two species differed significantly in mean δ^2H_f, which was unexpected; for the three sites at which both species were

sampled (Titran and Eidfjord in Norway, and Hanko, Finland), this was the only significant inter-specific difference in any of the isotope ratios. The trapping site at Eidfjord is approximately 25 meters above sea level, but is situated in a steep valley; 1.5 kilometres to the northeast, the altitude is 1000 meters above sea level. Whilst Blackbirds at this site are known to breed at low altitudes, most Redwings are found at higher altitudes, and it is not uncommon for them to breed more than 1000 meters above sea level (Alf Tore Mjøs, *pers. comm.*). This difference in breeding altitude between the two species may provide a possible explanation for their differing mean δ^2H_f. However, it does not explain why Blackbirds should have such a high mean δ^2H_f value. It seems unlikely that there is a strong marine influence at this site, since the distance to the nearest fjord (Hardanger Fjord) is approximately seven kilometres, but it is possible that Blackbirds at this site have an unusual feeding strategy; e.g. Blackbirds have been observed feeding on the intertidal zone on the Isle of Cumbrae, UK (Chris Redfern, *pers. comm.*). Blackbirds sampled at Eidfjord also had the lowest mean $\delta^{15}N_f$ value (which differed significantly from mean $\delta^{15}N_f$ value of Titran Blackbirds), which would not fit with a strong marine influence at this site.

Another possible explanation is that Blackbirds sampled at this site had dispersed there from other breeding sites. However, this also seems implausible, because although little is known about breeding dispersal, or dispersal of young birds prior to migration, the high δ^2H_f signatures would imply large-scale dispersal, over a relatively large distance (in a northerly direction). Furthermore, a number of the age 3 Blackbirds had not completed their post-juvenile moult at the time of sampling (Alf Tore Mjøs, *pers. comm.*) Since moult is energetically costly, it is unlikely that birds would be moving large distances at this time. Disappointingly, the study of (Hobson et al., 2004a) did not include any Norwegian sites. It would be interesting to obtain isotope values for other species that breed in Norway for comparative purposes, since two of the Norwegian sites sampled in the present study produced unusual δ^2H_f values. It is quite possible that the numerous mountains and fjords that characterise much of Norway result in strong local effects that confound any latitudinal variation in δ^2H_p.

Differences between age classes in the study species

For breeding Blackbirds there was a significant difference in δ^2H_f values between age groups, and significant interactions between the factors age and sex, as well as between age sex and population. There was also a significant difference in $\delta^{13}C_f$ values between age groups (but no significant interactions between main effects). For breeding Redwings, there was a significant difference in $\delta^{15}N_f$ values between age groups, and a significant interaction between the factors age and sex. There was also a significant difference in δ^2H_f values between age groups (but no significant interactions between main effects). Unfortunately, it is difficult to ascertain whether these results are a genuine difference between age groups, because of the limited numbers involved, particularly of birds hatched prior to the sample year. Indeed, some populations of breeding Blackbirds were composed entirely of first-year birds.

It is quite possible that dominance interactions may limit young birds and females to lower quality food (Smith & Metcalfe, 1997), and a recent study attributed diet as the most probable underlying cause of age-related differences in δ^2H_f values (Langin et al., 2007). As discussed previously, a number of studies have also documented differences in δ^2H_f values between age groups (e.g. Meehan et al., 2003; Smith & Dufty, 2005), or sexes (e.g. Newton et al., 2006), which could not be attributed to ecological differences (such as diet, or moult strategy). However, there were no significant differences in any isotope ratios between different age groups of winter birds, indicating that there are no broadscale age-related differences in the study species.

In fact there is some evidence of age-specific difference in diet in Redwings. A study by Redfern et al. (2002) examined the relationship between fat levels and intestine size in a large number of Redwings that died by flying into a lighthouse at Bardsey, UK, while on migration (and which included many of the birds used in the current study). A clear, size-independent correlation between claviculo-coracoid fat mass with intestinal length was found in first-year females only, and explained 25% of the variance in fat mass (independently of body size) for this group. The authors speculate that diet may vary according to experience and

dominance interactions within feeding flocks, affecting the ability of individuals to accumulate fat for migration (either prior to migration, or at stopover sites).

Winter sites

Feathers collected in the winter had a large range of δ^2H_f values. Blackbird feathers ranged, from around –60 to -95‰; the range of Redwing values from around –75 to –115‰. Thus, although there was some overlap between values for the two species, Redwings tended to have more negative values. This appears to support the predicted variation in δ^2H_f as a function of latitude, because Redwings tend to breed at more northerly latitudes than Blackbirds (Cramp & Simmons, 1988b). Whilst at first glance, this may seem to conflict with the results obtained from breeding populations (which suggested only a weak relationship between δ^2H_f values and latitude), this is likely to be a result of wintering birds originating from a much broader latitudinal range than that represented by the breeding sites sampled in this study.

Assignment tests

Discriminant functions are based on linear or quadratic combinations of the predictor variables (in this case, stable isotopes) that provide the best discrimination between the groups. The functions are generated from 'training data': a sample of individuals for which group membership is known (e.g. breeding birds). The functions can then be applied to individuals that have measurements for the predictor variables, but for whom group membership is unknown (e.g. wintering birds). The present study found that δ^2H_f values, and particularly $\delta^{13}C_f$ values, were useful for discriminating between UK-breeding (i.e. resident) and non-UK breeding Blackbirds (i.e. potential migrants). δ^2H_f values were useful for assigning Blackbirds to three breeding groups (UK, Fennoscandia, and Continent), but not when birds were assigned to just two groups (UK and non-UK), whereas $\delta^{13}C_f$ values were useful for both assignments. However, this is likely to be because UK $\delta^{13}C_f$ values were at one end (i.e. had the lowest values) of the total range of breeding site $\delta^{13}C_f$ values, but in the middle of the range of δ^2H_f values. Grouping all non-UK Blackbirds together would result in combining δ^2H_f values that are both lower and higher than those of the UK, so that values of the two groups (UK and non-UK) cannot

be discriminated. An alternative approach could be to use cluster analysis, which would classify or partition the data into different groups or subsets. However, this is a descriptive, rather than predicitve technique, and thus it was felt that a discriminant approach would be more appropriate for the present study.

A number of other studies have used discriminant analysis to predict group membership of migratory birds, with varying degrees of success (e.g. Caccamise et al., 2000; Wassenaar & Hobson, 2000b; Farmer et al., 2003; Farmer et al., 2004; Neto et al., 2006; Rocque et al., 2006). For example, Caccamise et al. (2000) analysed $\delta^{15}N_f$, $\delta^{13}C_f$ and $\delta^{34}S_f$ of Canada geese (*Branta canadensis*), and found that the latter two isotopes were useful for differentiating between resident and migrant birds. They were able to discriminate between three groups: migrants, inland residents, and coastal residents, with an accuracy of 92%.

Rocque et al. (2006) is critical of many of these earlier studies, because confidence in assignment was not assessed. Based on $\delta^{13}C_f$, $\delta^{15}N_f$ and $\delta^{2}H_f$, discriminant analyses correctly separated 80 to 82% of summer- and winter-grown feathers for two Plover species (although only 69% of winter-grown feathers were correctly classified). However, when data for the two species were combined, a three-way comparison to classify feathers as either winter-grown, or one of the two (interspecific) summer-grown groups, correct overall assignment dropped to 63% of samples. Rocque et al. (2006) also employed an "assignment with exclusion" method (adapted from Cornuet et al., 1999), whereby only individuals with a membership probability of $\geq$ 80% were accepted as group members, to impart a measure of confidence in assignment. Using this exclusion threshold, the performance of the three-way comparison method declined further, to just 41%; none of the winter-grown feathers could be assigned to their correct groups of origin, because none had a posterior probability of correct assignment higher than 75%. The authors concluded that it was not possible to confidently assign individual feathers to their known Continent of origin.

In the present study, non-UK birds were more often assigned to UK groups, than vice versa, which leads to disproportionate group assignments. This is clearly a problem when assigning birds of unknown origin, and assignments of winter

birds should be treated with caution. It seems likely that the 43.0% of winter Blackbirds (58/135 individuals) assigned to the UK may be slightly higher than the true proportion.

Differences between winter sites in feather isotope ratios

Despite the lack of clear overall isotopic structuring in either the winter or breeding data, there were significant differences in all three isotopic ratios between sites in non-breeding Blackbirds. Most notably, Tuam, Ireland differed in mean $\delta^{13}C_f$ between a number of other sites, and Preston differed in mean $\delta^{2}H_f$ from several other sites. It is important to consider that Blackbirds sampled at each site are likely to consist of both residents and migrants, and that proportions of these two migratory classes may vary between sites. This will likely confound any site-specific differences in origins of migrant birds, (particularly if there is local variation in UK and Ireland isotope values that has not been revealed by the limited number of UK breeding sites sampled). In other words, in winter, different parts of the UK and Ireland may receive migrants from different breeding origins, but it may be difficult to reveal such patterns if it is not possible to distinguish between birds that bred at the sample site, and those that migrated in from elsewhere. Any differences between winter sites could be attributed to different proportions of the two migratory classes, rather than differences in the origins of migrants. Thus, it is difficult to elucidate the extent of migratory connectivity in this species.

There was a significant difference in mean $\delta^{2}H_f$ between groups of non-breeding Redwings, but pairwise comparisons failed to elucidate where these differences occurred. Given the small number of winter sample sites from which Redwings were sampled during the course of their migration, birds originating from a vast area must have subsequently converged into flocks, indicating considerable mixing of populations on the wintering grounds, and possibly relatively low migratory connectivity. This is perhaps not surprising, given the nomadic nature of this species (Reid, 1993), and is analogous to the suggestion of Newton et al. (2006), that invasions may occur when birds, faced with food shortage, move through regions, accumulating in numbers as they do so, to form large flocks. However, it was not possible to ascertain whether Redwings overwintering in

different parts of the UK and Ireland originate from different breeding populations, nor is it possible to determine how much mixing occurs on the breeding grounds. The species' migratory traits (hard-weather-induced movements, little evidence of site fidelity, and mixing on the non-breeding grounds), coupled with lack of genetic population structure, appear to suggest that considerable mixing also occurs on the breeding grounds, and that different parts of the UK and Ireland are unlikely to receive migrants from different breeding populations. Nonetheless it is known, for example, that parts of Ireland (including Tuam) and Scotland receive Redwings of both the nominate and the Icelandic race, whereas Icelandic-ringed Redwings have never been recovered anywhere in England or Wales (Milwright, 2002a) (but see an early account by Witherby et al., 1938). Thus, the possibility that the nominate race exhibits different migratory behaviours between breeding populations, cannot be excluded.

Outliers

A number of isotopic ratios of breeding individuals were identified as outliers and excluded from the analyses. It may be that these individuals were feeding on unusual diets (discussed further below). For example, four birds had very low $\delta^{15}N_f$ values (one Icelandic Redwing, and three Blackbirds from two different breeding sites), which may imply extreme frugivory (versus insectivory). Given that all four of these birds were age 3 (i.e. fledglings), this would be a plausible explanation, and may reflect inexperience, or subordinate status, affecting feeding behaviour. There were also a number of $\delta^{2}H_f$ outliers, which are somewhat more difficult to explain. The possibility that some of the adult birds were sampled prior to moult, or that birds may have dispersed into the sample site following moult, cannot be ruled out. This is also a consideration for the small number of adult Blackbirds that were sampled early or late in the breeding season: two were collected at Nyborg, Denmark late June, and two were collected at Schifflange, Luxembourg in mid-October.

Dietary variation

Blackbirds have been gradually colonising the urban environment since the early nineteenth century, and are now one of the most common bird species in central

European towns. A potential benefit for species such as the Blackbird that have settled in urban areas is the availability of anthropogenic food supplies in this type of environment (Partecke et al., 2006). Whether or not food is provided deliberately (through bird feeders), the majority of anthropogenic food supplies are unlikely to be sourced locally. It is therefore important to consider the effect this may have on isotope ratios of feathers from birds feeding in this type of environment. An early study of Blackbirds in suburban Oxford, UK Snow (1958), identified food provided by humans (either deliberately, or discarded as waste) as one of the five main categories of food sources for these birds. Snow (1958) points out the versatility of Blackbirds in terms of food and feeding methods, and cites some unusual anecdotal evidence including observations of Blackbirds taking tadpoles and small fish from streams.

This ability of Blackbirds to exploit a diverse range of food sources may explain the two winter Blackbird samples with unusually enriched (i.e. high, or less negative) $\delta^{13}C$ values that were excluded from analyses. These two samples were collected from the same site in Cornwall, and $\delta^{15}N/\delta^{13}C$ analysis was repeated twice to confirm repeatability of results. Mean $\delta^{13}C_f$ values were –17.8‰ and –19.0‰, which is indicative of a marine diet. Both birds had a similar $\delta^{2}H_f$ value of -72.3‰ and -71.0‰ respectively. Mean $\delta^{15}N_f$ values were 9.6‰ and 11.7‰ respectively. Although both these $\delta^{2}H_f$ and $\delta^{15}N_f$ values are within the range of the total winter Blackbird dataset, they are fairly high, and are therefore not inconsistent with the implication of a marine diet.

Conclusion

There was a strong correlation between mean study site $\delta^{2}H_p$ and latitude, but the relationship between mean study site $\delta^{2}H_p$ and $\delta^{2}H_f$ values was considerably weaker. Other variables, such as marine influence, longitude, and possibly altitude, appeared to be having a significant (and confounding) effect on isotopic signatures. Nonetheless, $\delta^{2}H_f$ values did prove to be a useful variable for discriminating between the three Blackbird breeding groups (UK, Fennoscandia and Continent). An unexpected finding was that $\delta^{13}C_f$ appeared to be extremely useful for discriminating between resident and migrants Blackbirds (i.e. UK

versus non-UK breeding birds). The underlying reason for the apparent geographic differences is unknown.

Redwings from a vast range of breeding sites appear to be mixing en route to wintering grounds, indicating little migratory connectivity. This is supported by field evidence, which frequently reports the nomadic nature of this species. In contrast, within the UK and Ireland, there appear to be regional differences in either proportions of residents (versus migrants), or proportions and origins of migrant Blackbirds (or both), as indicated by significant pairwise differences in stable isotope signatures between a number of sites. Since the level of confidence in assignment was not tested, however, assignment of winter birds should be treated cautiously.

While there is also some evidence for age-specific differences in isotope ratios, small sample sizes mean that these findings are inconclusive, and further investigation of possible age differences would be extremely useful. Regardless of the aim of isotopic studies, careful consideration of sample size is recommended. Typically, small numbers of samples are collected to represent the stable isotope 'signature' of a particular site, yet the amount of within-site variation is often high, suggesting that a reliance on small numbers of samples is not a sufficiently rigorous approach.

As pointed out by Rocque et al. (2006), although correlating isotopic signatures along latitudinal gradients is useful in some species, a clear understanding of the ecology and physiology of the study species is essential. Unexpected differences between species (as occurred in this study), or between individuals within a study site are increasingly being reported; in particular, a number of recent studies have reported within-site variation in δ^2H_f values (e.g. Meehan et al., 2003; Smith & Dufty, 2005; Newton et al., 2006; Langin et al., 2007). This suggests that δ^2H values are more useful for predicting origins of birds at a broad geographical scale, and that it may only be possible to predict the relative proportions of birds in a given sample that originated from different sites, rather than pinpointing the precise origin of an individual. The results of the present study lend support to the prediction of Bowen et al. (2005) that, in general, there may be less potential

for determining the origins of individuals within the European continent than within North America. Indeed, it seems likely that δ^2H_f values may be most useful for those species whose breeding range spans a broad latitudinal range, or those with discrete, widely separated breeding sites (e.g. Berthold, et al., 2005).

CHAPTER 5: INTRASPECIFIC BODY SIZE VARIATION IN *TURDUS* SPECIES OVERWINTERING IN THE UK AND IRELAND

Introduction

Wing length variation in Turdus merula *and* T. iliacus

Blackbirds *Turdus merula* are partial migrants, and during the autumn, the resident British and Irish population is amplified by a huge influx of migrant birds (Snow, 1986a). Ringing data have revealed that these migrants originate from the Netherlands, Germany and southern Scandinavia. Based on recovery data, some, particularly those arriving in southeast England, are known to pass through Britain on to more southerly wintering areas (Chamberlain & Main, 2002). Observations by bird ringers in Britain suggest that there is a tendency for migrant Blackbirds (of both sexes) arriving in autumn to have longer wings than year-round resident birds. It is possible that only Blackbirds breeding at higher latitudes (i.e. Fennoscandian birds) have longer wings than UK-residents, and that wing lengths of birds breeding elsewhere in Continental Europe do not differ significantly from those of UK- and Irish-breeding birds (possible reasons for this are discussed below). This is supported by data provided by Cramp & Simmons (1988b), although the dataset is rather small (Table 5.1).

Table 5.1 Measurements taken from museum skins of Blackbirds sampled in three different European countries: UK, Germany and Sweden. All data were taken from (Cramp & Simmons, 1988b). The first line of data provides the mean wing length (and number of individuals measured); the second line provides the range of wing lengths. Note that skins are known to shrink, due to drying of the skin, and wing lengths of skins are ~2% shorter than measurements taken from live birds.

Location	**Adult ♂**	**Adult ♀**	**First-year ♂**	**First-year ♀**
Southern England	129.0 (n = 10) 127-132	123.3 (n = 10) 121-127	125.3 (n = 10) 124-128	120.7 (n = 9) 116-126
Germany	131.9 (n = 65) 124-136	126.4 (n = 33) 119-131	128.0 (n = 107) 120-134	125.6 (n = 43) 116-128
Sweden	133.9 (n = 9) 126-140	128.2 (n = 2) 125-131	130.2 (n = 13) 125-136	127.7 (n = 10) 124-131

Wing length is routinely recorded by bird ringers, and is a good indicator of overall body size. For example, in a study of 26 species ranging in mean mass from 5.3g (Goldcrest *Regulus regulus*) to 100.3g (Blackbird), amongst linear measures, wing length was consistently the best predictor of body size (Gosler, 1998). Similarly, a study of museum skins (n = 949) of American Robins (*Turdus migratorius*) throughout North America, revealed that wing length was strongly correlated with six other measures of general size (Aldrich & James, 1991), and a study of fat and body condition in migrating Redwings (n > 600) found wing length to be the best single linear measure of size (Redfern, 2000). Body weight is also frequently recorded by ringers, but intra-individual changes occur both diurnally and seasonally (e.g. Cresswell, 1998; Macleod et al., 2005).

It is possible that Redwings also exhibit a geographical cline in body size, although it has not been possible to test this, because the Redwing is only a winter visitor to Britain & Ireland (although a small number have bred in the UK), and therefore little can be inferred about the likely origin of a particular individual based on body size alone. In addition, males tend to have longer wings than females (Redfern, 2000; Svensson, 2005), but since the two sexes are monomorphic, and cannot be sexed outside the breeding season, sex-specific differences in body size cannot be routinely adjusted for. If evidence for geographical clines in wing length were found for either Blackbirds or Redwings (but particularly Blackbirds because they can be sexed in the hand, and thus sex-related differences in wing length can be controlled for), this would potentially be very useful information, because it may be indicative of breeding origin.

Geographical clines in body size: Bergmann's rule

Bergmann's rule refers to the observation of Carl Bergmann in 1847 that, in general, birds and mammals living in cooler climates tend to have larger bodies than congeners living in warmer climates (Bergmann, 1847). This ecographic rule has since been revised and clarified in various ways. Although originally referring to interspecific comparisons, it has been reformulated to include populations within species (Blackburn et al., 1999; Meiri & Dayan, 2003). In addition, latitude is often used as a proxy for temperature in tests of Bergmann's rule (Ashton, 2002). Adherence to Bergmann's rule has been documented for a

range of avian species (e.g. James, 1970; Martin, 1991; Bried & Jouventin, 1997; Graves et al., 2002; Jones et al., 2005), and two recent (independent) reviews of the literature found a strong overall tendency for avian species to adhere to Bergmann's rule (Ashton, 2002; Meiri & Dayan, 2003).

Nevertheless, there are a number of avian species that do not conform to Bergmann's rule. For example, a study of overwintering warblers of the genus *Phylloscopus* found a negative association of body size with latitude, both across the genus, and within a single species (*Phylloscopus trochiloides*) (Katti & Price, 2003). Furthermore, there appears to be considerable variation around the relationship. In his investigation of geographical variation in *Parus* species, Snow (1954) found that, although most populations conformed to Bergmann's rule, individuals of some populations endemic to higher altitudes of North Africa and southwest Asia tended to be larger than conspecifics inhabiting higher latitudes of Europe, even though winter temperatures were similar in the two regions. Snow suggested that shorter day length in the higher latitudes of Europe limited foraging hours, and thus body size. In a more recent study by Wiedenfeld (1991), morphological measurements (taken on study skins) of breeding Yellow Warbler *Dendroica petechia* collected from near the Arctic Circle to the Equator found that individuals were generally larger in the south than in the north. The reverse trend was displayed in populations of (non-migratory) Yellow Warblers from the Caribbean, where wing lengths were shorter and more rounded than in the far north. As suggested by Hamilton (1958), it is likely that there are many different selective forces affecting the evolution of body sizes in populations, so that regional or global deviations from Bergmann's rule are to be expected (Dayan et al., 1991).

Mechanisms underlying geographical clines in body size

Whether or not a general ecographic rule exists, the underlying mechanism(s) behind any observed latitudinal clines or patterns in body size remain controversial. Bergmann suggested that the observed pattern was based on heat conservation, with larger body size being selected for in cooler environments because the lower surface area to mass ratio results in a lower relative heat loss (Ashton, 2002). This has been challenged on the grounds that the thermal

properties of an animal's body surface (e.g. its insulation, exposure and vascularisation) are more important than body size *per se*, in determining rates of heat loss and gain (Scholander, 1955).

More recently, other mechanisms underlying latitudinal clines in body size have been proposed, pertaining to resource seasonality, migration distance and phylogeny (Jones, 2005). For example, in a recent meta-analysis of published data on body size variation in avian species, it was suggested that fasting endurance was probably a more important factor contributing to geographical clines in body size than the traditional hypothesis of heat conservation (Ashton, 2002). The fasting endurance hypothesis proposes that in seasonal environments, larger body size is selected for, because larger animals can store more fat, which can be used in times of seasonal stress (e.g. Brodie, 1975; Boyce, 1979; Lindstedt & Boyce, 1985). It has also been suggested that, on average, species with a larger body size, have higher dispersal ability than smaller-bodied species, and tend to occupy a larger geographical range. Consequently, larger-bodied species may be more likely to occupy high latitudes (Blackburn & Gaston, 1996).

Wing length and migratory status

Although an increase in body size appears to result in some increase in wing length (and area), the selective forces responsible for clines in wing length may be different to those responsible for clines in body size *per se*. For example, wing structure may be influenced by migratory habit (Hamilton, 1961; Zink & Remsen, 1986). In their study of age-related variation in partially migratory Blackcap (*Sylvia atricapilla*) populations, Pérez-Tris (2001) found that migratory Blackcaps had longer and more pointed wings than sedentary Blackcaps. These traits improve endurance in migratory flight. More recently, Fiedler (2005) analysed wing and tail biometrics of Blackcaps (n = 700) from eight different populations classed as sedentary to long-distance migrants. One finding was that wing length (as well as aspect ratio, and wing pointedness) increased with increasing migration distances of populations. In general, traits for energy-efficient flight (such as wing length and pointedness) were more strongly developed, whilst those for manoeuvrability were less developed, in birds travelling longer distances.

In contrast, reviews by Zink & Remsen (1986) and Meiri & Dayan (2003) found that sedentary species conform more strongly to Bergmann's rule than do migrant species (but see also Ashton, 2002). It has been suggested that this may be because migratory species do not encounter the harsher environmental conditions of their breeding ranges during winter, so the selective pressures acting on migrants may be lower than those acting on residents (Hamilton, 1961). Redwings wintering in the UK and Ireland are virtually all long-distance migrants (Milwright, 2002b), and therefore, it may be expected that selection on wing length acts more homogeneously in Redwings than in Blackbirds, resulting in consistent wing length (or overall body size) across its geographical range. Although the Redwing has a vast breeding range, and therefore it could be argued that latitudinal clines may still exist (as a result of populations inhabiting different ranges, and/or migrating over different distances), Redwings are highly nomadic (Reid, 1993; Snow, 1986b), and are not thought to exhibit strong breeding site fidelity (Cramp & Simmons, 1988a), so that selective pressures on populations are likely to differ between years. Interestingly, the Icelandic Redwing race *coburni* is described as "slightly larger" (up to 5% longer and visibly bulkier) than the nominate *iliacus* (e.g. Cramp & Simmons, 1988a; Svensson, 2005), yet this cannot be attributed to differences in either latitude or migratory distance.

Aims

1) The first aim of this part of the study was to determine whether there was any evidence for geographical clines in wing length in either the Blackbird or the Redwing. This was tested in two ways:

i) For Blackbirds, the data collected by bird ringers, as part of the national bird-ringing scheme for Britain & Ireland (Redfern & Clark, 2001) for the years 1996 to 2005 were obtained from the British Trust for Ornithology (BTO). These data were analysed with particular reference to seasonal changes in mean wing length, as well as regional (within Britain & Ireland) variation.

ii) Since the results of stable isotope analyses of breeding Blackbirds and Redwings (combined) indicate that there may be a latitudinal trend in both δ^2H

and $\delta^{13}C$ of feathers (reported in Chapter 4), the association between isotope ratio and wing length (in both species) was tested.

2) If geographical clines in wing length were found to exist in the Blackbird, a second aim was to determine whether this could be a useful criterion for discriminating between British- and Irish-breeding birds (i.e. residents), and those from the Continent (i.e. winter migrants).

The following hypotheses were tested:

- Mean wing length of Blackbird populations will change seasonally, and will be highest in autumn months, with the arrival of longer-winged migrants, and lowest during the breeding season, when mainly sedentary individuals are present in the population.

- Mean wing length of breeding Blackbirds will differ significantly between the UK and Fennoscandia, but there will be no significant difference between mean wing length of UK-breeding birds and those from the rest of the continent (i.e. excluding Fennoscandia).

- In Blackbirds, wing length is negatively correlated with both $\delta^{2}H_f$ and $\delta^{13}C_f$ values, because there is a geographic cline in wing length, with migrants originating from higher latitudes (i.e. those with more negative $\delta^{2}H_f$ and $\delta^{13}C_f$ values) having longer wings than UK and Ireland residents.

- There will be no such correlations between wing length and $\delta^{2}H_f$ or $\delta^{13}C_f$ values in Redwings, or if there are, they will be weaker than those in Blackbirds.

Methods

Determination of moult period for Blackbirds

Adult Blackbirds (defined as those birds in their second autumn or older) undergo a complete postbreeding moult, during which all feathers are replaced, including their primary wing feathers (Snow, 1958). Passerines have ten primaries (on each wing), which can be numbered descendantly, i.e. from the carpal joint (wrist) outwards from the body, as P1, P2 etc. The majority of passerine species (including the Blackbird) moult descendantly, dropping the innermost primary (P1) first, followed by the adjacent primary (P2) and so on, generally in a regular order towards the wing tip (Ginn & Melville, 1983). When birds are moulting their outermost primary feathers, an accurate wing length cannot be measured. It was therefore important to define the moult period for Blackbirds breeding in Britain & Ireland, so that birds caught during this period could be excluded from analyses.

For birds with primaries in active moult, each new primary feather is given a score of 1 to 5, according to its stage of growth. Thus, each individual bird can be given a total primary score of 1 to 49 (birds that have completed primary moult would score 50) (Snow, 1969). This information (along with the date) can be used to calculate the start date and duration of moult for a particular species. Within the BTO dataset, moult scores had been recorded for 640 individuals (n = 284 females; n = 356 males). Using the data from these individuals, the mean start date and duration of primary moult of Blackbirds breeding in Britain & Ireland was calculated (separately for males and females), using the maximum likelihood method developed by Underhill & Zucchini (1988). Data (Type 3) were analysed using a program written in R (Becker et al., 1988) by Walter Zucchini (*pers. comm.*).

Analysis of Blackbird ringing data

Data from the first capture (i.e. excluding retrap records) of Blackbirds between 1st January 1996 and 31st December 2005 in Britain & Ireland were used. Only birds of known capture date, location, age, sex and wing length were included. All birds were placed into two age categories: age group 3: birds with juvenile

primary feathers (i.e. those in their first year), or age group 4: birds with non-juvenile primary feathers (i.e. 'adult' birds – those that have moulted their juvenile primary feathers). Svensson, (2005) suggests a typical wing length of 119-138mm in Europe, and the few birds with recorded wing lengths of <110mm and >149mm were excluded, as these were considered to be extreme, and probably erroneous. In total, data from 113,934 individual Blackbirds were used (n = 34,201 age 3 females; n = 22,296 age 4 females; n = 35,823 age 3 males; n = 21,614 age 4 males).

Data from all years were pooled, and mean wing length calculated for 14-16 day periods throughout the year, excluding the moult period, in order to describe seasonal changes in mean wing length (period length was selected arbitrarily, simply on the grounds that it should be sufficiently small to detect seasonal changes). This was calculated separately for each age/sex group. For purposes of comparison, mean wing length during periods immediately before and after the average moult period were included, even though they encompass part or all of the standard deviation of moult duration (17 days for females; 14 days for males).

To check for regional differences in mean wing length, birds were separated into 12 different groups according to where they were caught, as depicted in Figure 5.1. Data were divided according to month (September to June), and for each month, a General Linear Model (GLM) Univariate Analysis of Variance (ANOVA) was used to investigate whether mean residual wing length differed between birds of different regions, sex/age groups (3F: age category 3 females; 3M: age category 3 males; 4F: age category 4 females 4M: age category 4 males) and year (1996 to 2005). Note that during September, only birds recorded at the end of the month (from 22nd September for males; from 28th September for females) are included, because birds caught earlier in the month may still be moulting their primary feathers. For the same reason, data from July and August were also excluded from the analysis. For the months October to June, data from birds caught during the entire month were analysed. For each month, Levene's test was used to check for homogeneity of wing length variance between regions.

Analysis of breeding data

Breeding Blackbirds for which wing length was available (n = 333) were assigned to one of three groups: UK (n = 58), 'Fennoscandia' (birds caught in Finland and Norway, n = 28), and 'Continent' (birds caught in Germany, Luxembourg, Kaliningrad and Denmark n = 247). Note that these groupings, although appearing somewhat arbitrary, were designated upon exploration of the available wing length data. Wing length residuals were then calculated for each age and sex group (3F; 3M; 4F and 4M), and a one-way ANOVA was used to test for differences in mean residual wing length between the three groups. Where significant differences occurred, post hoc testing (Tukey's HSD test) was used to determine between which groups these differences occurred. Spearman's rho was used to determine whether there was a correlation between wing length residual and latitude.

Analysis of stable isotope and biometric data

Stable isotope ratios of carbon, nitrogen and hydrogen in the feathers of Blackbirds (n = 136, 140 and 139 respectively) and Redwings (n = 164, 165 and 165 respectively) collected at sites in the UK and Ireland during the autumn/winter (described in Chapter 4) were tested for correlation with wing length. Where both variables were normally distributed, Pearson's correlation coefficient was used; where data deviated from a normal distribution, Spearman's rho was used. Only birds of known age and sex were included in the analysis. As with the BTO data, birds were aged as either 3 (those with juvenile primary feathers) or 4 (birds with non-juvenile primary feathers). Due to the relatively small dataset for this part of the study, wing length residuals were used so that different age and sex categories could be pooled.

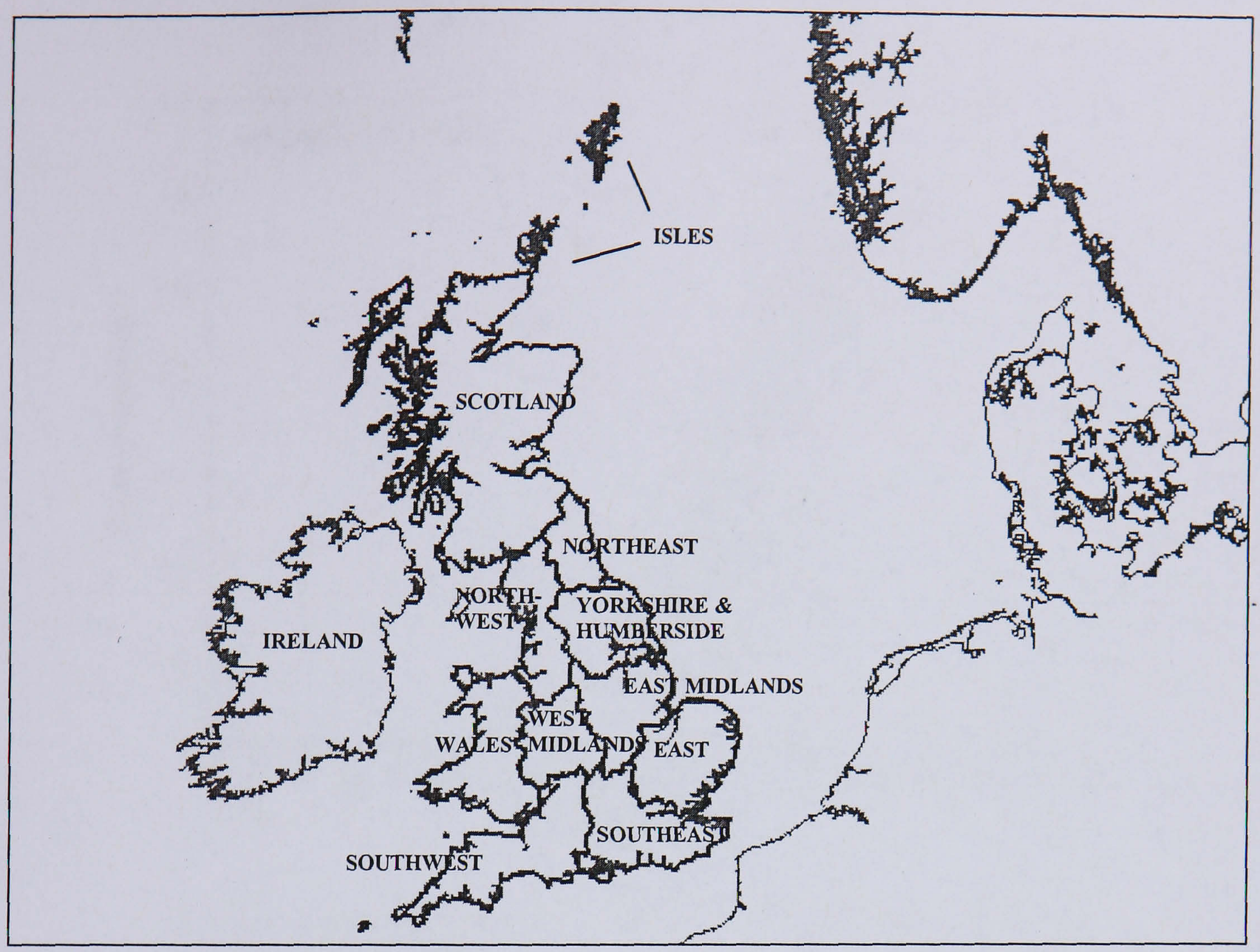


Figure 5.1 BTO ringing data from the first capture of Blackbirds between 1st January 1996 and 31st December 2005 in Britain & Ireland was divided into twelve regions, as illustrated here, in order to allow pairwise comparisons of mean wing length between different regions.

Results

Determination of moult period for Blackbirds

The mean start date of moult for British and Irish Blackbirds between 1996 and 2005 was day 192 for females (11th July) and day 185 for males (4th July). Mean duration of moult was 78 days for females (± 17 days), and 79 days for males (± 14 days) (Figure 5.2).

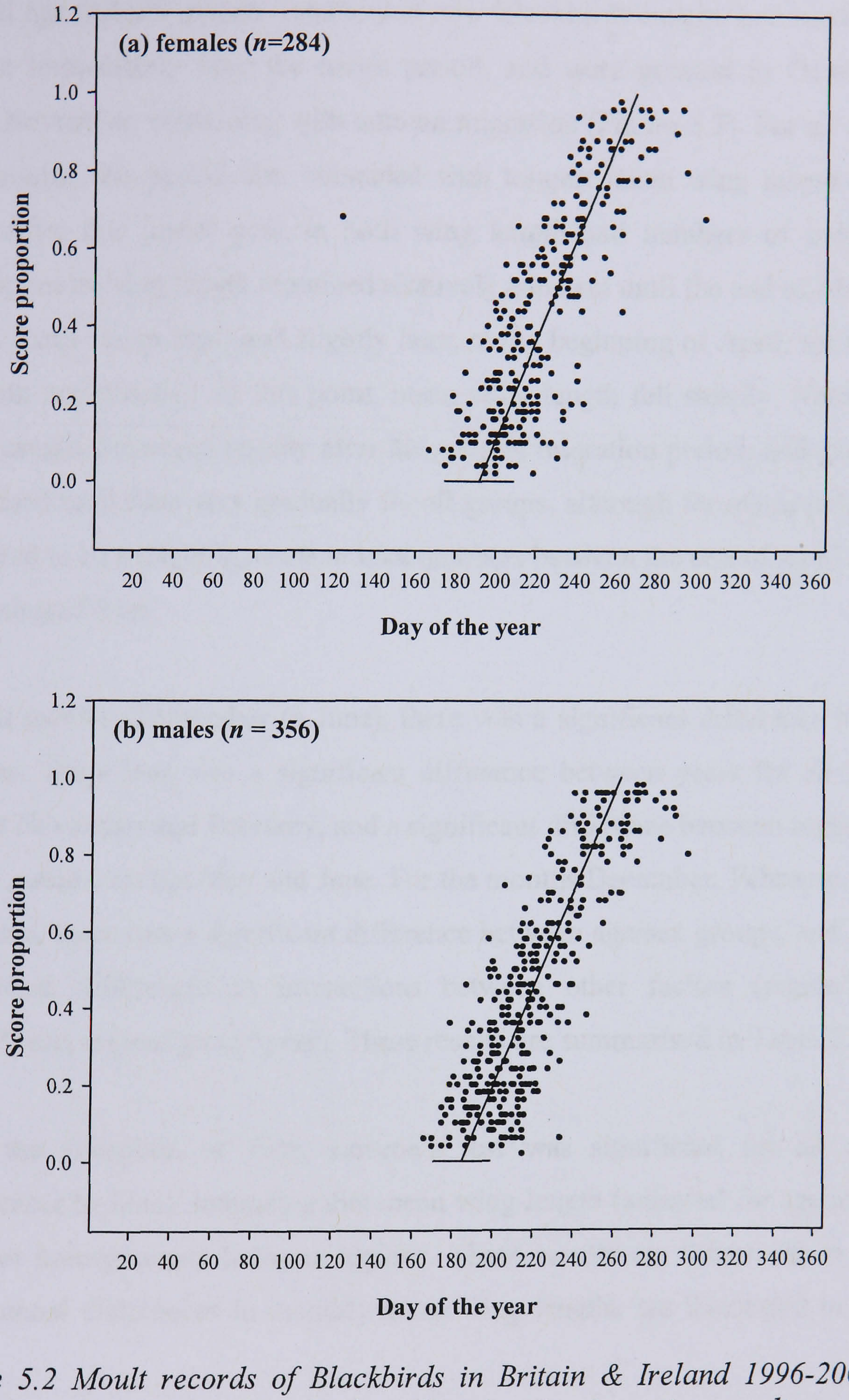


Figure 5.2 Moult records of Blackbirds in Britain & Ireland 1996-2005, (a) females and (b) males. For birds with primaries in active moult, each new primary feather is given a score of 1 to 5, according to its stage of growth. Thus, each individual bird can be given a total primary score of 1 to 49. Moult scores (as a proportion of 50) are plotted against day of the year. The line is fitted by maximum likelihood estimation from the start date (day 192, 11th July, for females; day 185, 4th July, for males) and duration (78 days for females; 79 days for males). The horizontal line at score 0 represents start day ± one standard deviation (17 days for females; 14 days for males).

Blackbird ringing data

For all age and sex groups, numbers of new Blackbirds caught increased rapidly almost immediately after the moult period, and were greatest in October and early November, coinciding with autumn migration (Figure 5.3). For all age and sex groups, this period also coincided with longest mean wing length (Figure 5.4). After this initial peak in both wing length and numbers of individuals caught, mean wing length remained relatively constant until the end of March for males (both age groups) and slightly later, at the beginning of April, for females (of both age groups). At this point, mean wing length fell steeply. Numbers of birds caught decreased rapidly after the autumn migration period, and generally, continued to decline very gradually for all groups, although for males only, there appeared to be a slight increase in total numbers between the end of April and the beginning of June.

For all months (September to June), there was a significant difference between regions. There was also a significant difference between years for all months except November and February, and a significant difference between region*year for all months except May and June. For the months December, February, March and June, there was a significant difference between age/sex groups, and several significant differences in interactions between other factors (region*group; group*year; region*group*year). These results are summarised in Table 5.2.

With the exception of June, Levene's test was significant for all months (September to June), indicating that mean wing length (adjusted for age and sex) was not homogeneous between regions. These results are illustrated in Figure 5.5. Annual differences in monthly mean wing lengths are illustrated in Figure 5.6.

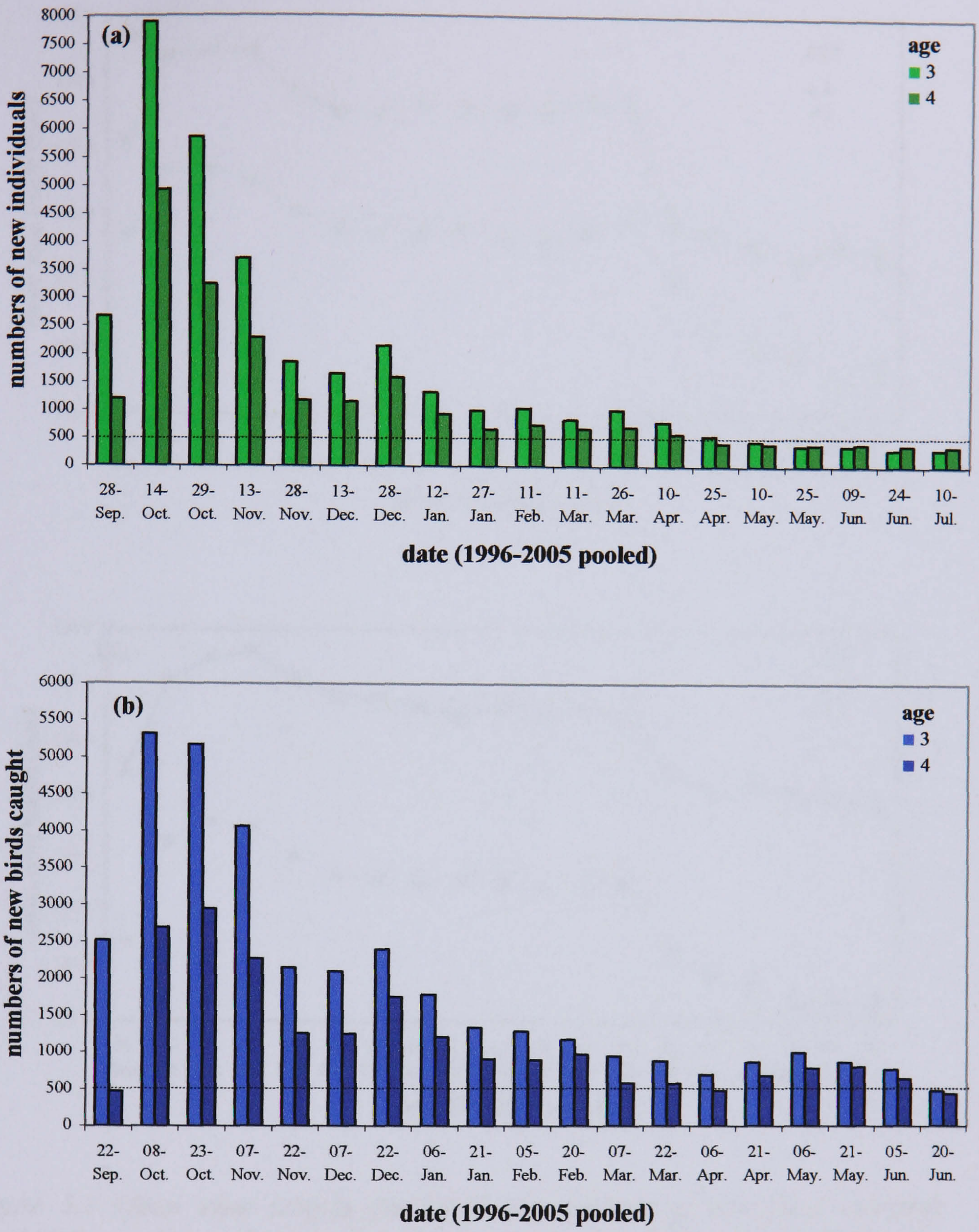


Figure 5.3 Numbers of new (i.e. first capture) Blackbirds caught in Britain & Ireland outside the moult period, during the years 1996-2005 (pooled). (The dashed line is for reference purposes.) Birds are divided into (a) females and (b) males and age group 3: birds with juvenile primary feathers, and age group 4: birds with non-juvenile primary feathers. Note that the dates differ slightly between the two sexes. This is because the moult period occurs slightly earlier in males.

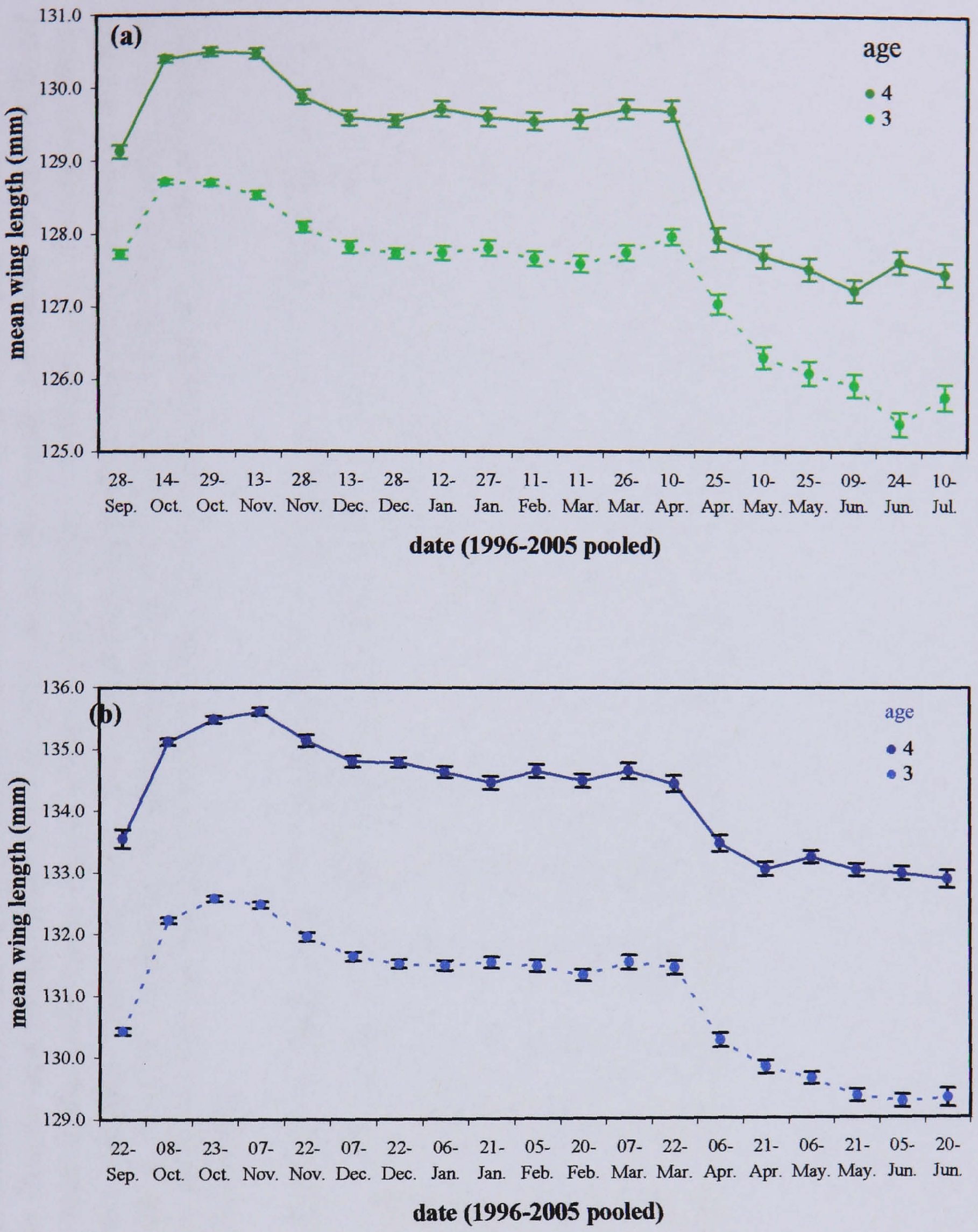


Figure 5.4 Mean wing lengths for 14-15 day periods of new (first capture) Blackbirds caught in Britain & Ireland outside the moult period, during the years 1996-2005 (pooled). Birds are divided into (a) females and (b) males and age group 3: birds with juvenile primary feathers, and age group 4: birds with non-juvenile primary feathers.

Table 5.2 Results of General Linear Model (GLM) Univariate Analyses used to test whether mean wing length (adjusted for age and sex) of first-capture Blackbirds caught in Britain and Ireland from 1996 to 2005 during the months September to June differed between twelve different regions. Factors used in the model were: region, group (defined as four combinations of sex and age: first-year females, first-year males, adult females and adult males) and year. Note that during September, only birds recorded at the end of the month (from 22nd September for males; from 28th September for females) are included, because birds caught earlier in the month may still be moulting their primary feathers. For the months October to June, birds caught during the entire month were analysed. Significant results are depicted in red type.

	region (n = 12)		group (n = 4)		year (n = 10)		region * group			region * year			group* year			region * group * year		
month	F	Sig.	F	Sig.	F	Sig.	df	F	Sig.	df	F	Sig.	df	F	Sig.	df	F	Sig.
SEP	2.752	0.002	0.376	0.771	3.118	0.001	31	1.505	0.037	80	2.193	<0.001	27	1.761	0.009	105	1.397	0.006
OCT	46.312	<0.001	0.979	0.401	4.836	<0.001	33	2.822	<0.001	95	5.608	<0.001	27	1.152	0.267	273	1.487	<0.001
NOV	54.858	<0.001	2.281	0.077	1.686	0.086	33	1.253	0.151	97	5.012	<0.001	27	0.989	0.481	287	1.096	0.129
DEC	14.957	<0.001	4.16	0.006	4.744	<0.001	33	1.274	0.135	97	2.348	<0.001	27	1.369	0.096	285	0.984	0.567
JAN	11.475	<0.001	0.717	0.542	5.217	<0.001	33	1.637	0.012	95	1.766	<0.001	27	0.920	0.583	271	1.033	0.343
FEB	7.84	<0.001	3.661	0.012	1.605	0.108	33	0.772	0.822	97	1.736	<0.001	27	1.186	0.232	269	1.280	0.002
MAR	17.27	<0.001	2.897	0.034	3.424	<0.001	33	1.418	0.057	98	1.832	<0.001	27	1.504	0.045	264	1.079	0.186
APR	13.027	<0.001	1.476	0.219	2.991	0.001	33	0.839	0.728	96	1.913	<0.001	26	0.777	0.781	243	0.951	0.693
MAY	10.144	<0.001	2.553	0.054	2.459	0.009	33	0.91	0.615	98	1.044	0.366	27	1.449	0.062	266	0.973	0.609
JUN	4.769	<0.001	2.948	0.032	2.264	0.016	33	1.542	0.025	95	1.053	0.345	27	1.072	0.364	250	0.895	0.876

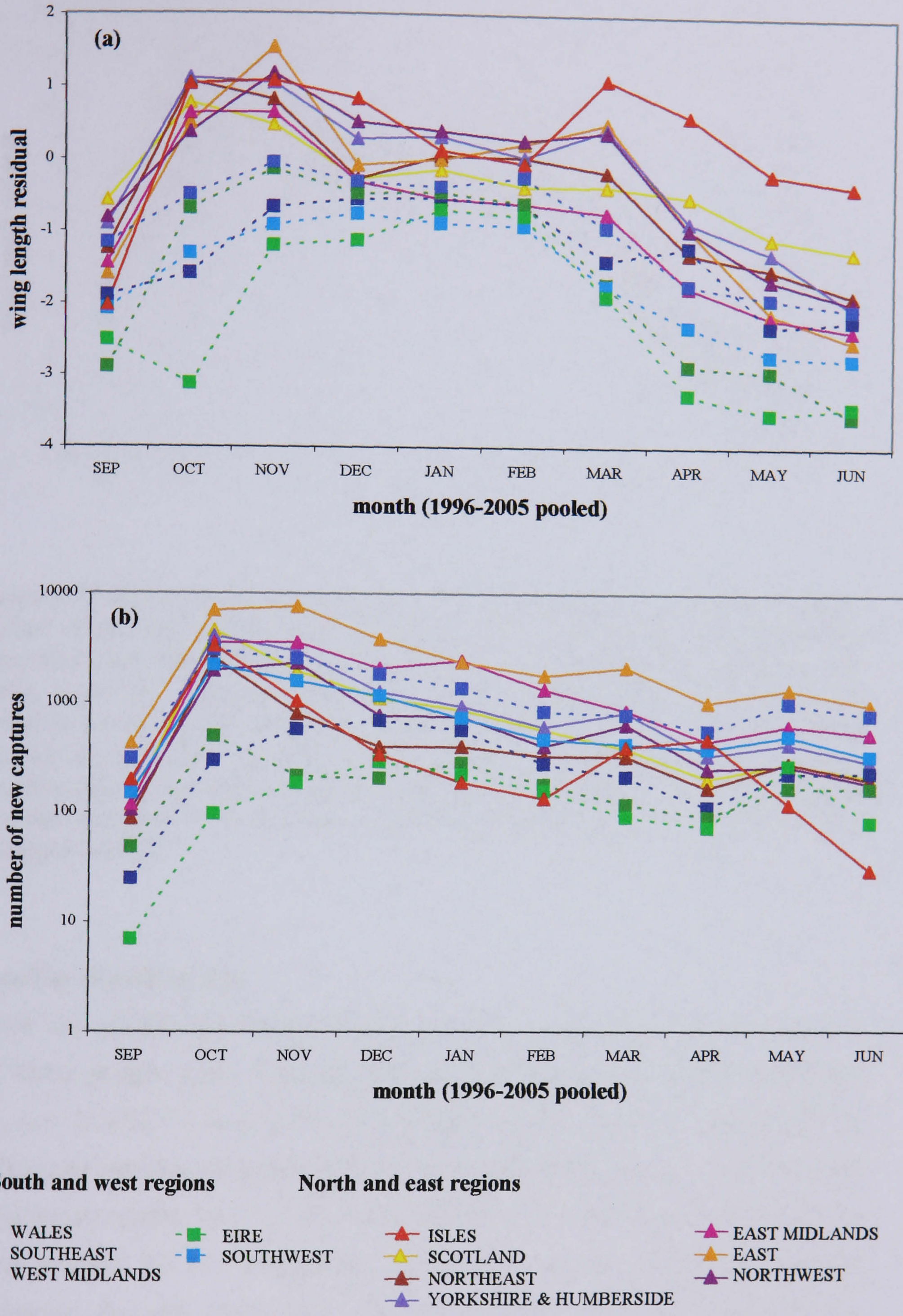


Figure 5.5(a) Monthly mean wing length; (b) monthly numbers, of new (first capture) Blackbirds caught in twelve different regions of Britain & Ireland, during the months September to June, between 1996 and 2005 (pooled). Note that during September, only birds recorded at the end of the month (from 22nd September for males; from 28th September for females) are included, because birds caught earlier in the month may still be moulting their primary feathers. For the months October to June, birds caught during the entire month were analysed. Wing lengths were adjusted for age (classed as either category 3: birds in their juvenile plumage, or 4: birds in their adult plumage) and sex.

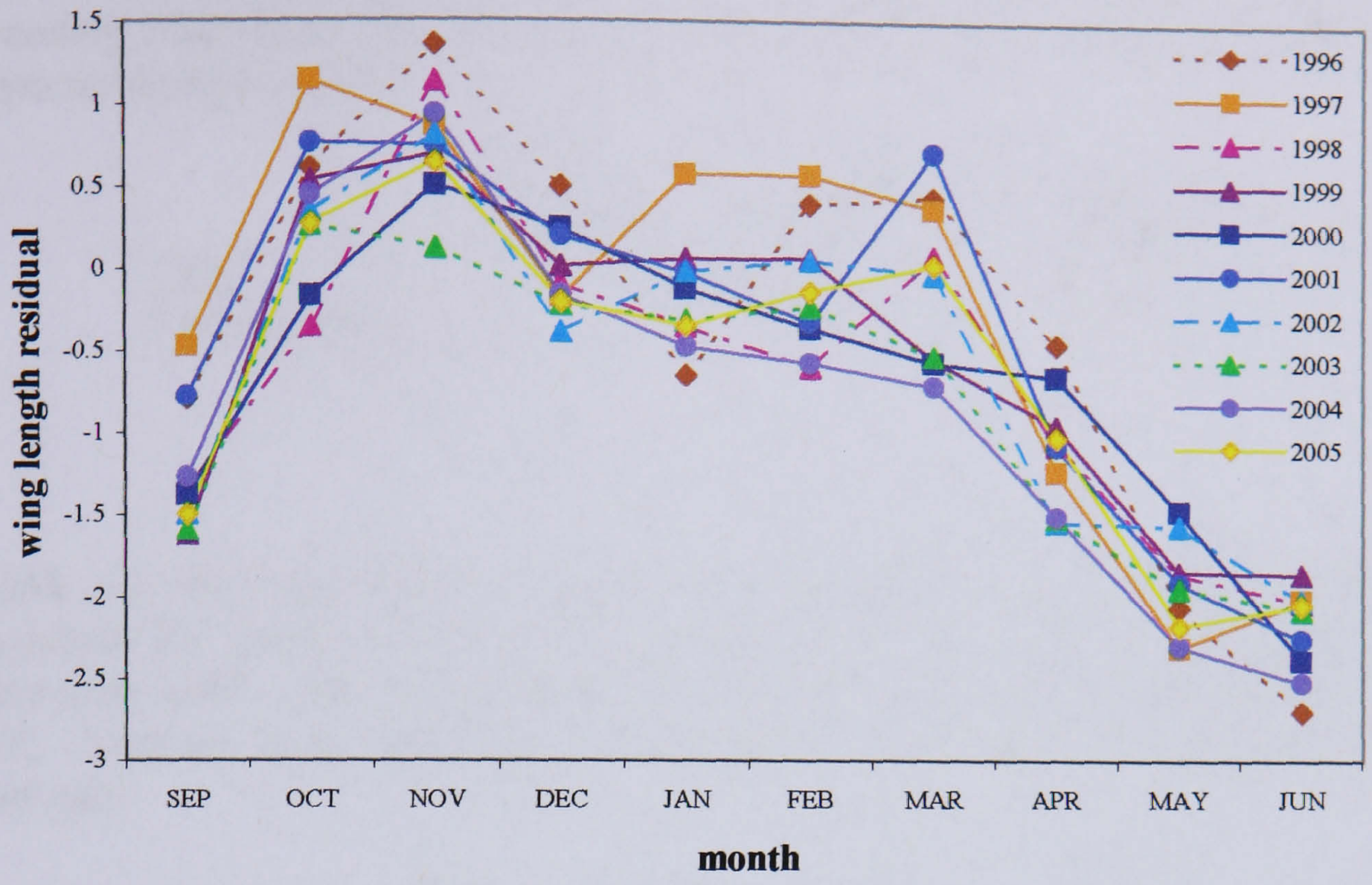


Figure 5.6 Monthly mean wing length of new (first capture) Blackbirds caught in Britain & Ireland, during each of the years 1996 to 2005. Note that during September, only birds recorded at the end of the month (from 22nd September for males; from 28th September for females) are included, because birds caught earlier in the month may still be moulting their primary feathers. For the months October to June, birds caught during the entire month were analysed. Wing length residuals were used in order to control for differences due to age (classed as either category 3: birds in their juvenile plumage, or 4: birds in their adult plumage) and sex.

Breeding Blackbird data

There was a highly significant difference in mean wing length residual between the three groups (UK, Fennoscandia and Continent) of breeding Blackbirds ($F_{2, 154} = 26.012$; $P < 0.001$). Post hoc testing revealed that there were significant differences between all groups (Table 5.3). Fennoscandia had the highest mean wing length residual (4.23), followed by UK (0.65), and Continent had the lowest mean residual (-0.57). Intriguingly, when each age/sex group was analysed separately, the only significant difference in mean wing length residual was between first-year males (although difference in mean wing length residual approached significance in adult males) (Table 5.4). There was also a significant association between wing length residual and latitude ($r_{1,332} = 0.303$; $P < 0.001$).

Table 5.3 Post hoc comparisons between wing length residuals of three groups of breeding Blackbirds: UK, Continent and Fennoscandia. Significant values are depicted in bold red type.

	UK ***n*** **= (58)**	**Fennoscandia** ***n*** **= (26)**	**Continent** ***n*** **= (247)**
UK	-	**<0.001**	**0.041**
Fennoscandia		-	**<0.001**

Table 5.4 One-way ANOVAs used to test for differences in mean wing length residuals for each of four age/sex groups (adult males 4M, adult females 4F, first-year males 3M, and first-year females 3F), between breeding Blackbirds: UK, Continent and Fennoscandia. Significant associations are depicted in bold red type.

	4M	**4F**	**3M**	**3F**
n	82	49	120	82
F	3.048	2.459	**25.090**	2.386
P	0.053	0.097	**<0.001**	0.099

Stable isotope and biometric data

For Blackbirds, combining wing length residuals for all four age/sex groups, there was a significant correlation between wing length and δ^2H_f values ($r_{1, 137} = -0.391$; $P < 0.001$) (Figure 5.7). When each group was analysed separately, there was a significant correlation between wing length and δ^2H_f values for all groups, with the exception of adult females (Table 5.5). There was also a significant correlation between wing length and $\delta^{15}N_f$ values ($r_{1, 139} = -0.254$; $P = 0.002$) (Figure 5.8). When each group was analysed separately, however, the association between wing length and $\delta^{15}N_f$ values was only significant for first-year males (Table 5.6). There was no significant association between wing length residuals and $\delta^{13}C_f$ values ($r_{1, 139} = 0.119$; $P = 0.168$).

For Redwings, combining wing length residuals for all four age/sex groups, there were no significant correlations between wing length and any of the three isotopes, although there was a trend for longer-winged Redwings to have more negative δ^2H_f values (δ^2H_f: $r_{1, 159} = -0.148$; $P < 0.062$; $\delta^{13}C_f$: $r_{1, 160} = 0.084$; $P = 0.287$; $\delta^{15}N_f$: $r_{1, 160} = -0.111$; $P = 0.161$).

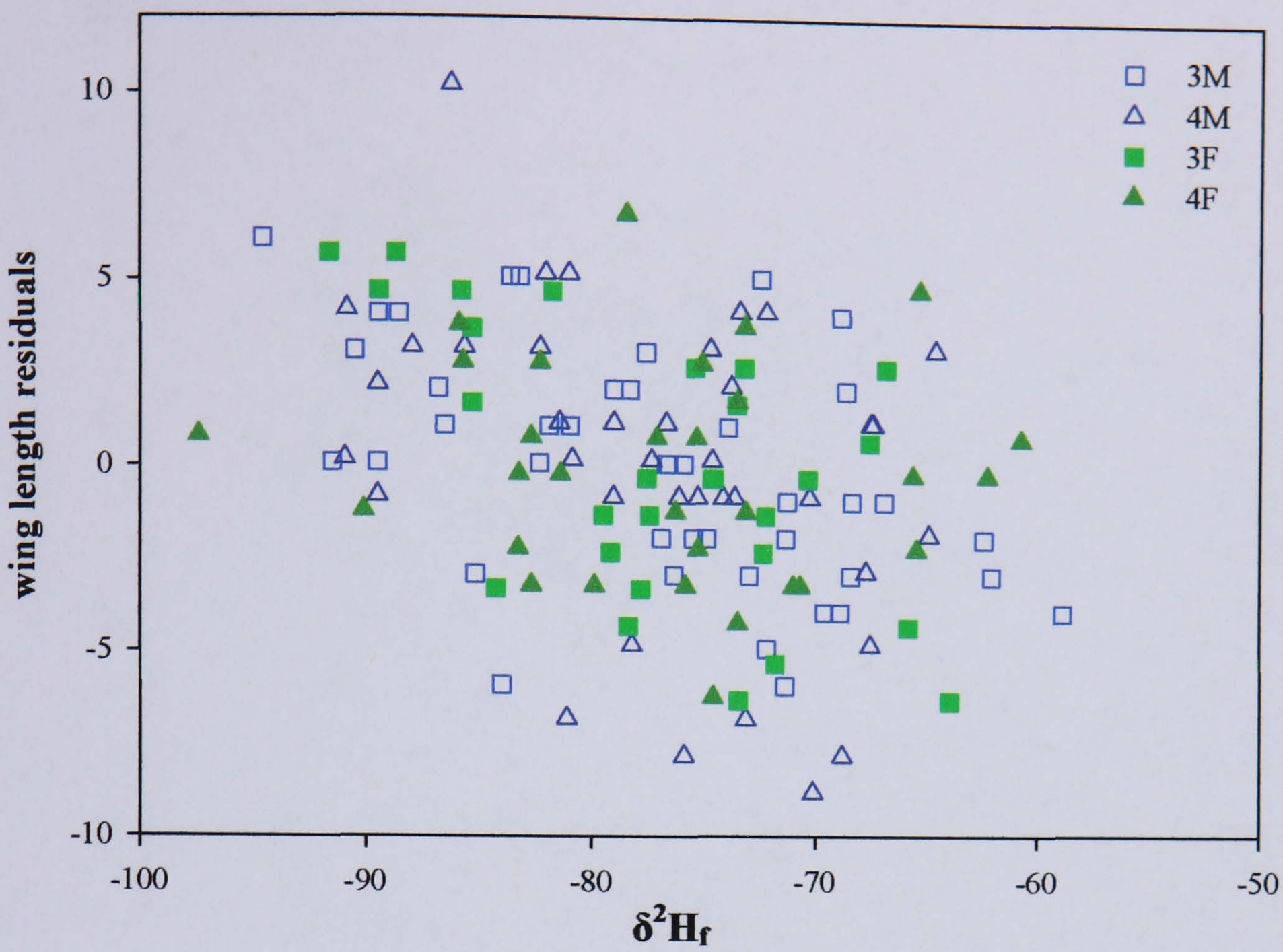


Figure 5.7 Residuals from mean Blackbird wing lengths for adult males (4M n = *38), adult females (4F;* n = *30), first-year males (3M* n = *42) and first-year females (3F* n = *28) plotted against hydrogen stable isotope ratios of feathers (δ^2H_f). Combining wing length residuals for all groups, there was a highly significant correlation between wing length and δ^2H_f ($r_{1,\ 137} = -0.391$; P < 0.001).*

Table 5.5 Correlations between hydrogen stable isotope ratios of Blackbird feathers (δ^2H_f) and mean wing lengths for adult males (4M), adult females (4F), first-year males (3M) and first-year females (3F). Significant associations are depicted in bold red type.

	4M	**4F**	**3M**	**3F**
n	38	30	42	28
r	**-0.366**	-0.084	**-0.501**	**-0.524**
P	**0.024**	0.659	**0.001**	**0.004**

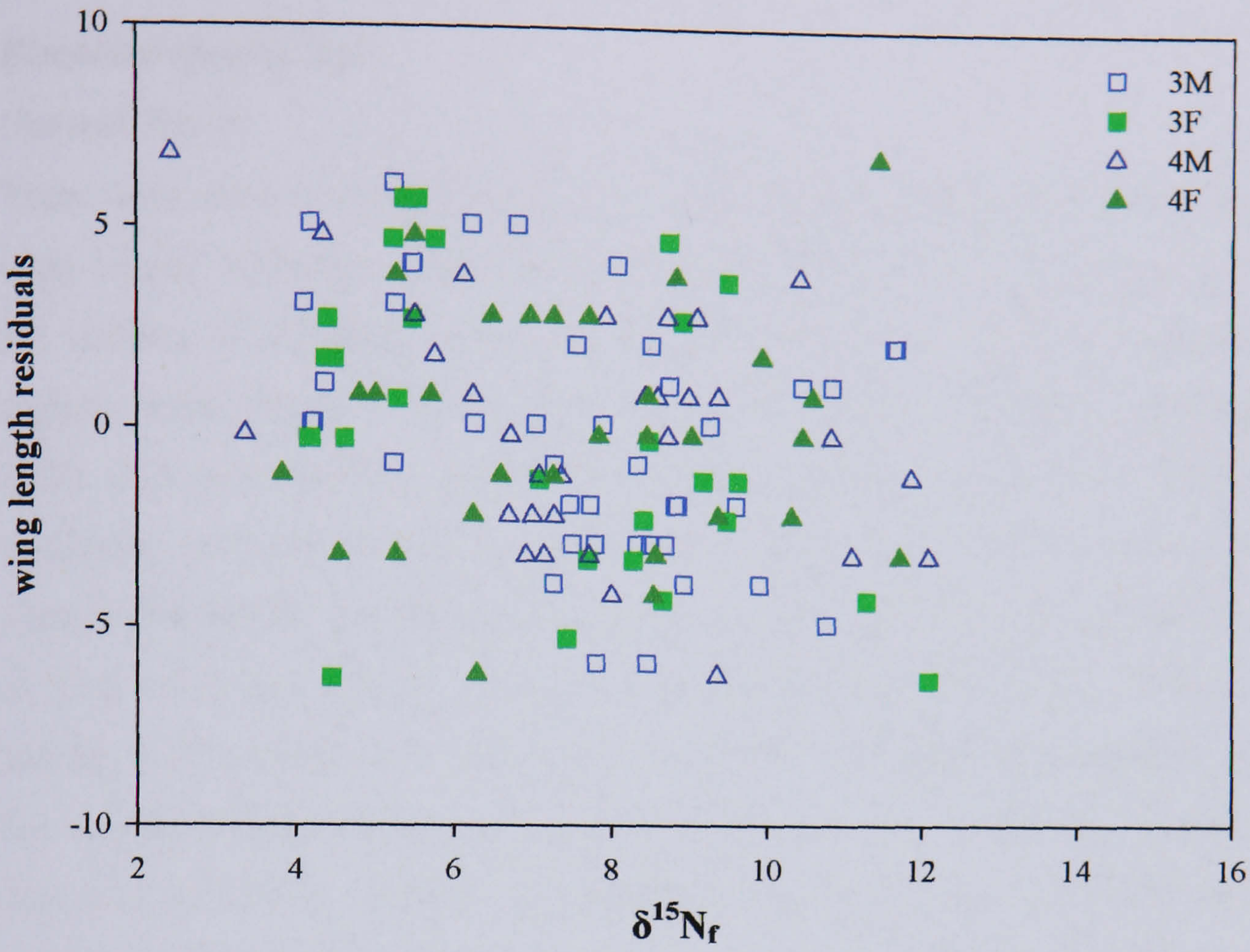


Figure 5.8 Residuals from mean Blackbird wing lengths for adult males (4M n = *39), adult females (4F;* n = *31), first-year males (3M* n = *42) and first-year females (3F* n = *28) plotted against nitrogen stable isotope ratios of feathers ($\delta^{15}N_f$).*

Table 5.6 Correlations between nitrogen stable isotope ratios of Blackbird feathers ($\delta^{15}N_f$) and mean wing lengths for adult males (4M), adult females (4F), first-year males (3M) and first-year females (3F). Significant associations are depicted in bold red type.

	4M	**4F**	**3M**	**3F**
n	39	31	42	28
r	-0.194	0.045	**-0.388**	-0.313
P	0.237	0.811	**0.011**	0.105

Discussion

Blackbird ringing data

General Trends

There were some striking trends both in total numbers of birds caught, and mean wing length. Although these results need to be interpreted with caution, due to the method of sampling, which cannot be standardised between periods and regions (since sample sites are determined by the location of ringers, and ringing effort will vary between individuals), these patterns appear to be relatively consistent between all four age and sex groups. The monthly totals of first-capture Blackbirds, for the years 1996-2005 pooled, illustrates a massive influx of birds of all sex and age groups, but particularly age 3 birds (both males and females) in the months of October and November. Although the increase in age 3 birds could, at least partly, be due to an increase of age 3 birds in the population (i.e. hatch-year birds that have completed their post-juvenile moult), this cannot explain the increase in numbers of adult birds. This suggests that the influx (of both adults and first-year birds) is largely due to autumn migrants arriving in Britain & Ireland. The proportion of migrants relative to residents caught during these months is likely to be higher due to increased movements by the former, and possibly also naivety by migrants, i.e. lack of local knowledge such as experience of mist net positions. The data indicate that many of these birds may be passing through Britain, rather than wintering there, as numbers drop substantially by mid-November.

For first year birds (of both sexes) and adult females, it appears that migration may closely follow completion of post-breeding moult, because, immediately after the defined moult period, numbers of these groups are relatively high, and peak in mid-October. For adult males, however, peak migration appears to occur slightly later, and also over a more extended period. There is some indication that first-year birds of both sexes (but particularly males) may begin autumn migration slightly earlier than adults, because the relative proportions of first-years (versus adults) appear to be highest in late September. Numbers of first-years of both sexes is highest in October.

The changes in monthly totals of Blackbirds (for all age and sex groups) clearly correspond with changes in mean wing length (Figure 5.4). Highest mean wing lengths were recorded in October and November, and then decrease at the end of November, remaining relatively constant until the end of March (males) or the beginning of April (females). At this point, mean wing lengths again decrease, this time more substantially, and remain at their lowest until the beginning of the moult period, starting in early July.

Feather abrasion

Changes in mean wing length may differ seasonally due to different levels of primary feather abrasion. Unfortunately, however, rates of abrasion appear to be reported infrequently in the literature. One study of variation of weight and wing lengths of Blackbirds at a UK site reported a decrease in wing length of first-year males, by approximately 4mm, between September (when feathers are new) and the following June (Prater, 1970), which seems rather high. A study investigating the migratory strategies of Dunlins (*Calidris alpina*) caught in Eilat, Israel, before and after crossing the Sinai, Sahara and Sahel deserts, found that mean wing length differed by only 1.4-1.9mm between birds caught in autumn and spring (which the authors attributed to abrasion of the outer primaries during over-wintering in Africa), although this difference, whilst comparatively small, was significant (Yosef & Meissner, 2006). A more recent study of wing length changes in Blackbirds, reported that, in the majority of birds (particularly adults), feather abrasion did not appear to have an important effect on wing length data until June or July (Leverton, 1989). Even in the majority of first-year Blackbirds (whose primary feathers are older than adults, since these are not moulted during the post-juvenile moult), abrasion did not appear to be a serious factor until the end of March, after which, abrasion proceeded rapidly.

Whilst primary feather abrasion should be considered as a contributing factor to seasonal changes in mean wing length in Blackbirds in the present study, trends in the data strongly indicate that this is not the most important reason for the variation. The largest changes in mean wing length occur in autumn (between the end of September and mid-October) and spring (between the beginning of April to the beginning of May). Although, in adult birds, an increase in mean wing

length might be expected in late summer, due to the appearance of new, post-breeding feathers that have not yet been subjected to abrasion, the peak in wing length occurs later, during the autumn migration period. In addition, age 3 birds do not moult their primary feathers during the post-juvenile moult, so the rise in mean wing length of this group of birds during the autumn cannot be attributed to the growth of new feathers. The fact that changes in mean wing length closely mirror numbers of birds caught also suggests that these changes are due to the movements of longer-winged (i.e. migrant) birds.

Since mainly resident birds should be present in the UK and Ireland during the breeding season, it seems likely that the difference in mean wing length between September and the following June *can* be attributed to feather abrasion. What cannot be predicted is whether the rate of primary feather abrasion is linear with time. It might be expected that abrasion does not occur at a constant rate, but instead varies according to seasonal changes in vegetation density and activity (e.g. relatively high levels of abrasion may occur during the breeding season, when vegetation is dense, and levels of activity are high).

Regional trends

Whilst the general seasonal trends of mean wing length are relatively consistent between the sexes and age classes, there appears to be a great deal of variation at a regional scale. Highly significant differences between regions occurred for every month analysed (September-June) (Table 5.1). In general, there appeared to be a tendency for birds caught in the north and east to have longer mean wing lengths from October to March, than those caught in the south and west (Figure 5.5(a)). Given that migrants from mainland Europe arrive in northern and eastern parts of Britain this is not surprising, at least for the autumn migration period. The data also suggest that those migrants overwintering in Britain tend to remain in more eastern regions (East, Southeast and West Midlands), because numbers of birds caught in these areas remain relatively high throughout the winter, although some appear to be moving as far west as Ireland.

It is also apparent that mean wing lengths of Blackbirds caught in Wales, Ireland and the Southwest region are shorter throughout almost the entire non-moult

period. This may be indicative of regional variation in mean wing length of *resident* (i.e. UK- and Irish-breeding) Blackbirds. However, mean wing length is not consistent during this period, suggesting that these regions are receiving migrants, but that these migrants are also shorter-winged than those arriving in more easterly and northerly regions. It is possible that some British-breeding Blackbirds make short distance movements into the south and west of Britain, and across to Ireland, during the winter. This could possibly be in response to the influx of longer-winged migrants, which may increase competition for resources. Such movements do occur - for example, one Blackbird used in this study was recorded in Thetford, Norfolk in spring, and south Devon in winter (a distance of ~480km), during three consecutive years (2003-2005). However, movements of more than 20km are few in number, although the proportion varies markedly with breeding area, being greatest for the northwest (11%) and lowest in the south (3%) (Chamberlain & Main, 2002).

It is notable that total numbers of birds caught in Wales and Ireland remain lower than other regions (although Ireland has relatively few bird ringers operating, compared with the UK) throughout the non-moult period, suggesting few movements of individuals, but this is not the case for the Southwest region. Ringing recoveries suggest a gradual decline in the numbers of Blackbirds migrating west to Ireland in recent years (Chamberlain & Main, 2002). However, the total numbers of birds caught in Ireland between 1996 and 2005 (n = 1494) is too small to determine whether any significant changes have occurred within this ten-year period.

The twelve regional divisions used in this study are both broad and fairly arbitrary, yet the regional differences in patterns of wing length may be indicative of relatively strong migratory connectivity. Analyses of seasonal wing length at a finer geographical scale, perhaps coupled with recovery data, may help to determine the strength of connectivity, and to further unravel the complex patterns of movement exhibited by Blackbirds.

Comparison of migration patterns in autumn versus spring

There is clearly a huge increase in the numbers of birds caught in Britain during the autumn migration period, yet a similar magnitude of increase is not apparent during the period encompassing spring migration. Nor is there an increase in mean wing length during the spring migration period of a similar gradient to that apparent in the autumn. Assuming that a significant proportion of birds are simply passing through Britain in the autumn, as the data indicate, the different trends exhibited between the two migration periods may be interpreted in several different ways. Firstly, during the autumn, birds may be migrating within a small time window, relative to the spring migration. Thus, huge numbers of birds arrive in Britain within a very short space of time, but their return migration is much more gradual. This may be expected, because during the autumn, climatic conditions are (typically) more severe, so that either periods suitable for migratory movements are constrained, and/or harsher weather initiates a migratory response.

An alternative explanation is that birds may be following different migratory routes between autumn and spring. Ringing recoveries indicate that autumn and spring migration routes differ in a number of species, such as the Wheatear and Willow Warbler (Alerstam, 1996). Another possibility is that birds are experiencing high mortality during the winter, so there are simply less birds returning in the spring. The huge numbers of first-year birds (of both sexes), and to a lesser extent adult females, arriving in the autumn (with no corresponding peak in the spring) might suggest that these groups are most susceptible to winter-induced mortality. Hard winters affect most bird species by reducing food availability, and subordinate individuals, such as juveniles, may be most affected by adverse weather conditions (Newton, 1998).

Of course, these different possibilities are not mutually exclusive, and are probably all operating, at differing extents, to produce the observed trends. Certainly spring migrants are passing through Britain, as indicated by the increase in wing length variation between regions during the spring (Table 5.2). Also, in some regions ('Isles' and, to a lesser extent 'East'), there are notable peaks in mean wing length during the spring (Figure 5.6). However, it is not

possible to ascertain whether there are significant differences in migratory routes between seasons without further studies.

Wing length and latitude

Breeding Blackbird data

The differences in mean wing length between breeding groups, and latitudinal trend in wing length residual, indicate that wing length is a useful indication of migratory status in Blackbirds. However, when age/sex groups were analysed separately, the mean difference in wing length between breeding groups only remained significant for first-year males. This suggests that morphological variation in this species is more complex than previously thought. For example, it is possible that only migratory males and/or first-year birds differ in wing length between regions. Note however that sample sizes for the UK, and especially Fennoscandia, were relatively small; further investigation, via sampling of additional sites within Europe, would be very useful for illuminating the extent of geographical variation in morphology, and determining whether differences are indeed specific to certain age and/or sex categories.

Stable isotope and biometric data

The correlation between δ^2H_f values and residual wing length is indicative of a geographical cline in wing length of Blackbirds, because δ^2H_f values and latitude of breeding samples were correlated (Chapter 4). However, the correlation was weak, and was only significant when data for both species were pooled. This suggests that δ^2H_f does vary with latitude, but only over a broad geographical scale, and that the non-breeding Blackbirds sampled in the UK and Ireland originated from a much larger latitudinal range than that encompassed by the sampled breeding sites.

The results indicate that regional variation in wing length of Blackbirds is related to latitude. In addition, there is some indication that Redwings may also display latitudinal trends in wing length, since the association between δ^2H_f values and wing length residuals of non-breeding Redwings approached significance (δ^2H_f: $r_{1, 159} = -0.148$; $P < 0.062$). Nonetheless, while these results provide evidence that

there is a latitudinal trend in wing length, this does not preclude the possibility of an association between migratory habit and wing length.

Wing length and $\delta^{15}N_f$ values

The trend for longer-winged Blackbirds to have lower $\delta^{15}N_f$ values is puzzling. Although the relationship was only significant in first-year males, it also approached significance in first-year females (for which there was a smaller sample size). The most likely explanation would seem to be that the trend is in some way related to diet. For example, low $\delta^{15}N_f$ values are indicative of high levels of herbivory relative to insectivory (Jason Newton *pers. comm.*) There is evidence that a restricted diet results in a progressive increase in $\delta^{15}N$ in the tissues of birds (Hobson et al., 1993; Cherel et al., 2005), so it is possible that smaller first-year birds (wing length being indicative of overall body size) are more likely to be starving. Alternatively, this finding could simply be a statistical artefact, due to insufficient sample sizes. Certainly, there was no evidence for age-related differences in $\delta^{15}N_f$ values for either breeding or wintering Blackbirds (Chapter 4).

Conclusion

There is good evidence to suggest that Blackbirds exhibit a geographical cline in wing length. This is potentially useful for discriminating between UK- and Irish-breeding Blackbirds, and those from mainland Europe. The trends revealed by the ringing data are intriguing, and reveal apparently complex patterns of movements, that vary on a regional basis within the UK and Ireland. Data from birds sampled in the present study support the evidence for differences in wing length between breeding areas. On the other hand, there is no evidence that Redwings exhibit a similar geographical cline in wing length, although further sampling would be useful, because the association between $\delta^{2}H_f$ and wing length residuals in Redwings approached significance.

CHAPTER 6: INTEGRATING STABLE ISOTOPE RATIOS WITH MICROSATELLITE MARKERS AND MORPHOLOGY, TO ELUCIDATE MIGRATORY MOVEMENTS OF TWO SPECIES OF *TURDUS* THRUSHES OVERWINTERING IN THE UK AND IRELAND

Introduction

The feasibility of applying genetic and stable isotope techniques has been demonstrated in a number of studies attempting to trace migratory movements of birds (e.g. Chamberlain et al., 1997; Haig et al., 1997; Hobson & Wassenaar, 1997; Rubenstein et al., 2002). However, concerns over the scale of resolution achievable are beginning to arise (Kelly et al., 2005). For example, Rocque et al. (2006) analysed δ^2H in two generations of feathers from two Plover species and Northern Wheatears, which breed in North America and winter in South America, the South Pacific and Asia, and Africa. In the Wheatears, no differences in δ^2H_f values were detected between summer- and winter-grown feathers. In addition, they were unable to assign summer-grown Plover feathers to within better than several thousand kilometers of their true origins.

Although population-specific mtDNA markers have been found for some species, such as the Dunlin, it appears that, so far, mtDNA markers most often reveal only broadscale genetic structuring. Lovette et al. (2004) used mtDNA markers in an attempt to determine migratory connectivity in three Neotropical passerine species: the Yellow-breasted Chat (*Icteria virens*), Common Yellowthroat (*Geothlypis trichas*), and Nashville Warbler (*Vermivora ruficapilla*). They were able to assign overwintering individuals to eastern or western breeding lineages, but not to breeding populations of origin. A similar result was also found in another Neotropical passerine species Wilson's Warbler (Kimura et al., 2002). Even when marked differences in migratory behaviour occur between populations, if such traits differentiated relatively recently, they may not be reflected in the genetic structure of populations. (Bensch et al., 1999) found little genetic differentiation between two populations of Willow Warblers, showing different migratory directions (a 'migratory divide'). More recently,

Davis et al. (2006) analysed mtDNA and microsatellite variation in the Black-throated Blue Warbler, a species that shows pronounced differences in migratory behaviour and other traits between populations. Analyses revealed that most genetic variation occurred within rather than between populations, and indicated that the migratory and other differences between populations occurred relatively recently.

A number of studies have pointed out that the use of multiple markers may prove to be a more beneficial approach, allowing migratory movements to be traced at multiple scales, both at the regional and population level (e.g. Webster et al., 2002). Although several studies have utilised more than one isotope ratio, or type of genetic marker, to date, very few have attempted to combine these two types of data, and only two (those of Kelly et al., 2005, and Boulet et al., 2006) have actually tried to predict the breeding origins of individuals using the combined marker approach.

Integrating different types of data

A major challenge when using multiple markers to elucidate patterns of migratory movements is how to integrate information derived from different types of data, such as genetic and stable isotope data (Boulet & Norris, 2006). The first study to report both isotopic and genetic data for a migratory bird species was that of Wennerberg et al. (2002), who used mtDNA control region sequences, $\delta^{13}C_f$ values, and morphological measurements, to examine the population structure of the White-rumped Sandpiper sampled on its breeding grounds in the Canadian Arctic. Wennerberg et al. (2002) found that $\delta^{13}C_f$ values differed significantly between sites. Most of the mtDNA and morphological variation occurred within breeding sites (and there was no association between mtDNA and morphological measurements between breeding sites), although some differences between sites were found. The relationship between $\delta^{13}C_f$ values and morphological measurements (which consisted of five morphological measurements combined into two principal components) was investigated using multiple regression, but no significant association was found.

In 2003, Clegg et al. (2003) used variation in microsatellites and δ^2H_f values to characterise the population structure of Wilson's Warbler, sampled on both breeding and wintering grounds. Surprisingly, despite reporting that genetic markers revealed structure in an east-west orientation, and isotopic ratios revealed structure in a north-south orientation, no attempt was made to integrate these two types of data. More recently, Kelly et al. (2005) used both mtDNA and stable isotope ratios, to examine seasonal movements in Swainson's Thrush *Catharus ustulatus*. An earlier study on this species by Ruegg & Smith (2002) revealed that phylogenetic analysis of the mtDNA control region sequences can separate Swainson's Thrush into genetically distinct coastal and continental populations. Kelly et al. (2005) were able to integrate this information with δ^2H_f and $\delta^{34}S_f$ values. They used a two-step approach to assign individuals to specific breeding populations. Firstly, they divided birds (sampled from 12 breeding populations) into two groups, based on mtDNA data: those with coastal haplotypes, and those with inland haplotypes. They then applied quadratic discriminant function analysis to each separate haplotype group, using δ^2H_f and $\delta^{34}S_f$ values as the independent variables, and sample site as the dependent variable. From the total breeding ground dataset, 66% was randomly selected as training data, in order to construct discriminant functions. The remaining 34% of this dataset (the test data) was then classified, with a successful assignment of 80% for coastal birds, and 76% for inland birds.

A different approach was used by Boulet et al. (2006), who were able to assign wintering Northern Yellow Warblers *Dendroica petechia* (*aestiva* group of subspecies) to their region of breeding origin by overlaying geographic information system maps derived from mtDNA and δ^2H_f data. Boulet et al. (2006) constructed three δ^2H_f isoclines that defined four large isotopic regions. Previous studies by Milot et al. (2000) and Boulet & Gibbs (2006) had already identified three mtDNA lineages in this species: the eastern lineage breeding in eastern and central North America, the western lineage breeding in western and central North America, and the southern lineage breeding in the southwestern United States only. This allowed Boulet et al. (2006) to construct three genetic contours for the probability of observing each of the three mtDNA haplotypes. The genetic contours were overlaid on the isoclines to create lineage-specific

isotopic sub-regions. Individuals captured on the wintering and stopover sites were then assigned to a breeding sub-region according to their mtDNA lineage and feather isotopic value (four separate subregions of North America were defined for the eastern and the western lineages; two subregions of North America were defined for the southern lineage). Boulet et al. (2006) were also able to integrate ringing recapture data into their study, (although few records were available: $n = 57$ between 1921 and 2002). However, this approach required large numbers of samples ($n = 684$ samples from 56 breeding sites; $n = 999$ samples from 85 stopover/wintering sites), which is probably only feasible for large-scale studies.

Likelihood-based assignment methods

Another method designed to overcome the difficulties associated with using a single type of data, such as stable isotopes, which may reveal imprecise linkage between feather isotopic signatures and breeding origin, is likelihood-based assignment (Royle & Rubenstein, 2004). This method takes into account variations in stable isotopes on the breeding grounds, and integrates isotopic information with prior probabilities of assignment related to relative abundance of birds, using Bayes's rule (Boulet et al., 2006; Boulet & Norris, 2006). According to Royle & Rubenstein (2004), geographic variation in abundance is an important consideration in developing assignment rules, regardless of the type of marking technique used. A few studies using this approach have already been published (Royle & Rubenstein, 2004; Wunder et al., 2005; Norris et al., 2006a). However, a major limitation in using abundance information to improve the accuracy of individual assignments of wintering birds is that, for many species, distribution of the breeding population is unknown (Royle & Rubenstein, 2004). Furthermore, this approach is likely to be of little value for species with widespread breeding distributions, such as the Blackbird. In addition, combining genetic data, which is discrete, with continuous data, such as stable isotopes and morphology, remains a significant problem.

Aims

The aim of this part of the study was to combine genetic and stable isotope data, as well as wing length data for Blackbirds, in order to: (i) determine whether

Blackbirds from particular geographic subdivisions of the breeding grounds can be characterised, in terms of their stable isotope 'signature', genotype and morphology; (ii) determine whether Redwings can be classified to subspecies level, using stable isotope ratios and genotypic data; (iii) to use this approach to assign wintering/migrating individuals caught in the UK and Ireland back to their breeding origins in the case of Blackbirds, or race in the case of Redwings (race being indicative of breeding origin, albeit on a broad geographical scale). The following hypotheses were tested:

- Combining genetic and isotopic data, along with morphology (in the case of Blackbirds), is more useful for breeding population discrimination than a single type of data used in isolation.

- Using this approach, it will be possible to assign winter Blackbirds back to their breeding origin, but only on a broad geographical scale.

- Combining genetic and isotopic data, it will be possible to improve the discrimination between the two Redwing subspecies, *iliacus* and *coburni*, already possible using microsatellite markers (see Chapter 3).

Methods

Sampling

Sampling of feathers is described in Chapter 3, and individual analyses (genetics, stable isotopes, and wing length residuals) are also described in preceding chapters (Chapters 3, 4 and 5 respectively). Breeding Blackbirds were assigned to three different geographical breeding groups: UK, Continent and Fennoscandia (Table 6.1(a)); breeding Redwings were assigned to the two races *iliacus* and *coburni* (Table 6.1(b)). Numbers of non-breeding Blackbird and Redwing samples used in this part of the study are provided in Table 6.2.

Table 6.1(a) Sampling location of twelve breeding populations of Blackbird for which stable isotope, genetic and morphological data were used as training data to discriminate between birds grouped into UK, Continent and Fennoscandian breeding birds.

Sample site	**Latitude**	**Longitude**	***n***	**Assignment group**
Skye, UK	57.15N	06.20W	5	UK
Northumberland, UK	54.56N	01.54W	14	
Norfolk, UK	52.24N	00.43E	14	
Annaberg-Bucholz, Germany	50.56N	13.00E	5	Continent
Radolfzell, Germany	47.45N	9.00E	4	
Ozzano, Italy	44.42N	10.08E	3	
Schifflange, Luxembourg	49.30N	06.01E	12	
Kaliningrad, Russia	55.00N	20.34E	12	
Nyborg, Denmark	55.20N	10.48E	10	
Hanko, Finland	59.49N	22.54E	11	Fennoscandia
Eidfjord, Norway	60.25N	07.08E	15	
Titran, Norway	63.40N	08.19E	7	

*Table 6.1(b) Sampling location of eight breeding populations of Redwings for which stable isotope and genetic data were used as training data to discriminate between birds grouped into nominate and Icelandic races (*iliacus *and* coburni *respectively).*

Sample site	**Latitude**	**Longitude**	***n***	**Race**
Selfoss, Iceland	64.07 N	20.29W	19	*coburni*
Troms, Norway	68.45N	19.40E	16	*iliacus*
Rovaniemi, Finland	66.31N	25.45E	5	
Titran, Norway	63.40N	08.19E	14	
Handol, Sweden	63.16N	12.27E	13	
Konnevesi, Finland	62.37N	26.26E	9	
Hanko, Finland	59.49N	22.54E	3	
Eidfjord, Norway	60.25N	07.08E	10	
Espoo, Finland	60.11N	24.48E	2	

Table 6.2(a) Sampling locations of Blackbirds wintering in the UK and Ireland, from which stable isotope, genetic and morphological data were collected for assignment purposes.

Region	**Sample sites**	***n***	**Latitude**	**Longitude**
Scotland	Tain, Ross & Cromarty, UK	7	57.48N	04.03W
	Monifieth, Angus, UK	12	56.29N	13.00W
Northeast	Low Newton, Northumberland, UK	11	55.30N	01.37W
	Mickley Square, Northumberland, UK	8	55.20N	02.41W
Ireland	Tuam, Galway, Ireland	12	54.57N	01.53W
North West	Preston, Lancashire, UK	14	53.30N	08.51W
Midlands	Rampton, Nottinghamshire, UK	7	53.17N	00.47W
South	Thetford, Norfolk, UK	5	52.24N	00.43E
	Colyford, Devon, UK	1	50.43N	03.03W
	Arreton, Isle of Wight, UK	6	50.40N	01.14W
	Cornwall*, UK	5	50.21N	04.56W

*Note that birds from Cornwall were sampled from three sites, all within close proximity, therefore longitude and latitude provided in the table are means of the three sites.

Table 6.2(b) Numbers of non-breeding Redwing samples collected in the UK during four different periods, from which stable isotope and genetic data were collected for assignment purposes.

Sample site	**Latitude**	**Longitude**	***n***	**Collection date**
Bardsey, North Wales, UK	52.45N	04.46W	76	Oct 1995
			26	Mar 1998
			5	Oct 1998
			27	Oct 1999

Statistical analyses

Multinomial logistic regression analyses were used to combine the different types of data and predict source regions (Blackbirds), or race (Redwings) of wintering birds. Logistic regression can be used in a similar way to discriminant function analysis, and according to Dytham (2003), is a very powerful technique. Logistic regression is suitable when variables do not follow a Gaussian distribution, as is the case in this study.

Breeding Blackbirds

All variables listed in Table 6.3 were entered into a multinomial logistic regression main-effects model. Results of the likelihood ratio tests and the Wald statistic (the square of the ratio of each variable to its standard deviation) provided an indication of which variables had the greatest discriminatory power. Those that had less effect were removed sequentially, and the effect tested, until the most optimum combinations were identified. Two reduced models were thereby created: (a) excluding $\delta^{15}N_f$ values; (b) excluding, all genetic variables and $\delta^{15}N_f$ values (i.e. including only $\delta^{13}C_f$, $\delta^{2}H_f$ and wing length residuals), and their effectiveness was compared with the complete model.

Only breeding Blackbirds with two or more of the three different types of data (isotopes, genetics and morphology) were included in the analyses. Where isotope data were missing, these were replaced with the mean value for the original breeding population; missing wing length residuals were replaced with the mean for the group of membership (UK, Continent or Fennoscandia); where sex and age was known, the mean for this subgroup (specific to the relevant geographic region, i.e. UK, Continent or Fennoscandia) was used, to improve accuracy. Individuals with missing genetic variables were excluded from the analysis.

Model performance

In order to test the prediction performance of each model, breeding data was subsequently partitioned into 'training' and 'test' sets. Approximately 20% of the data (23 individuals) were randomly selected as 'test data'. Selection was weighted to represent proportions of individuals from the three breeding groups. Firstly, this allowed accuracy of the model when including the complete dataset (i.e. *all* breeding individuals) to be compared with performance when a smaller sample size was used (i.e. the training dataset, consisting of 80% of breeding individuals). Secondly, accuracy of the model in assigning *test* data (calibrated to the training data) to breeding groups could then be compared to performance when assigning training data to populations. This entire operation was repeated five times for each model (i.e. different, randomly selected data were used for training and testing purposes) (Manel et al., 1999b).

An additional measure of reliability may be to consider the overall proportional representation of each of breeding region, as predicted by a particular model. This is because, while it may not be possible to assign an individual to its most likely region of breeding origin with a high degree of accuracy, it may be feasible to determine the relative proportions of birds from different regions represented in a given group of samples. Therefore, the overall proportional representation of each of the three groups as predicted by the multinomial linear regression models, was compared with proportions predicted by discriminant analysis (based solely on the stable isotope ratios of $\delta^{13}C_f$ and $\delta^{2}H_f$) as reported in Chapter 4.

Winter Blackbirds

The model judged to provide the most consistently accurate assignments was used to predict region of breeding origin (UK, Continent or Fennoscandia) of Blackbirds sampled in the UK and Ireland during the winter (n = 87), with the complete breeding dataset used for calibration (i.e. to train the model).

Table 6.3 Variables used in multinomial logistic regression analysis to assign breeding Blackbirds to one of three groups: UK, Continent and Fennoscandia.

Genetic data (refer to Chapter 3 for methodology)	**Stable isotopes** (refer to Chapter 4 for methodology)	**Morphology** (refer to Chapter 5 for methodology)
-log likelihood of belonging to UK and non-UK groups -log likelihood of belonging to UK, Continent and Fennoscandian groups likelihood probabilities of belonging to UK and non-UK groups likelihood probabilities of belonging to UK, Continent and Fennoscandian groups 4 Factorial Correspondence Analysis (FCA) co-ordinates	$\delta^{13}C_f$ $\delta^{15}N_f$ $\delta^{2}H_f$	Wing length residuals (calculated separately for first-year males, first-year females, adult males and adult females, then pooled, in order to maximise sample size)

Redwings

Breeding Redwings were divided by race (*iliacus* and *coburni*). All variables listed in Table 6.4 were entered into a multinomial logistic regression main-effects model. Variables that had the lowest discriminatory power were removed sequentially, and the effect tested, until the most optimum combination was identified. A reduced model was thereby created, excluding δ^2H_f and the -log likelihood values. This latter model was used as calibration data to assign Redwings sampled in the UK outside the breeding season (n = 134) to their most likely race.

Model performance

As with Blackbirds, the prediction performance of the reduced model was tested via random partitioning of the breeding data into 'training' (~80%) and 'test' (~20%) datasets. Partitioning was weighted to represent proportions of individuals of the two races. Assignment of each dataset was then tested. The entire operation was repeated five times (Manel et al., 1999b).

Table 6.4 Variables used in multinomial logistic regression analysis to discriminate between two subspecies of Redwings: iliacus *(nominate race) and* coburni *(Icelandic race).*

Genetic data (refer to Chapter 3 for methodology)	**Stable isotopes** (refer to Chapter 4 for methodology)
-log likelihood of belonging to *coburni* and *iliacus* likelihood probabilities of belonging to *coburni* and *iliacus* 4 Factorial Correspondence Analysis (FCA) co-ordinates	$\delta^{13}C_f$ $\delta^{15}N_f$ δ^2H_f

Results

Breeding Blackbirds

Comparative model performance

The model-fitting criterion for the complete model (i.e. including all variables) was significant (-2 log likelihood = 78.024; χ^2 = 169. 950; df = 36; $P < 0.001$). Performance of individual variables (assessed using likelihood ratio tests and the Wald statistic) is summarised in Table 6.5. Model fitting criteria for both reduced models were also significant (model (a): -2 log likelihood = 81.920; χ^2 = 166.054; df = 34; $P < 0.001$; model (b): -2 log likelihood = 156.891; χ^2 = 91.084; df = 6; $P < 0.001$).

Prediction performance (% of accurate assignments) of the three models, while of a similar range, was highest for reduced model (a) (i.e. including all variables except $\delta^{15}N_f$ values), with an overall prediction performance of 84.2%. The complete model performed equally well for all groups except Fennoscandia, producing an overall prediction performance of 81.6%. Prediction performance of reduced model (b) (i.e. excluding $\delta^{15}N_f$ values and all genetic variables) was almost always poorer than the other two models for all three breeding groups, with an overall accuracy of 76.3%. Results are summarised in Table 6.6.

Consistency of model performance

For both the complete model and reduced model (a), the training dataset, although reduced to ~80% of the original data, performed with a higher level of accuracy than the complete dataset (93.2% and 92.7% respectively). Once again, reduced model (b) produced the lowest level of accurate predictions, although performance was only marginally lower than the complete dataset (73.4% for the training dataset versus 76.3% for the complete dataset; Table 6.7(a)). The improved accuracy of the training dataset for the first two models (the complete model and reduced model (a)) may be a statistical consequence of 'sorting' the data by a large number of variables, because the fewer the data, the easier it is to fit the model.

The most striking result was the dramatic decrease in prediction performance of both the complete model and reduced model (a) when assigning test datasets; total accuracy was just 56.5% and 55.6% respectively. In contrast, reduced model (b) was the most consistent model, resulting in the highest overall percentage of correct assignments at 72.2% overall (only marginally lower than the complete and training datasets), and lowest overall standard deviation (± 3.9; Table 6.7(b)). Thus, although the complete model and reduced model (a) appeared to produce a far greater predictive performance when the datasets were relatively large, this appears to be at the expense of consistency. For this reason, the variables used in reduced model (b) were selected for assignment of the winter Blackbirds to their most probable breeding origin, calibrated against all breeding Blackbirds (n = 114).

Comparative misassignments between breeding groups

The overall proportional representations of each of the three groups as predicted by the multinomial linear regression models, were all very similar (the reduced models were perhaps marginally better overall; Table 6.8). This indicates that misassignments appear to be consistent between groups (e.g. a wrongly classified UK-breeding bird is equally likely to be assigned to the Continent group as it is to the Fennoscandia group). However, when compared with proportions predicted by discriminant analysis based on $\delta^{13}C_f$ and $\delta^{2}H_f$ values (reported in Chapter 4), the linear regression models clearly perform better. Although the discriminant analysis accurately assigned a high percentage of UK-breeding birds (90.9%, Chapter 4), it also wrongly assigned a relatively large number of non-UK breeding birds to the UK breeding group. Thus, although a model may appear to perform well (in terms of predictive performance), consideration of relative proportions of misassignments between different groups is also important.

Table 6.5 Performance of individual variables used in a multinomial regression analysis on Blackbirds (n = 114) assessed using likelihood ratio tests and the Wald statistic (the square of the ratio of each variable to its standard deviation). Individuals were assigned to three breeding groups: UK (group 1), Continent (group 2), and Fennoscandia (group 3), with group 1 (UK) set as the reference category against which the remaining two groups are compared. Significant values are depicted in bold red type.

	Likelihood ratio tests				**Parameter estimates for group 2**			**Parameter estimates for group 3**		
Variable	**-2 Log Likelihood**	**Chi-Square**	**df**	***P***	**Wald Statistic**	**df**	***P***	**Wald Statistic**	**df**	***P***
$\delta^{13}C_f$	**145.844**	67.820	2	**<0.001**	**14.743**	1	**<0.001**	**14.992**	1	**<0.001**
$\delta^{15}N_f$	81.920	3.896	2	0.143	2.387	1	0.122	0.058	1	0.810
$\delta^{2}H_f$	**98.766**	20.742	2	**<0.001**	**9.598**	1	**0.002**	0.036	1	0.850
wing length residual	**117.560**	39.536	2	**<0.001**	0.942	1	0.332	**4.913**	1	**0.027**
-log likelihood of belonging to 'UK' (versus 'non-UK') group	**86.141**	8.117	2	**0.017**	0.839	1	0.360	0.648	1	0.421
-log likelihood of belonging to 'non-UK' (versus 'UK') group	**87.704**	9.680	2	**0.008**	1.499	1	0.221	**4.612**	1	**0.032**
-log likelihood of belonging to 'UK' (versus 'Continent' or Fennoscandia') group	79.403	1.379	2	0.502	0.928	1	0.336	0.102	1	0.749
-log likelihood of belonging to 'Continent' (versus 'UK' or 'Fennoscandia') group	81.834	3.810	2	0.149	1.863	1	0.172	2.556	1	0.110
-log likelihood of belonging to 'Fennoscandia' (versus 'UK' or 'Continent') group	**91.060**	13.036	2	**0.001**	1.277	1	0.258	**6.184**	1	**0.013**
likelihood probability of belonging to 'UK' (versus 'non-UK') group	79.254	1.230	2	0.541	0.473	1	0.492	0.405	1	0.525
likelihood probability of belonging to 'non-UK' (versus 'UK') group	78.839	0.815	2	0.665	0.002	1	0.960	0.619	1	0.431
likelihood probability of belonging to 'UK' (versus 'Continent' or Fennoscandia') group	**85.166**	7.142	2	**0.028**	1.992	1	0.158	1.717	1	0.190
likelihood probability of belonging to 'Continent' (versus 'UK' or 'Fennoscandia') group	81.844	3.820	2	0.148	0.738	1	0.390	1.331	1	0.249
likelihood probability of belonging to 'Fennoscandia' (versus 'UK' or 'Continent') group	79.067	1.043	2	0.594	0.592	1	0.442	1.126	1	0.289
FCA1	80.530	2.506	2	0.286	2.187	1	0.139	0.710	1	0.400
FCA2	78.227	0.203	2	0.903	0.170	1	0.680	0.119	1	0.730
FCA3	**86.657**	8.633	2	**0.013**	0.113	1	0.737	**3.291**	1	**0.070**
FCA4	**86.486**	8.463	2	**0.015**	0.004	1	0.949	**4.121**	1	**0.042**

Table 6.6 Prediction performance (% of accurate assignments) of multinomial regression analysis used to separate three breeding groups of Blackbirds: UK, Continent and Fennoscandia, using (1) a complete model, in which all available variables (listed in Table 6.3) were included; (2a) a reduced model, in which the variable $\delta^{15}N$ was excluded; (2a) a reduced model, in which the variables $\delta^{15}N$, Factorial Correspondence Analysis (FCA) coordinates and likelihood probabilities were excluded. Results presented are for the complete dataset of breeding birds (n = 114).

Model	**UK** ($n = 33$)	**Continent** ($n = 36$)	**Fennoscandia** ($n = 45$)	**Total** ($n = 114$)
(1) Complete: including all variables	78.8	76.1	91.4	81.6
(2a) Reduced (a): excluding $\delta^{15}N$	78.8	82.6	91.4	84.2
(2b) Reduced (b): excluding $\delta^{15}N$, & all genetic variables	75.8	76.1	77.1	76.3

Table 6.7 Prediction performance (% of accurate assignments) of multinomial regression analysis used to separate three breeding groups of Blackbirds: UK, Continent, and Fennoscandia. In order to test the consistency of results, the dataset was randomly partitioned into (a) training data (approximately 80% of individuals); (b) test data (approximately 20% of individuals), with weighted representation of the three breeding groups. This procedure was repeated five times, and the means and standard deviations are presented here. Three models were tested: (1) a complete model, in which all available variables (listed in Table 6.3) were included; (2a) a reduced model, in which the variable $\delta^{15}N$ was excluded; (2a) a reduced model, in which the variables $\delta^{15}N$, and likelihood probabilities were excluded.

(a) training data

Model	**UK** ($n = 26$)		**Continent** ($n = 37$)		**Fennoscandia** ($n = 28$)		**Total** ($n = 91$)	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
(1) Complete: including all variables	89.2	7.4	91.9	5.1	98.6	3.2	93.2	4.2
(2a) Reduced (a): excluding $\delta^{15}N$	86.2	8.0	93.0	5.3	98.6	3.2	92.7	4.8
(2b) Reduced (b): excluding $\delta^{15}N$, & all genetic variables	74.6	4.4	71.38	3.1	75.0	2.6	73.4	1.4

(b) test data

Model	**UK** ($n = 7$)		**Continent** ($n = 9$)		**Fennoscandia** ($n = 7$)		**Total** ($n = 23$)	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
(1) Complete: including all variables	51.4	19.2	48.9	12.7	66.3	12.4	56.5	9.2
(2a) Reduced (a): excluding $\delta^{15}N$	45.7	21.2	51.1	16.9	71.4	14.3	55.6	9.4
(2b) Reduced (b): excluding $\delta^{15}N$, & all genetic variables	80.0	7.8	62.2	12.7	77.1	7.8	72.2	3.9

Table 6.8 Overall proportional representation of three Blackbird breeding groups (UK, Continent, and Fennoscandia) as predicted by three multinomial linear regression models: (1) a complete model, in which all available variables (listed in Table 6.3) were included; (2a) a reduced model, in which the variable $\delta^{15}N$ was excluded; (2a) a reduced model, in which the variables $\delta^{15}N$, Factorial Correspondence Analysis (FCA) coordinates and likelihood probabilities were excluded, compared with proportions predicted by discriminant analysis based on feather $\delta^{13}C$ and $\delta^{2}H$ values (reported in Chapter 4).

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		UK	**Continent**	**Fennoscandia**
	Actual proportions	**29.0% (*n* = 33)**	**40.4% (*n* = 46)**	**30.7% (*n* = 35)**
Predicted proportions	Complete model	30.7% (*n* = 35)	38.6% (*n* = 44)	30.7% (*n* = 35)
	Reduced model (a)	28.1% (*n* = 32)	41.2% (*n* = 47)	30.7% (*n* = 35)
	Reduced model (b)	28.9% (*n* = 33)	41.2% (*n* = 47)	29.8% (*n* = 34)
	Discriminant analysis	38.6% (*n* = 44)	26.3% (*n* = 30)	35.1% (*n* = 40)

Winter Blackbirds

Based on reduced multinomial linear regression model (b) (derived from the variables $\delta^{13}C_f$, $\delta^{2}H_f$, and wing length residuals), 29.9% of winter Blackbirds (26/87 individuals) were assigned to the UK breeding population; 44.8% (39 individuals) were assigned to Fennoscandia, and the remaining 25.3% (22 individuals) to the Continent (Table 6.9).

*Table 6.9 Classification matrix for Blackbirds wintering in different regions of the UK and Ireland (*n *= 87), derived from multinomial logistic regressions (MLR) based on the stable isotope ratios of $\delta^{13}C$ and $\delta^{2}H$ in body feathers, combined with wing length.*

Region	**UK**	**Continent**	**Fennoscandia**
Scotland (*n* = 18)	16.7 (*n* = 3)	38.9 (*n* = 7)	44.4 (*n* = 8)
Northeast (*n* = 19)	21.1 (*n* = 4)	31.6 (*n* = 6)	47.4 (*n* = 9)
Ireland (*n* = 12)	66.7 (*n* = 8)	8.3 (*n* = 1)	25.0 (*n* = 3)
Northwest (*n* = 14)	57.1 (*n* = 8)	14.3 (*n* = 2)	28.6 (*n* = 4)
Midlands (*n* = 7)	0	42.9 (*n* = 3)	57.1 (*n* = 4)
South (*n* = 17)	17.6 (*n* = 3)	17.6 (*n* = 3)	64.7 (*n* = 11)
Totals	**29.9% (*n* = 26)**	**25.3 (*n* = 22)**	**(44.8 (*n* = 39)**

Breeding Redwings

Model performance

The model-fitting criterion for the complete model (i.e. including all variables) was significant (-2 log likelihood = 21.722; χ^2 = 71.526; df = 11; $P < 0.001$). Performance of individual variables (assessed using likelihood ratio tests and the Wald statistic) are summarised in Table 6.10. Model fitting criteria for the reduced model was also significant (-2 log likelihood = 24.687; χ^2 = 68.561; df = 24; $P < 0.001$). Overall prediction performance (% of accurate assignments) of the complete model was 93.4%; that of the reduced model was a slightly higher 95.6% (Table 6.11). Reducing the model further (i.e. excluding additional variables) resulted in a substantial decrease in prediction performance.

Consistency of model performance

Accurate assignment of birds using the training dataset (~80% of the original data), was slightly lower than when using the complete dataset (89.3% versus 95.6%). This was largely due to misassignments of Icelandic birds (*coburni*) to the nominate race; in each of the five trials, 2-3 Icelandic birds were misassigned. Similarly, when using the test dataset, the percentage of correctly assigned birds was consistently high, particularly for birds of the nominate race. It appears that the performance of the model is both accurate and consistent. Icelandic birds were more likely to be misassigned to the nominate race, than vice versa.

*Table 6.10 Performance of individual variables used in a multinomial regression analysis on Redwings (*n *= 91) assessed using likelihood ratio tests and the Wald statistic. Individuals were assigned to two races:* iliacus *(group 1) and* coburni *(group 2). Significant values are depicted in bold red type.*

Likelihood Ratio Tests					**Parameter Estimates**		
Variable	**-2 Log Likelihood**	**Chi-Square**	**df**	***P***	**Wald**	**df**	***P***
Intercept	**29.993**	**8.271**	1	**0.004**	3.194	1	0.074
d13C	**31.668**	**9.947**	1	**0.002**	3.256	1	0.071
d15N	**25.927**	**4.205**	1	**0.040**	3.204	1	0.073
d2H	24.107	2.385	1	0.123	1.717	1	0.190
- log likelihood of belonging to *iliacus*	22.372	0.65	1	0.420	0.627	1	0.428
- log likelihood of belonging to *coburni*	21.727	0.005	1	0.943	0.005	1	0.943
likelihood probability of belonging to *iliacus*	22.192	0.47	1	0.493	0.447	1	0.504
likelihood probability of belonging to *coburni*	**34.078**	**12.356**	1	**<0.001**	**4.491**	1	**0.034**
FCA1	22.153	0.432	1	0.511	0.376	1	0.540
FCA2	22.154	0.432	1	0.511	0.411	1	0.521
FCA3	25.337	3.615	1	0.057	2.564	1	0.109
FCA4	21.936	0.214	1	0.644	0.205	1	0.651

Table 6.11 Classification matrices for two races of Redwings: iliacus *and* coburni, *based on multinomial regression analysis, using two different models: (1) a complete model, which included in Table 6.4; (2) a reduced model, in which the variables* $\delta^2 H_f$ *and both –log likelihood probabilities were excluded.*

(a) Complete model

	iliacus	***coburni***
iliacus	95.8% (*n* = 69)	4.2% (*n* = 3)
coburni	15.8% (*n* = 3)	84.2% (*n* = 16)
Overall accuracy: 93.4%		

(b) Reduced model

	iliacus	***coburni***
iliacus	98.6% (*n* = 71)	1.4% (*n* = 1)
coburni	15.8% (*n* = 3)	84.2% (*n* = 16)
Overall accuracy: 95.6%		

Table 6.12 Prediction performance (% of accurate assignments) of a multinomial regression model used to separate two subspecies of Redwings: iliacus *and* coburni. *In order to test the consistency of results, the dataset was randomly partitioned into (a) training data (approximately 80% of individuals); (b) test data (approximately 20% of individuals), with weighted representation of the two races. This procedure was repeated five times, and the means and standard deviations are presented here. Variables included in the model were $\delta^{13}C$, $\delta^{15}N$ and likelihood probabilities (results of genetic assignment tests based on eight microsatellite loci) of belonging to each of the two races.*

(a) Training data			
	iliacus	***coburni***	**total**
mean	98.58	80.00	**89.30**
SD	3.18	20.92	**9.71**

(b) Test data			
	iliacus	***coburni***	**total**
mean	98.30	81.34	**94.78**
SD	0.00	3.00	**0.63**

Non-breeding Redwings

Based on the reduced multinomial linear regression model (derived from the variables $\delta^{13}C_f$, $\delta^{15}N_f$, and likelihood probabilities, and of belonging to each of the two races), the majority of Redwings sampled in the non-breeding season (92.5%; 124/134 individuals) were assigned to the nominate race *iliacus*. The remaining 7.5% of birds (10 individuals) were assigned to the Icelandic race *coburni* (Table 6.13).

Table 6.13 Classification matrix for Redwings sampled in Bardsey, UK during different periods, derived from multinomial logistic regression.

Group	***n***	***iliacus***	***coburni***
Oct 1995	76	92.1%($n = 70$)	7.9% ($n = 6$)
Mar 1998	26	92.3% ($n = 24$)	7.7% ($n = 2$)
Oct 1998	5	80% ($n = 4$)	20% ($n = 1$)
Oct 1999	27	96.3% ($n = 26$)	3.7% ($n = 1$)
Total	**134**	**92.5% ($n = 124$)**	**7.5% ($n = 10$)**

Discussion

Breeding Blackbirds

Relative performances of different variables

The results suggest that the most useful variables for discriminating between UK-resident and migrant Blackbirds are stable isotope ratios ($\delta^{13}C_f$ and $\delta^{2}H_f$), followed by wing length residuals. This supports the findings of Chapter 4, that a quadratic discriminant function based solely on $\delta^{13}C$ can correctly assign 79.3% of birds to two breeding groups (UK and non-UK), and the addition of $\delta^{2}H_f$ can correctly assign 71.2% of birds to three breeding groups (UK, Continent and Fennoscandia). The UK breeding birds had the lowest mean $\delta^{13}C_f$ values, and Fennoscandia had the highest (least negative) values. This finding is important because the total range of $\delta^{13}C_f$ values is fairly restricted (presumably due to the absence of large amounts of C_4 biomass within the European breeding range, Still et al., 2003; Jason Newton *pers. comm.*), yet there appears to be sufficient geographical variation (over a relatively small distance) to provide useful discrimination between European sites. The reason for the variation is unclear; it seems unlikely that it can be attributed to marine influence (marine habitats are comparatively more $\delta^{13}C$-enriched than terrestrial habitats), since many of the breeding sites were coastal.

Wing length is clearly a very useful variable for discriminating (albeit on a broad scale) between birds of different breeding origin, and is probably the least expensive and most practical data to collect. It would be interesting (and potentially very useful) to determine whether other morphological variables, particularly those that are flight-related such as wing shape and tail-length, vary between birds of different migratory status. For example, Copete et al. (1999) found differences in wing shape between sedentary and migratory Reed Buntings *Emberiza schoeniclus*. Pérez-Tris et al. (1999) took this approach a step further, using wing-length, wing shape and tail-length to differentiate between sedentary and migratory Blackcaps *Sylvia atricapilla*. Using a discriminant function based on these morphological variables, they were able to accurately assign > 90% of birds to the two groups. Similarly, variation in migratory distance between subspecies of Chiffchaffs *Phylloscopus collybita collybita* and *P. c. brehmii* was

inferred by analysing differences in flight-related morphology (wing-length, wing shape and tail-length) (Pérez-Tris et al., 2003). It was found that *P. c. brehmii* had more pointed wings (more concave wing shapes and pointed wing tips) and a shorter tail than *P. c. collybita*. These traits are thought to give a higher speed and lower energy consumption during long non-stop flights (e.g. Monkkonen, 1995; Norberg, 1995), suggesting that *P. c. brehmii* are better suited than *P. c. collybita* to long-distance migration.

The genetic data had poor discriminatory power, as indicated by the relatively large number of variables tested in the analysis. It was difficult to derive suitable (i.e. quantifiable) variables from the genetic data to include in the model, since they are discrete rather than continuous. These results suggest that the most fruitful approach for similar studies may be to concentrate on morphology and possibly stable isotopes. Populations of migratory bird species, even the partially migrant Blackbird, are likely to have relatively high levels of gene flow, and thus weak genetic structure.

Model Performance

Clearly the prediction performance of the multinomial logistic model is not of sufficient accuracy to assign individual birds to their most likely breeding origin with a strong degree of certainty (no more than ~70-75%), even on a broad geographical scale. However, the consistency of the model indicates that its performance is at least reliable, which makes it useful for providing a broad indication of the proportional representation of different breeding groups in a given sample site. The apparent lack of consistent and predictable patterns of geographic variation in genetic population structure, and even stable isotope ratios, makes it very difficult to produce a more accurate means of assigning birds to their most likely breeding origin. Several factors may be useful for improving this situation. Increased sample sizes (for both breeding and wintering birds) would allow more accurate testing of the model, and may also improve the fit of the model to the data. Obviously division of birds into groups encompassing distinct geographical regions is somewhat arbitrary; sampling of additional breeding sites would be useful for identifying any hidden patterns in the variables.

Winter Blackbirds

General trends

Although the dataset is rather small, the results for winter Blackbirds suggest that 70% were migrants. This supports the findings in the previous chapter that the UK appears to be an important wintering and stopover site for migrating Blackbirds. Ringing recoveries from 1909-1997 suggest that most of these overwintering birds have originated from Norway, Germany, Sweden, Denmark and the Netherlands. Subsequent recoveries in France, Spain and Portugal demonstrate that some birds merely pass through Britain to more southerly wintering areas. Evidence suggests that movement patterns may vary on a regional basis: few birds ringed in northwest England and Ireland outside the breeding season migrate further south, while most birds recovered in southern Europe were ringed in southeast England (Chamberlain & Main, 2002). Based on ringing recoveries, Chamberlain & Main (2002) were able to derive a figure of 12% as the minimum proportion of Blackbirds present in Britain & Ireland outside the breeding season that originate from mainland Europe. Findings presented here, as well as those reported in Chapter 5, strongly suggest that the true figure is far higher.

The data suggest that most of the migrants arriving in Britain and Ireland in the autumn are from Norway and Finland, rather than more central regions of Europe. This is an interesting finding, because ringing data indicate that the majority of Norwegian Blackbirds that move to Britain & Ireland are obligate migrants, and migrate every year. In contrast, ringing data suggest that Blackbirds belonging to the partially migratory populations further south, are facultative migrants, and may not migrate to Britain & Ireland in all years (Main, 2000; Chamberlain & Main, 2002). In addition, the huge influx of long-winged Blackbirds (reported in Chapter 5) also indicates that the majority of these migrants are of Fennoscandian extraction (since only birds breeding at more northerly latitudes were found to have longer wings than UK residents), rather than more southerly latitudes of mainland Europe.

Regional patterns

Despite the limitations of such a small dataset, some interesting regional variations are apparent. Ireland appeared to have the highest proportion of residents, which is not surprising, and supports the findings of the previous chapter. However, a third of the birds sampled at the Irish site were apparently migrant birds, which is somewhat higher than expected. The Northwest of Britain also appeared to receive a relatively high proportion of residents, which again is unsurprising, and fits with the findings of the previous chapter. All other regions had substantially lower proportions of residents than Ireland and the Northwest. Perhaps surprisingly, none of the birds from the Midlands were assigned as UK-breeders (although admittedly this region has the smallest sample size, $n = 7$). The remaining three regions (Scotland, the Northeast and the South) all appeared to have very similar proportions of resident birds, ranging between 16 and 22%. Of these, the South of Britain appears to receive the highest proportion of Fennoscandian migrants. The proportion of winter birds sampled at this site that were assigned to the Continent appears rather low, given the close proximity of this region to mainland Europe. Scotland and the northeast had comparable proportions of migrants from Fennoscandia, and both appear to receive similar proportions of migrants from the Continent.

An analysis of Song Thrush *Turdus philomelos* recovery data in Europe indicated that birds breeding in the Netherlands and northwest Germany appeared to have two migration patterns: a short-distance group dispersed through Belgium to northwest France in autumn/early winter, moving on to England and Wales in late winter. A second long-distance group migrated to southeast Biscay in October, with many progressing to west Iberia in midwinter (Milwright, 2006). It is possible that Blackbirds similarly show differences in migratory routes, as well as overwintering regions.

Breeding Redwings

Relative performances of different variables

In contrast to Blackbirds, it appears that the most useful independent variables for distinguishing between the two races of Redwings were the genetic assignment probabilities. The additional level of resolution provided by stable isotopes of $\delta^{13}C_f$ and $\delta^{15}N_f$ (but not $\delta^{2}H_f$) was only marginal; predictions based just on genetic assignment tests (particularly likelihood probabilities; Chapter 3) were almost as accurate. The lack of genetic structure between birds of the nominate race, and the difficulty in obtaining sufficient biometric data meant that there was insufficient data to try and discriminate between different populations within the nominate race. Increased sampling and additional biometric data may be useful for resolving this, but it seems unlikely that genetic data would be of further benefit in this regard.

Non-breeding Redwings

Consistent with the findings reported in Chapter 3, a very small number of Icelandic Redwings were identified amongst the Bardsey birds. The indication that Icelandic Redwings may be mixing with flocks of the nominate race on migration is interesting, although the numbers involved are so small, that this result must be treated with caution. It is difficult to draw firm conclusions about the reliability of the identification of Icelandic individuals without testing additional Icelandic populations. Nonetheless, in all tests of the logistic regression models, Icelandic birds were more likely to be misassigned as birds of the nominate race, than vice versa. A single nominate Redwing (from Rovaniemi, Finland) was misassigned as Icelandic (this same individual was misassigned in every test in which it was included). Therefore, it is also possible that in fact there were more Icelandic birds present amongst the non-breeding birds, and that these were misidentified as belonging to the nominate race.

Alternative modelling approaches

In an ecological context, logistic regression is often used with presence/absence data to predict species distributions (e.g. Green et al., 1994; Hinsley et al., 1995, 1996; Manel et al., 2000; Sinclair, 2001; Gunnarsson et al., 2006), whereas discriminant function analysis is typically used for classification purposes

(frequently, in avian species, for predicting sex) (e.g. Borras et al., 1993; Vanfraneker & Terbraak, 1993; Counsilman et al., 1994; Tomkovich & Soloviev, 1996; McCloskey & Thompson, 2000; Kentish et al., 2001; Palma et al., 2001; Genovart et al., 2003; Bavoux et al., 2006; Neto et al., 2006). However, it has been suggested that these conventional methods have limited application because they assume a linear relationship between variables, which is often not the case in ecology (Lek et al., 1996).

More recently, artificial neural networks (ANNs) have been applied to ecological studies because this method can accommodate non-linear interactions (e.g. Mastrorillo et al., 1997). However, ANNs are computationally expensive compared with linear models (e.g. Manel et al., 1999b; Tan et al., 2006). Relatively few ecological studies have compared the performance of these different modelling approaches. Exceptions to this include two (related) studies by Manel et al. (1999a; b), who found only marginal differences in performance between discriminant analysis, logistic regression, and ANNs, for predicting species distributions. Other criticisms of ANNs include the possibility of 'overtraining', a situation in which the model fits the training data so precisely, that it loses the ability to generalise to new datasets (e.g. Tan et al., 2006). In addition, ANNs have sometimes been labelled as a "black box" approach to modeling, because they provide little explanatory insight into the contributions of independent variables in the prediction process (e.g. Olden et al., 2004).

Another alternative for determining breeding origins might be to use the Bayesian method, by incorporating the posterior probability of origin. For example, the probability of capturing a migrant (versus resident) Blackbird is higher during the autumn migration period, than during the breeding season. Several genetic software packages have utilised a Bayesian approach for clustering purposes (e.g. Pritchard et al., 2000; Corander et al., 2003; Guillot et al., 2005), as have a number of studies that used stable isotope ratios to determine breeding origins of migrant birds (Royle & Rubenstein, 2004; Wunder et al., 2005; Norris et al., 2006a). However, incorporating different types of data (such as continuous variables with discrete genetic data) into a Bayesian approach is a substantial hurdle that remains to be overcome.

Conclusions

For Blackbirds, the overall pattern is one of complexity. The results presented here indicate that there is considerable variation in numbers and origins of migrants overwintering in (or passing through) different regions of the UK and Ireland, which, in turn, may suggest moderate levels of migratory connectivity in this species. However, due to the difficulty of obtaining genotype data from the small amount of genetic material present in feathers, sample sizes were small; more extensive sampling of non-breeding birds would be required before any real conclusions could be reached regarding regional variation (between wintering/stopover sites) in breeding origin. Although it was possible to discriminate between the two Redwing races with a high level of certainty, lack of genetic structure, and insufficient biometric data, meant that distinguishing between birds of different breeding origin was not possible beyond the subspecies level.

Clearly, combining different markers may not always be the best approach for determining origins, and the type of marker that is most useful varies between species. Nonetheless, the ability to consistently determine the origin of an individual with an accuracy in the region of 70-75% (as was the case with Blackbirds) is potentially very useful, particularly if applied to other species about which less is known concerning its migratory movements.

CHAPTER 7: GENERAL DISCUSSION

Introduction

Elucidating the links between breeding, stopover and wintering sites used by a migratory species throughout its annual cycle is fundamental to our understanding of the ecology and evolution of migration (Hobson, 1999a; Hobson, 2005a). Empirical studies have demonstrated that events occurring at one stage of the annual cycle clearly impact on populations in subsequent stages (e.g. Marra et al., 1998; Sillett et al., 2000; Bearhop et al., 2004). Since population declines of many avian species, particularly passerines, have been documented in recent years, an understanding of the links between different geographic regions utilised by migratory species is imperative; the recent trend in global climate change can only add to the urgency. Farmer et al. (2003) identify two specific aspects of the biogeography of avian populations that require clear understanding in order for conservation measures to be effective. These are (i) the location of specific breeding, wintering and stopover grounds for each species under consideration, and (ii) the extent to which populations of individual species are geographically subdivided on these locations, i.e. the degree of migratory connectivity.

Much of the current information about migration has been gathered over the last 100 years or so, largely as a result of observations, and ringing of individual birds (Marchant, 2002). Clearly, these conventional means of monitoring movements have made significant contributions to our understanding of migratory movements, and continue to do so. Recoveries and resightings of ringed birds provide the only irrefutable evidence of movements of individuals; the main drawback of this approach is that, after ringing, birds have to be caught or sighted (where rings are conspicuous enough to identify individuals) a second time. Although there is a wealth of ringing data available for ubiquitous species such as the Blackbird, the same cannot be said of many avian species, particularly those of conservation concern. Recently, intrinsic markers, such as stable isotope ratios and genetic markers, have been implemented, with the aim

of providing supplementary information about migratory movements. The crucial advantage of such techniques is that they do not require resighting or recapturing of individuals (Rubenstein & Hobson, 2004).

Stable isotopes

An escalating number of studies are employing stable isotope ratios to trace movements of migratory birds, and the technique has proved to be informative for a number of species, particularly in the Americas, where the first studies were carried out. In the present study, the usefulness of feather $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values for determining the breeding origins of Blackbirds and Redwings overwintering in Britain & Ireland was tested. It was predicted that across Europe, $\delta^{2}H_p$ values would vary on a latitudinal scale, as it does in the Americas, which would then be reflected in $\delta^{2}H_f$ values, thus providing a useful means of discriminating between populations breeding at different latitudes. It was predicted that $\delta^{13}C_f$ and $\delta^{15}N_f$ values would also vary between geographic regions, although to a lesser extent than $\delta^{2}H_f$ values, providing an additional level of discrimination between breeding sites.

Trends in $\delta^{2}H_f$ values

Although there was a strong correlation between collection site $\delta^{2}H_p$ values and latitude, the association between site $\delta^{2}H_f$ and $\delta^{2}H_p$ values was much weaker, and when each of the two study species was considered separately, there was no correlation between $\delta^{2}H_f$ and $\delta^{2}H_p$ values for either species. This is likely to be partly a statistical effect, caused by the small numbers of feathers sampled (relative to the amount of $\delta^{2}H_p$ data), which showed comparatively high variation within sites. For example, the average standard deviation in Blackbird breeding site $\delta^{2}H_f$ values ranged from ±1.0‰ to ±13.8‰, with a mean of ± 7.07‰. Similarly, a recent study by Hobson et al. (2004a) of comparative $\delta^{2}H_f$ values at a number of European sites (n = 38) found a relatively high level of within-site variation. As with the present study, Hobson et al. (2004a) used small sample sizes ($n \leq 8$ per site); even when a number of individuals of the same species were sampled at the same site, the variation was often between ± 8 and 12.5‰.

Thus, while small sample sizes and large within-site variation may not present a problem for some Nearctic or Neotropical species with breeding ranges that encompass a wide latitudinal range (e.g. Wassenaar & Hobson, 2000b; Hobson et al., 2001; Farmer et al., 2004), this approach may be less useful for species whose range lies within the European continent.

In addition, other factors may have confounding effects on hydrogen isotope ratios, particularly where a species' breeding range, encompasses a relatively narrow latitudinal range, as is the case for both Blackbirds and Redwings. There was certainly evidence that geographical features at two Norwegian sites, Titran and Eidfjord, had a strong influence on feather isotopic ratios, resulting in outlying δ^2H_f values for Blackbirds breeding at both sites, and outlying δ^2H_f values for Redwings breeding on Titran. This latter site is located on the tip of an island, and there appeared to be a strong marine influence on stable isotope ratios of feathers sampled here, resulting in less negative isotopic values (although several of the other breeding sites sampled were coastal, yet did not appear to be influenced by their proximity to the marine environment). At the second site (Eidfjord), Blackbirds had significantly more negative δ^2H_f values than Redwings (but there was no difference in either $\delta^{13}C_f$ or $\delta^{15}N_f$ values). Since this site is located in a valley, one might expect δ^2H_f values to vary with altitude: δ^2H_p tends to decrease with increasing altitude. However, the difference between the two species was not in the direction expected, because Redwings, which tend to breed at a much higher altitude than Blackbirds, had the highest (least negative) δ^2H_f values. This discrepancy was thus both unexpected, and difficult to explain.

A number of recent studies have found other problems associated with δ^2H_f values, such as unexpected (and unexplained) variation between different age or sex groups (Meehan et al., 2003; Smith & Dufty, 2005; Newton et al., 2006), and even variation between different types of feathers on the same individual (Langin et al., 2007). Moreover, these studies are not restricted to Europe (Rocque & Winker, 2005; Wunder et al., 2005). Nonetheless, δ^2H_f values were useful for discriminating between UK and non-UK breeding sites in Blackbirds. In addition, wing length, which in breeding Blackbirds was found to vary between

geographic regions, was correlated with δ^2H_f values in winter Blackbirds. For some European species, δ^2H_f values may be more useful, particularly those with discrete, widely separated breeding sites (e.g. Berthold, et al., 2005), or with a breeding range that encompasses a large latitudinal range.

Trends in $\delta^{13}C_f$ values

An important, and unexpected, finding was the high discriminatory power of $\delta^{13}C_f$ values for distinguishing between UK and non-UK breeding Blackbirds. Birds from the three UK sites had the lowest mean $\delta^{13}C_f$ values of all Blackbird breeding sites sampled. Using $\delta^{13}C_f$ values alone, 79.3% of birds were correctly assigned to the two breeding groups. There was a longitudinal trend in Blackbirds $\delta^{13}C_f$ values, which may explain why this isotope was useful for discriminating between the two regions, although the underlying reasons for the geographical trend remain unclear, particularly in view of the relatively restricted range of $\delta^{13}C_f$ values encountered. For Redwings, $\delta^{13}C_f$ values were found to vary with latitude, which was also unexpected.

The majority of the global vegetation coverage consists of C_3 plants (e.g. Ehleringer, 1991), which vary in $\delta^{13}C$ values (due to differences in mechanisms associated with water-use efficiency), with the overall effect that more xeric habitats generally have higher $\delta^{13}C$ values than cooler or more mesic environments. In addition, marine biomes have lower $\delta^{13}C$ values than terrestrial or freshwater biomes (e.g. Hobson, 2005a). According to Hobson (2005a) geographical patterns in $\delta^{13}C$ values patterns are therefore more useful for inferring habitat use, rather than for tracking migrants *per se*. In the light of this, the patterns observed in the study species remain difficult to explain, particularly for Blackbirds (which showed such a strong discrimination between birds breeding in the UK, and those breeding on mainland Europe).

Genetic markers

If a species shows substantial genetic structuring between populations, as might be predicted in a species displaying high migratory connectivity, this could

provide another means of discriminating between birds from different breeding populations. The present study employed microsatellite loci to investigate levels of genetic structure between breeding populations of Blackbirds and Redwings. The two Redwing races, *iliacus* and *coburni*, appeared to be genetically distinct, and microsatellite markers were thus invaluable for distinguishing between the two. This is potentially useful because, although there are morphological differences between the two races, these differences are relatively cryptic, and it is not always possible to differentiate between the two in the field (or even in the hand, Chris Benson, *pers. comm.*). Within the nominate race (*iliacus*), however, there was no genetic structuring.

Blackbird populations showed weak levels of genetic structuring; the most interesting finding was the apparent distinction of birds breeding on Skye. A number of subspecies of passerines endemic to Scottish islands (including Skye) have been described (McGowan et al., 2003), and it is quite feasible that the founder effect and/or random genetic drift may have led to genetic differentiation of Skye Blackbirds from those breeding on the mainland.

Possible reasons for lack of genetic structure

Population structure is the result of recent processes, such as drift, dispersal, mating and selection, as well as historical events, such as Pleistocene glacial cycles across temperate regions (e.g. Hewitt, 1999). Both are likely to have contributed to the lack of genetic structure in the two species studied here. Firstly, the very fact that they are so mobile, can lead to widespread dispersal and immigration in bird species, resulting in weak genetic differentiation between populations (Webster et al., 2002). This seems particularly likely to be the case for Redwings, which tend to be nomadic, and are not thought to display strong site fidelity. Secondly, in the northern temperate zone, lack of genetic diversity within species might reflect relatively recent postglaciation expansion of species ranges. As habitats contracted during the last Ice Age, loss of allelic diversity due to genetic drift in bottlenecked refugial populations would have been followed by rapid range expansions northward as ice sheets receded (e.g. Hewitt, 1996; Merilä et al., 1997; Mila et al., 2000).

Bensch et al. (1999) found little genetic differentiation between two populations of Willow Warblers, showing different migratory directions (a 'migratory divide'). This suggests that, for some European passerines, glacial refugia may not have played a role in the evolution of migratory differences between populations. Similarly, Davis et al. (2006) found little genetic variation between populations of Black-throated Blue Warblers, despite differences in migratory and other behaviours between populations. Results of their phylogeographic analyses indicated that extant populations expanded from a single glacial refugium, and thus, the migratory and other differences between them occurred relatively recently.

Alternative genetic markers

Use of additional microsatellites and increased sample sizes may reveal greater genetic structuring, particularly in Blackbirds (since results indicate that level of genetic structure is higher in this species than in Redwings). Alternatively, use of a different suite of markers such as mtDNA, amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995) or other nuclear markers, such as major histocompatibility complex (MHC) pseudogenes (which are thought be free of selective forces that constrain functional genes) (Aguilar et al., 2005), may provide additional insight.

For example, Clegg et al. (2003) were able to compare the level of population genetic structure in Wilson's Warbler revealed with microsatellites to that obtained in an earlier study using mtDNA (Kimura et al., 2002), using samples from the same geographical regions. In contrast to the microsatellite results, Kimura et al. (2002) detected a significant amount of mtDNA variance in the western populations. It is difficult to determine whether such comparisons of variation are biologically meaningful, due to differences between the two types of marker, including the different modes of inheritance, mutation rates, and the fact that mtDNA represents a single locus, whereas each microsatellite represents a different locus. Nonetheless, employing a combination of genetic markers may be useful, because they may improve resolution (Webster et al., 2002), or a particular marker may be informative where others are not (e.g. Bensch et al., 2002). Preliminary investigations into mtDNA sequence variation indicated that

there may be some differences between Blackbird populations, but did not reveal any differences between Redwings, suggesting that this type of marker would be of little use in the latter species. Time constraints meant that this investigation could not be pursued any further, but initial results are summarised in Appendix 3.

One of the few studies to have utilised AFLPs in vertebrates is that of Bensch et al. (2002b), who used this method to identify hybrids between two subspecies of Chiffchaffs, the Common Chiffchaff *Phylloscopus collybita collybita*, and the Iberian chiffchaff *P. c. brehmii*. This method was selected after a previous study using more traditional genetic markers (mtDNA and microsatellites) was unable to differentiate between the two subspecies (Bensch et al., 1999). Bensch & Åkesson (2005) advocate the use of this method, because of its relatively short start-up time, and cost effectiveness. However, they also point out that a common problem with AFLPs is their apparent sensitivity to DNA quality and the presence of DNA inhibitors, relative to standard PCR-based applications, which was the main reason that this approach was not selected for the present study.

A different type of marker that may prove suitable are MHC pseudogenes, although this approach is relatively new, and its utility has only been tested in a small number of avian species. Aguilar et al. (2005) investigated genetic variation of an MHC pseudogene (*Anvi*-DAB1) in the Little Greenbul *Andropadus virens* from several localities in West Africa, and found high levels of genetic variation between Ivory Coast and Cameroon, comparable to those found with microsatellite loci. Aguilar et al. (2005) suggest that pseudogenes may be a valuable tool for quantifying genetic variation and differentiation, but recommend comparison of their genetic variability to selectively neutral markers to determine if levels of genetic variability are influenced by selection.

Morphology

Trends in ringing data

Trends in Blackbird ringing data revealed a wealth of information about this species. For first-year birds and adult females, autumn migration appeared to begin towards the end of September, immediately after the moult period, with numbers peaking in October. For adult males, migration appeared to begin a little later, and perhaps over a slightly more extended period. For all age and sex groups, changes in monthly totals of birds clearly corresponded with changes in mean wing length; highest mean wing lengths were recorded in October and November. Monthly totals and mean wing length data combined suggest that a huge number of migrants arrive in the UK and Ireland in autumn, but dramatic falls in both numbers and mean wing length at the end of November, indicate that the majority of these birds are merely passing through on their way to more southerly overwintering areas. This is also supported by recovery data (Chamberlain & Main, 2002). Alternatively, it could be speculated that migrants are less likely to be caught as the season progresses, and birds recover from the stress associated with migration, and become more familiar with their new environment. In light of the finding that only migrant Blackbirds from more northerly latitudes had longer wings than UK-residents (Chapter 5), one could speculate that the majority of the migrants arriving in the UK and Ireland in the autumn are likely to be from Norway, Sweden and Finland.

Regional variation

Comparison of data from different parts of the UK and Ireland revealed considerable regional variation, with a general tendency for birds caught in the north and east during the autumn and winter to have longer mean wing lengths, than those caught in the south and west. The data suggest that those migrants overwintering in Britain tend to remain in more eastern regions (East, Southeast and West Midlands), because numbers of birds caught in these areas remain relatively high throughout the winter. There is also some indication that wing length of *resident* Blackbirds may vary between regions, with those breeding in Wales, Ireland and the Southwest region apparently having shorter mean wing length than other regions. There is some suggestion that, on occasion, British-

breeding Blackbirds make short distance movements into the south and west of Britain, and across to Ireland, during the winter (possibly in response to the influx of longer-winged migrants, which may increase competition for resources). If mean wing length of British- and Irish- breeding birds does differ between regions, this phenomenon could thus provide some insight into small-scale movements in this species.

Comparison of autumn and spring migration patterns

Another interesting finding from the ringing data was the apparent discrepancy in numbers and mean wing length between the autumn and spring migration periods. The huge increase in numbers of birds caught, and the increase in mean wing length during autumn migration, is not reflected in a similar magnitude during spring migration. There are a number of interpretations for these differences, none of which are mutually exclusive. During the autumn, birds may be migrating within a small time window, relative to the more gradual spring migration, or birds may follow different migratory routes between autumn and spring. Another possibility is that birds are experiencing high mortality during the winter, so there are simply less birds returning in the spring.

Wing length

There are several different lines of evidence to support the prediction that wing length varies between Blackbirds breeding in different geographical regions (trends in ringing data supplied by the BTO, differences between mean wing length of breeding birds, and correlations between wing length and latitude/δ^2H_f values in breeding/winter birds respectively). This may be directly or indirectly related to migratory habit. Clearly, wing length is a useful variable for discriminating between Blackbirds of different breeding origin, and has the advantage of being the least expensive and most practical data to collect. Several studies have demonstrated that flight-related morphology such as wing shape and length, and tail length vary between birds of different migratory status (e.g. Leisler & Winkler, 1991; Monkkonen, 1995; Lockwood et al., 1998). Such differences have already been used to differentiate between sedentary and migratory birds in a number of species, including Reed Buntings *Emberiza schoeniclus* (Copete et al., 1999), Blackcaps *Sylvia atricapilla* (Pérez-Tris et al.,

1999), and Chiffchaffs *Phylloscopus collybita* (Pérez-Tris et al., 2003). Alternatively (or additionally), variation in wing length may be associated with latitude, with larger Blackbirds inhabiting higher latitude (and thus conforming to Bergmann's Rule). Thus, further investigation of morphological variables, both those that are related and unrelated to flight, may prove fruitful.

Combining techniques

It was predicted that combining techniques would prove substantially more useful for assigning individuals to their most likely population of origin, than any one technique used in isolation. The number of studies attempting to combine different types of data, such as ringing data or species abundance data with stable isotope ratios (e.g. Royle & Rubenstein, 2004; Mazerolle et al., 2005; Norris et al., 2006a), or morphology or ringing data with genetic data e.g. (Lopes et al., 2006; Seki et al., 2007) appears to be increasing, although surprisingly few attempts have been made to *fully* integrate stable isotope ratios with genetic data. This may be, at least in part, because combining these two different types of data (genetic data are discrete, whereas stable isotope ratios are continuous) is not straightforward.

Since several of the variables were not normally distributed, the simplest approach was to use multinomial logistic regression to predict group membership, based on all three types of data (genetic, isotopic and morphological). This proved to be a reasonably effective method for assigning Blackbirds to three broad geographical regions. As with the discriminant analyses (derived from stable isotope ratios), $\delta^{13}C_f$ proved to be the most useful variable for assigning Blackbirds, followed by wing length and $\delta^{2}H_f$. Using all variables, and the complete dataset of breeding Blackbirds (n = 114) resulted in a prediction performance (percentage of correct assignments) of 81.6%, whilst reducing the dataset to 80% resulted in an even higher prediction performance of 93.2%. This was probably because the smaller dataset allowed 'overtraining' of the model. However, testing the performance of this model on new data (i.e. the test dataset) resulted in a dramatic decrease in accuracy, with only 56.5% of birds

correctly assigned to their region of origin. Excluding all genetic variables, whilst resulting in a decrease in a substantial decrease in prediction performance, was the most consistent model, with 72.2% to 76.3% of birds being correctly assigned for all datasets (complete, training and test). For Redwings, combining different techniques only marginally improved the number of correct race assignments than the use of microsatellites alone.

The type of variable that is most useful for a study of this nature clearly varies, not only with species, but also on the nature of the investigation. For example, to distinguish between UK resident and migrant Blackbirds, the most effective approach appears to be the use of $\delta^{13}C_f$ values (in a discriminant analysis). Note that in this situation (i.e. assigning Blackbirds to just two groups), combining either wing length or $\delta^{2}H_f$ values with $\delta^{13}C_f$ values is unlikely to result in additional discriminatory power, because the geographical differences in these variables are more complex than $\delta^{13}C_f$ values. However, if additional geographic resolution is desirable (i.e. assigning Blackbirds to three geographic regions), stable isotopes of carbon coupled with hydrogen stable isotopes and wing length, would be the most useful combination of variables (in a multinomial logistic regression model); adding additional variables (such as genetic data) may have a confounding influence, by decreasing the consistency of the model performance. Thus, while combining different variables may, in some instances, be more useful than using a single variable, combining the maximum number of variables is not necessarily the best approach.

Comparable studies

Given the weakness of genetic population structure revealed with microsatellite loci, large within-site variation in $\delta^{2}H_f$ values, and relatively small dataset, the ability to consistently assign 72.2% to 76.3% of Blackbirds to their correct region of origin is perhaps a considerable achievement. Other studies attempting to integrate stable isotope and genetic data have had mixed success. Using mtDNA, $\delta^{13}C_f$, and morphological measurements, Wennerberg et al, (2002) found only limited differences between Arctic breeding sites of the White-rumped Sandpiper, and no correlations between variables. While Clegg et al. (2003) reported apparently complementary geographical variation across the American

continent in microsatellite DNA and δ^2H_f values of Wilson's Warbler populations, no attempt was made to integrate these two types of data.

Most recently, Boulet et al. (2006) overlaid geographic information system maps derived from mtDNA and δ^2H_f data to assign wintering Northern Yellow Warblers to a broad region of origin in North America, although this required a relatively large dataset. Perhaps the most successful study of this kind is that of Kelly et al. (2005), who used a combination of mtDNA, δ^2H_f and $\delta^{34}S_f$, to assign Swainson's Thrushes to site-specific origins (as described above). However, evidence suggests that Neotropical species are exceptionally suitable for this type of study. In both species, earlier studies identified differences in mtDNA between breeding lineages (Milot et al., 2000; Ruegg & Smith, 2002; Boulet & Gibbs, 2006), and the utility of stable isotopes for determining migratory origins has been reported for at least one similar species (the Black-throated Blue Warbler) (Chamberlain et al., 1997). Clearly, the development of effective approaches for integrating different variables (both intrinsic markers and ringing/ biometric data) is at an early stage; several studies have reported promising results, but there remains much progress to be made.

Alternative statistical approaches

Rigorous and statistical approaches to analyse stable isotope data are still lacking (Hobson, 2005a). A number of studies are following the lead of Royle & Rubenstein (2004), by integrating likelihood probabilities, based on species abundance, with stable isotope data, which should lead to improved accuracy of assignments. For example, an analysis of stable isotope ratios in Mountain Plover feathers found that the performance of inverse regression models for assignment purposes was no better than random assignment. Probability models performed much better, and Wunder et al. (2005) recommend using this approach over assumptive regression models, for inferring geographic origins. Bayesian statistical approaches have already proved to be a powerful means of examining genetic population structure (e.g. Pritchard et al., 2000; Corander et al., 2003; Guillot et al., 2005). As suggested by Norris et al. (2006b) a major challenge for the future is to combine data of different types (such as stable isotopes and genetics) in a single probability-based framework.

Proportions of migrant Blackbirds

Using discriminant functions based on stable isotopes of $\delta^{13}C_f$ and $\delta^{2}H_f$, 43.0% of Blackbirds in the UK and Ireland in winter were assigned to the UK breeding population; the multinomial logistic regression analysis, which incorporated wing length, estimated a much lower proportion of migrants in the winter sample, just 29.9%. The total sample size for the latter analysis was much smaller (87 individuals, versus 135 in the former analysis) because genetic data were only available for a small proportion of individuals (due to low quantity and/or quality DNA extractions, as discussed in Chapter 3). However, analysis of the breeding data suggested a tendency for the discriminant analysis to overestimate the proportion of UK (relative to non-UK) breeding birds, whilst this was not found to be a problem with the logistic regression. Thus it seems that the true proportion of migrants is closer to the lower estimate. This would also fit with the findings of the BTO ringing data, which indicated a high proportion of migrants in the UK and Ireland during the winter.

Migratory connectivity

The wide range of $\delta^{2}H_f$ values in winter birds of both species indicates that they originated from numerous sites within the breeding range. In Blackbirds, significant differences in isotopic ratios between winter sites, and the results of the logistic regression analysis, may be indicative of differences in (proportions of) origins of migrants received by different parts of the UK and Ireland. This in turn may be indicative of moderate migratory connectivity. However, this assertion can only remain speculative without further sampling. In contrast, it appeared that Redwings originated from a vast area, but subsequently converged into migratory flocks, indicating considerable mixing of populations on the wintering grounds. One can speculate that this may be suggestive of relatively weak migratory connectivity. Note, however, that there is some suggestion that the Icelandic race exhibits a migratory divide, with birds from the eastern half of Iceland more likely to winter in Scotland and Ireland than those from the western half (which presumably tend to fly directly to Iberia) (Milwright, 2002a). In addition, it is possible that Redwings of the nominate race may also exhibit

migratory connectivity to some extent, because birds wintering in the easternmost parts of the range (the Aegean, the Levant, and the Transcaucasus) are thought to be from Russia and Siberia, although birds breeding in Russia and Siberia also winter in the same western European areas as Fennoscandian birds (Cramp & Simmons, 1988a; Milwright, 2002b).

Differential migration

There is evidence to suggest that migratory tendency differs between age and sex groups in Blackbirds (e.g. Lundberg, 1985; Chamberlain & Main, 2002; Partecke & Gwinner, 2007). Similarly, age-specific differences in migratory distance of Redwings are thought to occur. The mean distance from breeding to wintering areas of *T. iliacus* is less in their first winter than in later winters, whereas among the Icelandic race *coburni*, the reverse situation appears to occur with first-winter birds tending to migrate further than the adults (Milwright, 2002a, b). For Blackbirds, it seems probable that social dominance and competitive exclusion (Catry et al., 2004) are the driving forces behind this, something that may also occur in Redwings (Redfern et al., 2002). Isotope data indicated that some differences occurred between the different age and sex groups in breeding birds of both species. However, it was not possible to determine whether these results reflected genuine differences, because sample sizes were insufficient to fully explore this. In addition, there were no significant differences in any isotope ratios between different groups of winter birds. The lack of any clear trends means that it is not possible to further elucidate possible differences between different groups in either species, based on results of this study.

Changes in migratory behaviour in response to climate change

Although historical changes in global climate, vegetation zones and sea level undoubtedly had an impact on migration systems (e.g. Sutherland, 1998), the extent to which migratory species are able to respond to more rapid changes in climate remains uncertain. Recent studies have demonstrated that many aspects of migratory behaviour in birds, such as timing, duration and direction of migration are genetically controlled (e.g. Berthold & Helbig, 1992; Berthold,

1999), yet there is also a large body of evidence indicating a high degree of flexibility in migratory behaviour (Fiedler, 2003). A number of studies have shown that migratory behaviour can be lost and gained relatively rapidly. Perhaps the classic example is that of the Blackcap *Sylvia atricapilla* breeding in central Europe, which, since the 1960s, evolved a novel westerly migratory route to overwinter in Britain (Langslow, 1979; Berthold et al., 1992). This microevolution of migratory behaviour occurred within 30 years, and breeding experiments demonstrated that it was genetically determined (Berthold et al., 1992).

In contrast, other species have shown considerable conservation of migratory behaviour; Swainson's Thrush underwent a range expansion in the Late Pleistocene, but continues to follow its ancestral circuitous migratory route, rather than taking the shortest path between overwintering and breeding areas (Ruegg & Smith, 2002). Still other species may be unable to respond to climate change, either because their migration strategy is unaffected by climate change, or because the climate in breeding and wintering areas are changing at different speeds, preventing adequate adaptation (Both & Visser, 2001). For example, Both & Visser (2001) reported that while the Pied Flycatcher *Ficedula hypoleuca*, has advanced its laying date to match the increase in spring temperatures in temperate regions that has occurred over the past 20 years, the species has not similarly advanced its spring arrival date. This may be because the timing of some species' spring migration relies on endogenous rhythms that are not affected by temperature (Gwinner, 1996).

Partial migrants

It seems likely that species demonstrating phenotypic flexibility of migratory behaviour, such as partially migrant species, will have substantial propensity for adapting to climate change. A trend for increased sedentariness in urban versus rural or forest populations has been revealed in several studies (e.g. Adriaensen & Dhondt, 1990; Yeh & Price, 2004). A ''heat island'' effect of cities has been described, whereby the increased cover of artificial surfaces (roof and paving materials) reflect less of the sun's rays during the day and trap more heat at night, resulting in significantly (1–4°C) warmer air and surface temperatures than the

surrounding countryside (Arnfield, 2003; Shochat et al., 2006). Partecke & Gwinner (2007) suggest that species phenotypic responses to urbanisation and its associated environmental changes, is thus somewhat analogous to the impact of global warming.

Recently, Partecke & Gwinner (2007) hand-raised Blackbirds from urban and forest populations, in an effort to determine whether sedentariness in this species represents local adaptation (i.e. whether it has a genetic basis), rather than simply representing phenotypic plasticity. It was found that migratory disposition (measured as the amount of nocturnal activity and fat deposition) was lower in urban than in forest males, particularly during their first year, whereas females from the two habitat types did not differ. These results indicate that, in males, migratory disposition is genetically determined. However, Partecke & Gwinner (2007) suggest that the finding that the difference in migratory disposition between captive and forest males diminished during the second year, also implies that non-genetic factors modify migratory behaviour.

According to Nice (1937) and Lack (1944), in a number of partially migratory species, fewer males migrate than females, and a smaller proportion of adult birds migrate than young birds. Sedentary birds may gain fitness benefits over migrants, because of prior access to prime breeding territory and nest sites, and earlier onset of reproduction (e.g. von Haartman, 1968; Adriaensen & Dhondt, 1990). Partecke & Gwinner (2007) suggest that selection in urban habitats operates differently on the migratory disposition of male and female Blackbirds, due to male-biased fitness consequences, which may favour selection for sedentariness in males more than in females, whereas an earlier study by Partecke et al. (2004) suggested that fitness benefits of migratory and resident females seem to be similar.

Turdus *thrushes*

Their successful adaptation to urban environments, as well as recent range expansions (Kalela, 1949; Spencer, 1975; Sharrock, 1976) and colonisations of European islands (Venables & Venables, 1952; Sacher et al., 2006), indicate that Blackbirds will adapt well to environmental change, and may even benefit, by

further extension of its range. While fewer Blackbird recoveries from Ireland and the Continent in recent years suggest that British-breeding birds are becoming increasingly sedentary (thought to be at least partially due to milder winters in recent years), ringing data for the years 1996 to 2005 did not indicate any general decline in the huge numbers of Blackbirds arriving in Britain in the autumn.

Milwright (2002b) suggests that the biggest long-term challenge faced by the Redwing may be the effect of global warming on its breeding biology. However, it seems likely that, as with the Blackbird, Redwings will be able to adapt to the changing climate. Although among the least robust of the Palearctic thrushes, and vulnerable to mass mortality, the Redwing is remarkably successful in maintaining high population numbers in cold northern and middle latitudes, by its ability to find vacant ecological niches, and to shift rapidly in response to environmental conditions (Cramp & Simmons, 1988a). According to Cramp & Simmons (1988a) the Redwings readiness to fly long distances in the middle and upper airspace facilitates this ability. Furthermore, It is highly likely that the Icelandic race (*coburni*) is derived from the nominate race (*iliacus*); Iceland would have been uninhabitable during the last Ice Age, and therefore, it seems likely that the two races have separated after this period. Since all known populations of the nominate race have a well-established west to southwest migration, separation of the two races appears to have rapidly resulted in the adoption of a novel and highly focused migratory direction (Milwright, 2002b).

However, other *Turdus* species spending part of their annual cycle in Britain & Ireland may be detrimentally affected by climate change. Beale et al. (2006) modelled the effect of temperature and rainfall on the breeding success and territory occupancy of Ring Ouzels *Turdus torquatus* in northern Britain, a species that continues to decline as a breeding bird in many parts of Britain & Ireland (Burfield, 2002). Whereas breeding success was not found to be strongly affected by climatic variables, there were significant climatic correlates of annual change in territory occupancy, and models based on these climatic trends suggested that increased summer temperatures might underlie declines in the British Ring Ouzel population. A serious lack of knowledge concerning the species' ecology, including its migratory movements, has hampered conservation

efforts. For example, it is not known whether British/Irish and Fennoscandian populations winter together or separately, nor whether factors operating on its migration route or wintering grounds are contributing to its decline (Beale et al., 2006).

Conclusion

Increasingly, intrinsic markers (genetic and chemical markers) are being used to complement more traditional approaches to tracing migratory movements (e.g. ringing recoveries and morphological measurements), and new insights into the migratory strategies of a number of avian species have been revealed. However, efforts to integrate different markers and methods, in order to fully exploit potential sources of information concerning movements, are still at an early stage. Lack of rigorous and statistical approaches to analyse and integrate different types of data remains a substantial hurdle (e.g. Hobson, 2005a; Norris et al., 2006b).

There is evidence to suggest that there may be less potential for determining the origins of individuals within the European continent than within North America; the range of δ^2H_p values in Europe appears to be more restricted than in North America (Hobson et al., 2004a; Bowen et al., 2005). Thus, the ability to consistently assign 72.2% to 76.3% of Blackbirds to their correct region of origin is a promising result, particularly in light of the small dataset, weak genetic population structure, and large within-site variation in δ^2H_f values.

There were a number of unexpected and interesting findings, such as the apparent usefulness of $\delta^{13}C_f$ for discriminating between UK and non-UK breeding birds, which warrant further exploration. Despite being the most practical and inexpensive type of data to collect and analyse, it is suggested that morphology and other ringing data are a greatly underexploited resource, with great potential for future studies of bird migration. The most useful variable/combination of different variables for a study of migratory connectivity will vary, both according to the species, its geographical range, and the scale of

resolution required. For example, the findings of the present study suggest that, for species whose range lies within the European continent, stable hydrogen isotopes may be most useful for separating discrete, and/or widely separated (in terms of latitude) breeding areas. A clear understanding of the ecology and physiology of the study species is thus essential (Rocque et al., 2006).

In addition, useful insights into the migratory strategies of the two study species were revealed. The lack of genetic variation within the nominate Redwing race indicates a lack of migratory connectivity, which is consistent with the ecology of this species (its nomadic nature, and lack of strong site fidelity, Snow, 1986b; Reid, 1993). In contrast, there was some evidence suggesting that migratory connectivity might be stronger in the Blackbird: weak genetic population structure, and differences in stable isotope ratios and wing length between birds sampled at different wintering sites, which suggests that different parts of the UK & Ireland may receive differing proportions of migrants originating from different breeding sites. Again, this is consistent with what is already known about the species' ecology, for example there is thought to be variation in migratory behaviour both between populations (e.g. Cramp & Simmons, 1988b; Mulsow & Tomialojc, 1997; Chamberlain & Main, 2002).

Attempts to find effective means of further elucidating links between the geographical regions used by migratory species remains of fundamental importance, not only to reveal further insight into the ecology and evolution of migration, but also to predict how migratory species will respond to our profoundly changing climate.

APPENDIX 1: Examples of Studies Investigating the Use of Stable Isotope Ratios to Link Breeding and Non-Breeding Grounds of Migratory Birds

(a) Studies conducted in the Americas

Authors	Stable isotopes used	Species	Brief summary
Chamberlain et al. (1997)	$\delta^{13}C$ $\delta^{2}H$	Black-throated Blue Warbler *Dendroica caerulescens*	Demonstrated that stable isotopes can be used for identifying the origins of birds breeding in North America. Suggested that using several stable isotopes will increase resolution for determining geographic origins.
Rubenstein et al. (2002)			Isotopic data indicated that populations segregated by North American breeding latitudes mixed on the Caribbean wintering grounds.
Royle & Rubenstein (2004)			The first study to integrate stable isotope data with prior probabilities of assignment related to relative abundance of birds (using Bayes's rule).
Hobson & Wassenaar (1997)	$\delta^{2}H$	Neotropical migratory passerines (*9 spp.*)	Demonstrated the utility of $\delta^{2}H$ for linking breeding and wintering grounds for Neotropical migrants in Guatemala.
Marra et al. (1998)	$\delta^{13}C$	American Redstart *Setophaga ruticilla*	Used $\delta^{13}C$ to infer winter habitat type, and found that quality of habitats determined physical condition and spring departure of Redstarts, which in turn influenced arrival dates and physical condition on the breeding grounds.
Norris et al. (2006)	$\delta^{2}H$		Used a likelihood-assignment test incorporating with a prior probability of breeding abundance with isotopic data to identify most probable breeding regions. Data indicated that eastern populations originated from northwest breeding areas, and eastern populations were from eastern and southern breeding areas.
Caccamise et al. (2000)	$\delta^{13}C$ $\delta^{15}N$ $\delta\ ^{34}S$	Canada Geese *Branta canadensis*	Found that $\delta^{13}C$ and $\delta\ ^{34}S$ signatures provided a reliable means of discriminating between resident and migrant birds.
Wassenaar & Hobson (2000b)	$\delta^{13}C$ $\delta^{2}H$	Red-winged Blackbirds *Agelaius phoeniceus*	Demonstrated that $\delta^{13}C$ and $\delta^{2}H$ provide a means of segregating breeding populations along a latitudinal gradient in North America.
Hobson et al. (2001)	$\delta^{13}C$ $\delta^{2}H$	Bicknell's Thrushes *Catharus bicknelli*	$\delta^{2}H$ data suggested that many different breeding populations in North America mix on the wintering grounds in the Dominican Republic.
Hobson et al. (2004b)	$\delta^{2}H$		Located possible locations of wintering populations unidentified in earlier study.
Hobson & Wassenaar (2001)	$\delta^{13}C$ $\delta^{2}H$	Loggerhead Shrikes *Lanius ludovicianus*	Determined the breeding and natal origins of endangered Loggerhead shrikes wintering in North America and Mexico.
Pérez Garrido (2006); Pérez & Hobson (2007)	$\delta^{2}H$		Determined breeding origins of western subspecies *L. l. excubitorides* wintering in Mexico.
Kelly et al. (2002)	$\delta^{2}H$	Wilson's Warbler *Wilsonia pusilla*	Isotopic data indicated that the species has a leapfrog pattern of migration within North and Central America.
Clegg et al. (2003)			Combined $\delta^{2}H$ and microsatellite analyses to infer migration patterns and population connectivity between breeding grounds in North America and overwintering areas in Mexico and Central America.
Paxton et al. (2007)			Differences in $\delta^{2}H$ values indicated that different breeding populations use different migratory pathways.
Wennerberg et al. (2002)	$\delta^{13}C$	White-rumped Sandpiper *Calidris fusicollis*	Used $\delta^{13}C$ along with mtDNA and morphology to investigate population structure on the breeding grounds. Found significant differences in $\delta^{13}C$ between sites, but this was not correlated with morphological differences.

(a) Studies conducted in the Americas

Authors	Stable isotopes used	Species	Brief summary
Farmer et al. (2003; 2004)	$\delta^{15}N$ $\delta^{13}C$ $\delta^{2}H$ $\delta^{18}O$ $\delta^{34}S$	Nine shorebird *spp.*	Preliminary studies to evaluate the use of stable isotopes to identify wintering areas of Neotropical migratory shorebirds in Argentina.
Hobson et al. (2003)	$\delta^{13}C$ $\delta^{15}N$ $\delta^{2}H$	Hummingbirds (8 *spp.*)	Found a significant relationship between tail feather $\delta^{13}C$ and $\delta^{2}H$ values and elevation in the Ecuadorian Andes. Thus $\delta^{13}C$ and $\delta^{2}H$ should prove useful for tracking altitudinal migrants.
Meehan et al. (2003)	$\delta^{2}H$	Cooper's Hawks *Accipiter cooperii*	Determined the latitudinal origins of immature birds migrating through the Florida Keys.
Smith et al. (2003)	$\delta^{2}H$	Sharp-shinned Hawks *Accipiter striatus*	$\delta^{2}H$ combined with recovery of ringed birds revealed that first-year birds used a chain migration pattern during their autumn migration through North America.
Mehl et al. (2004; 2005)	$\delta^{15}N$ $\delta^{13}C$	King Eiders *Somateria spectabilis*	Found that $\delta^{15}N$ and $\delta^{13}C$ were useful for broad-scale assignment to wintering grounds (i.e. discriminating between eastern and western- wintering populations), and that females were not strongly philopatric to wintering areas among years.
Atkinson et al. (2005)	$\delta^{15}N$ $\delta^{13}C$	Red Knot *Calidris canutus*	Found that $\delta^{15}N$ and $\delta^{13}C$ in flight feathers of birds passing through Delaware Bay, USA could be used to identify at least 3 discrete wintering areas.
Hebert & Wassenaar (2005a)		Northern Pintail	Used stable isotope ratios to identify geographic origins of birds shot by hunters, and infer areas of poor production.
Hebert & Wassenaar (2005b)	$\delta^{15}N$ $\delta^{13}C$ $\delta^{2}H$ $\delta\ ^{34}S$	Mallard *Anas platyrhynchos* and Northern Pintail *A. acuta*	Found that a multi-isotope approach was effective in determining natal origins of waterfowl in western North America
Clark et al. (2006)		Northern Pintail and Lesser Scaup *Aythya affinis*	Investigated whether predictable isotopic patterns in precipitation occur in wetlands. Found that $\delta^{2}H$ values in feathers of Scaup ducklings were correlated with those of precipitation.
Kelly et al. (2005)	$\delta^{2}H$ $\delta\ ^{34}S$	Swainson's Thrush *Catharus ustulatus*	Combined isotope ratios with mitochondrial DNA markers, enabling correct classification of 75% of birds of unknown origin to site-specific breeding locations.
Mazerolle & Hobson (2005)	$\delta^{2}H$	White-throated Sparrows *Zonotrichia albicollis*	Compared the utility of different tissue types (feathers, claws and blood) for inferring origins. Findings suggested that feathers were the most appropriate tissue to use in this context.
Mazerolle et al. (2005)			Used $\delta^{2}H$ in feathers to determine both breeding and wintering catchment areas of birds caught at a fixed migration monitoring station in Canada.
Smith & Dufty (2005)	$\delta^{2}H$	Northern Goshawk *Accipiter gentilis*	Found age-related and intra-individual differences in δ2H values of feathers, suggesting that movements of individual birds cannot be reliably described.
Wunder et al. (2005)	$\delta^{13}C$ $\delta^{15}N$ $\delta^{2}H$	Mountain Plover *Charadrius montanus*	Found that inverse regression models based on isotopic data were not useful for assigning latitudinal origins of birds, although probability models provided better (than random) results. Question the notion of a characteristic range of $\delta^{2}H$ values for any single location.
Boulet et al. (2006)	$\delta^{2}H$	Northern Yellow Warbler *Dendroica petechia*	Overlaid geographic information system maps derived from $\delta^{2}H$ and mtDNA data. Were able to identify several main flyways in spring and autumn.
Hobson et al. (2006)	$\delta^{2}H$	Sandhill Cranes *Grus canadensis*	Used $\delta^{2}H$ to provide new quantitative information concerning breeding origins of harvested Sandhill Cranes in North America.
Rocque et al. (2006)	$\delta^{2}H$	Intercontinental migrants (3 *spp.*)	Were unable to accurately assign feathers to origin of growth to within better than several thousand kilometres of their true origin.

(b) Studies conducted in Europe/Africa

Authors	Stable isotopes used	Species	Brief summary of study
Chamberlain et al. (2000);	$\delta^{13}C$ $\delta^{15}N$	Willow Warblers *Phylloscopus trochilus trochilus* & *P. t. acredula*	Consistent with differing morphology and migratory routes between the two subspecies, isotopic data changed abruptly at a presumed migratory divide in Sweden. Moreover, the isotopic signatures reflected wintering quarters.
Bensch et al. (2006)			Found differences in birds wintering in different regions of Africa, and it was possible to locate African wintering areas of Swedish-breeding birds.
Evans et al. (2003)	$\delta^{13}C$ $\delta^{15}N$	Swallow *Hirundo rustica*	$\delta^{13}C$ revealed that swallows breeding in distinct parts of Europe (England and Switzerland) overwinter in different regions of Africa.
Hobson et al. (2004a)	$\delta^{2}H$ $\delta^{18}O$	Migratory European birds (27 *spp.*)	Isotopic values of precipitation suggested that isotope gradients occurred across Europe, and that there was a correlation between isotope ratios of precipitation and feathers.
Pain et al. (2004)	$\delta^{15}N$ $\delta^{13}C$ $\delta^{2}H$	Aquatic Warbler *Acrocephalus paludicola*	Significant differences in mean $\delta^{13}C$ were found between subpopulations, suggesting a strong relationship between European breeding and African breeding latitudes.
Bearhop et al. (2005)	$\delta^{2}H$	4 passerine species (both resident and migrant)	Found that $\delta^{2}H$ values in the claws of several passerine species grown in two European regions were significantly different. This subsequently allowed the winter origins of Blackcaps (*Sylvia atricapilla*) caught on breeding grounds in southern Europe to be inferred.
Yohannes et al. (2005)	$\delta^{15}N$ $\delta^{13}C$ $\delta^{2}H$	Palearctic warblers (3 *spp.*)	Used stable isotopes of feathers to help identify autumn stopover sites in northeast Africa. Whilst this was possible on a broad scale, the authors found that identification of more precise locations would not be possible without additional regional isotopic maps of Africa.
Newton et al. (2006)	$\delta^{2}H$	Northern Bullfinches *Pyrrhula p. pyrrhula*	Investigated the origin of an invasion of northern Bullfinches into parts of western Europe in 2004. Measurements of $\delta^{2}H$ in feathers suggested the birds might have come from a wide area of northern Europe eastward into Russia.

(c) Studies conducted in Australasia/Antarctica

Authors	Stable isotopes used	Species	Brief summary of study
Cherel et al. (2000)	$\delta^{13}C$ $\delta^{15}N$	Black-browed Albatrosses *Diomedea melanophrys*	Used isotopic together with ringing data to identify moulting (interbreeding) foraging areas. Found that birds from Kerguelen Islands, southern Indian Ocean wintered in subtropical waters off southern Australia.

APPENDIX 2: Allele Frequencies

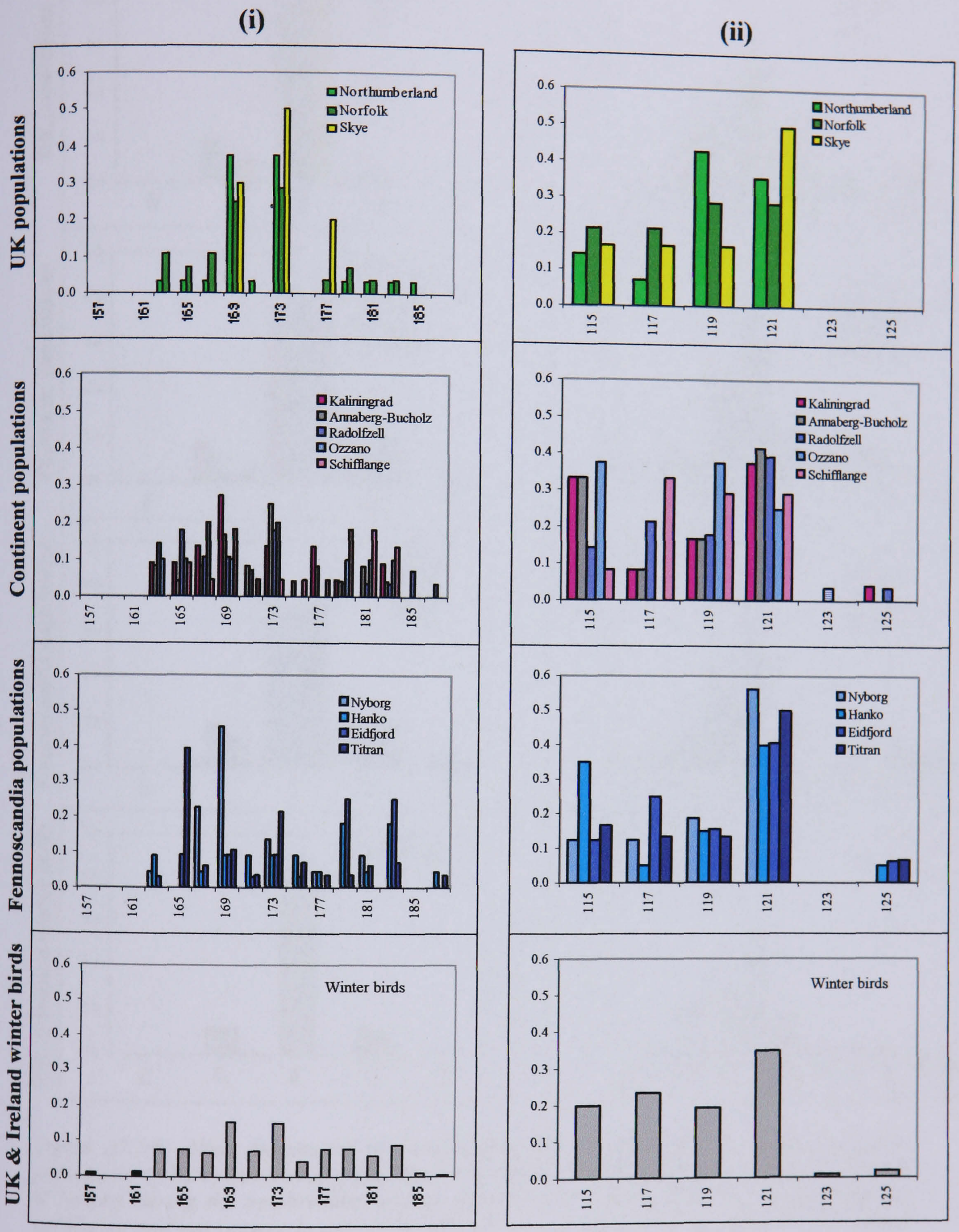


Figure A2.1(a) Allele frequencies at microsatellite loci (i) Cuμ28 and (ii) Cuμ32, in twelve populations of European breeding Blackbirds (top three rows), and Blackbirds caught in the UK & Ireland during the non-breeding season (i.e. winter birds; bottom row). Alleles found in a single breeding population and not present in any of the other breeding populations are shaded with vertical lines; alleles present in the non-breeding birds but not found in any of the breeding populations are shaded in black.

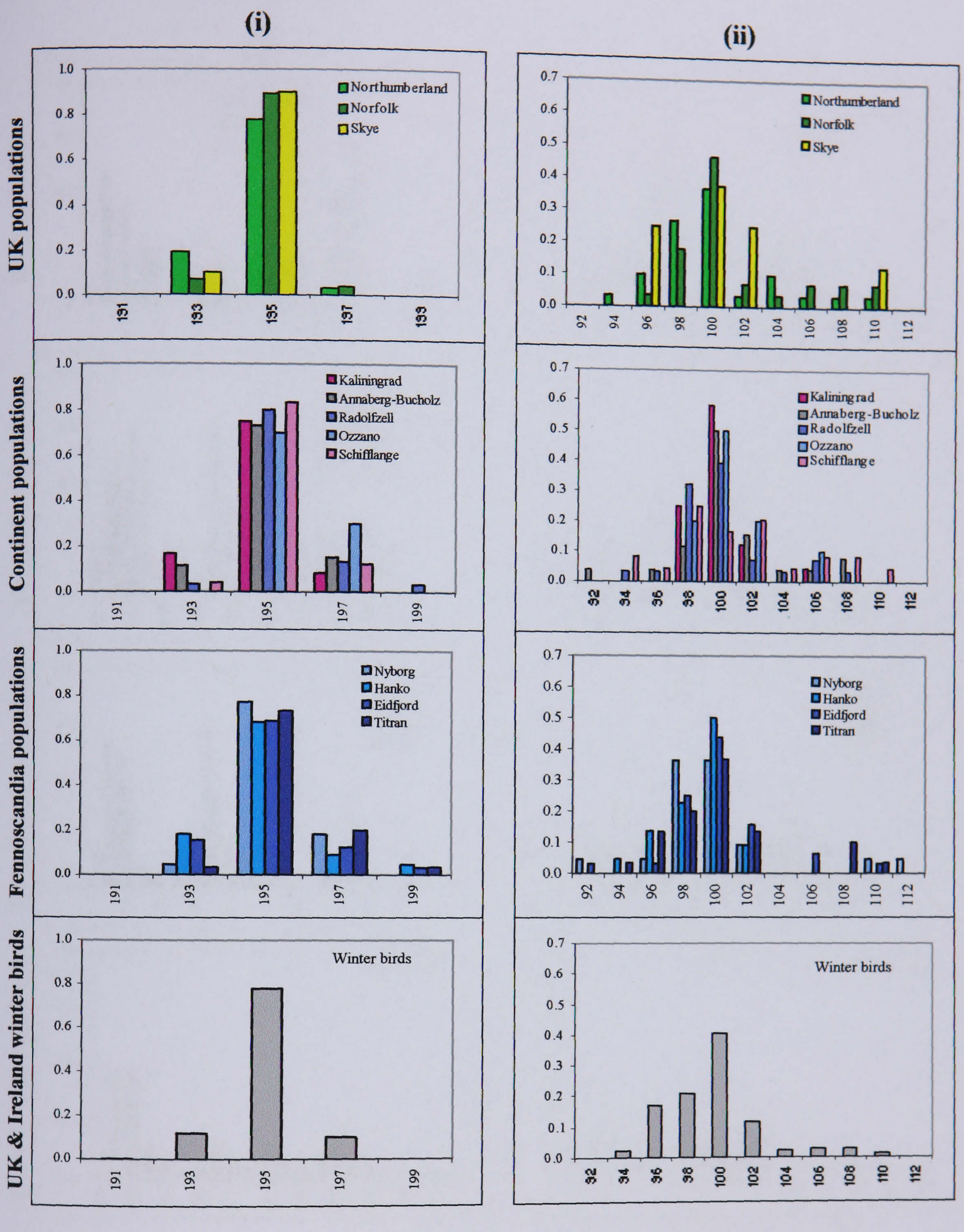


Figure A2.1(b) Allele frequencies at microsatellite loci (i) BB3 and (ii) TGU07, in twelve populations of European breeding Blackbirds (top three rows), and Blackbirds caught in the UK & Ireland during the non-breeding season (bottom row). Alleles found in a single breeding population and not present in any of the other breeding populations are shaded with vertical lines.

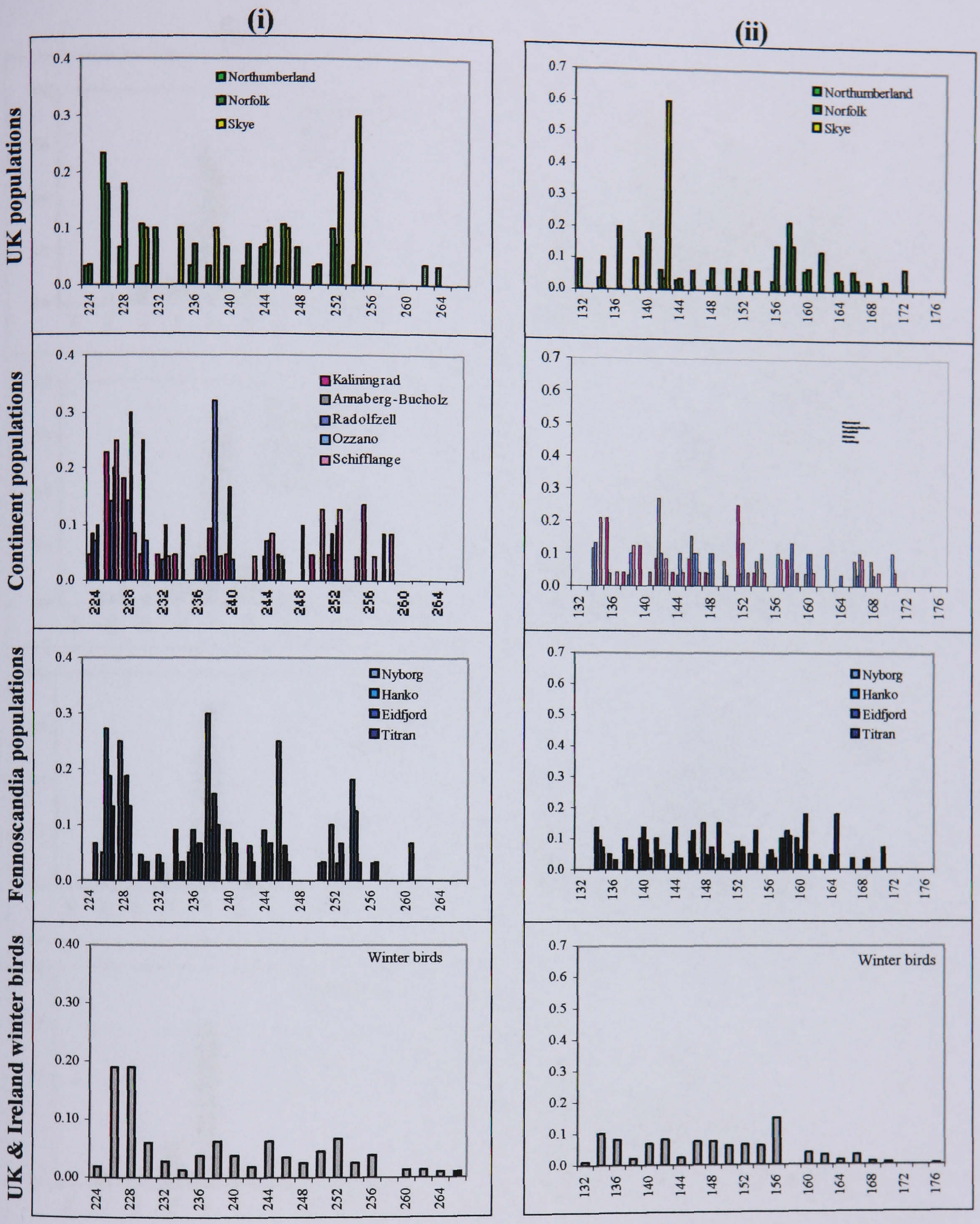


Figure A2.1(c) Allele frequencies at microsatellite loci (i) Ase40 and (ii) PAT43, in twelve populations of European breeding Blackbirds (top three rows), and Blackbirds caught in the UK & Ireland during the non-breeding season (i.e. winter birds; bottom row). Alleles found in a single breeding population and not present in any of the other breeding populations are shaded with vertical lines; alleles present in the non-breeding birds but not found in any of the breeding populations are shaded in black.

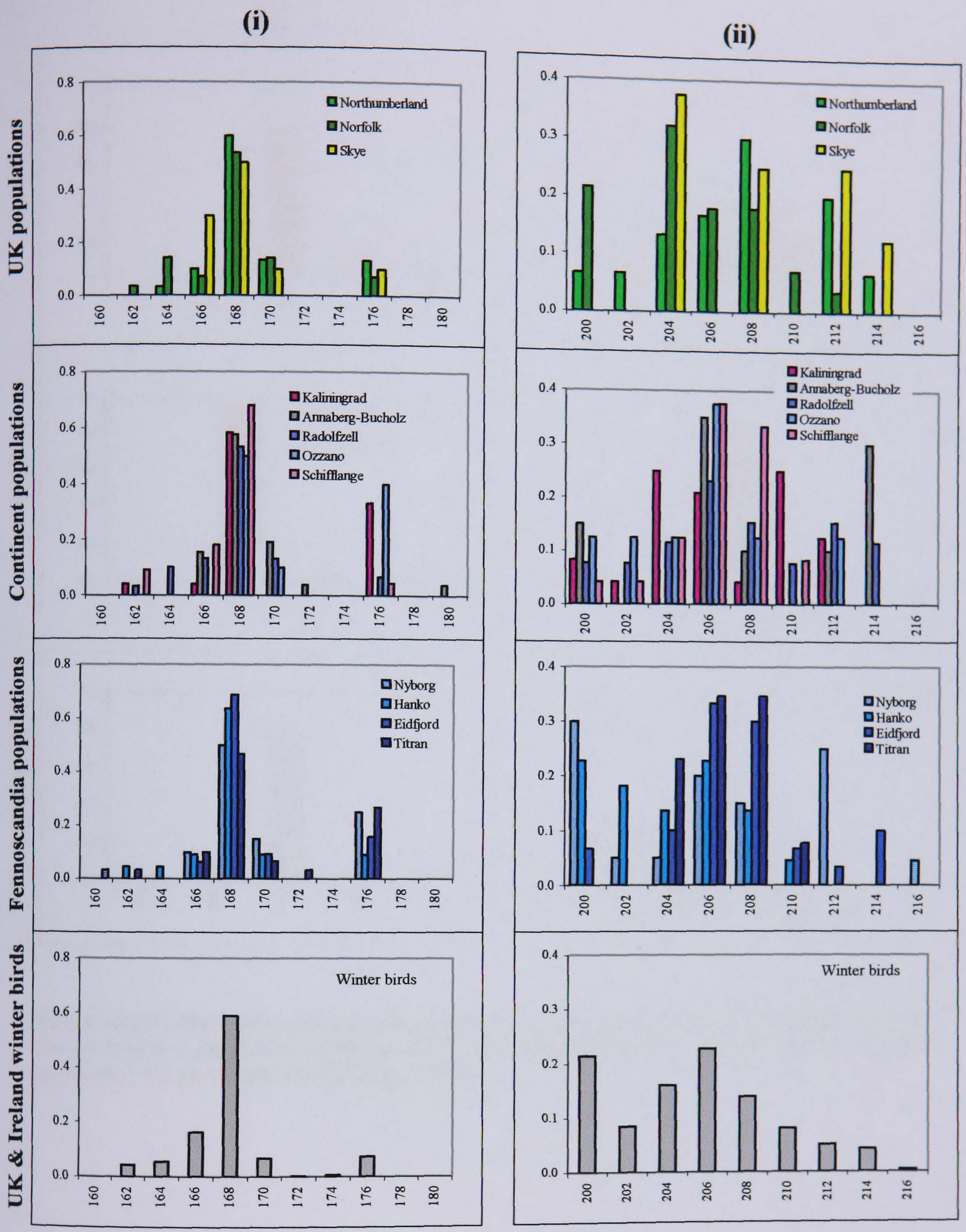


Figure A2.1(d) Allele frequencies at microsatellite loci (i) RW4 and (ii) RW9, in twelve populations of European breeding Blackbirds (top three rows), and Blackbirds caught in the UK & Ireland during the non-breeding season (i.e. winter birds; bottom row). Alleles found in a single breeding population and not present in any of the other breeding populations are shaded with vertical lines; alleles present in the non-breeding birds but not found in any of the breeding populations are shaded in black.

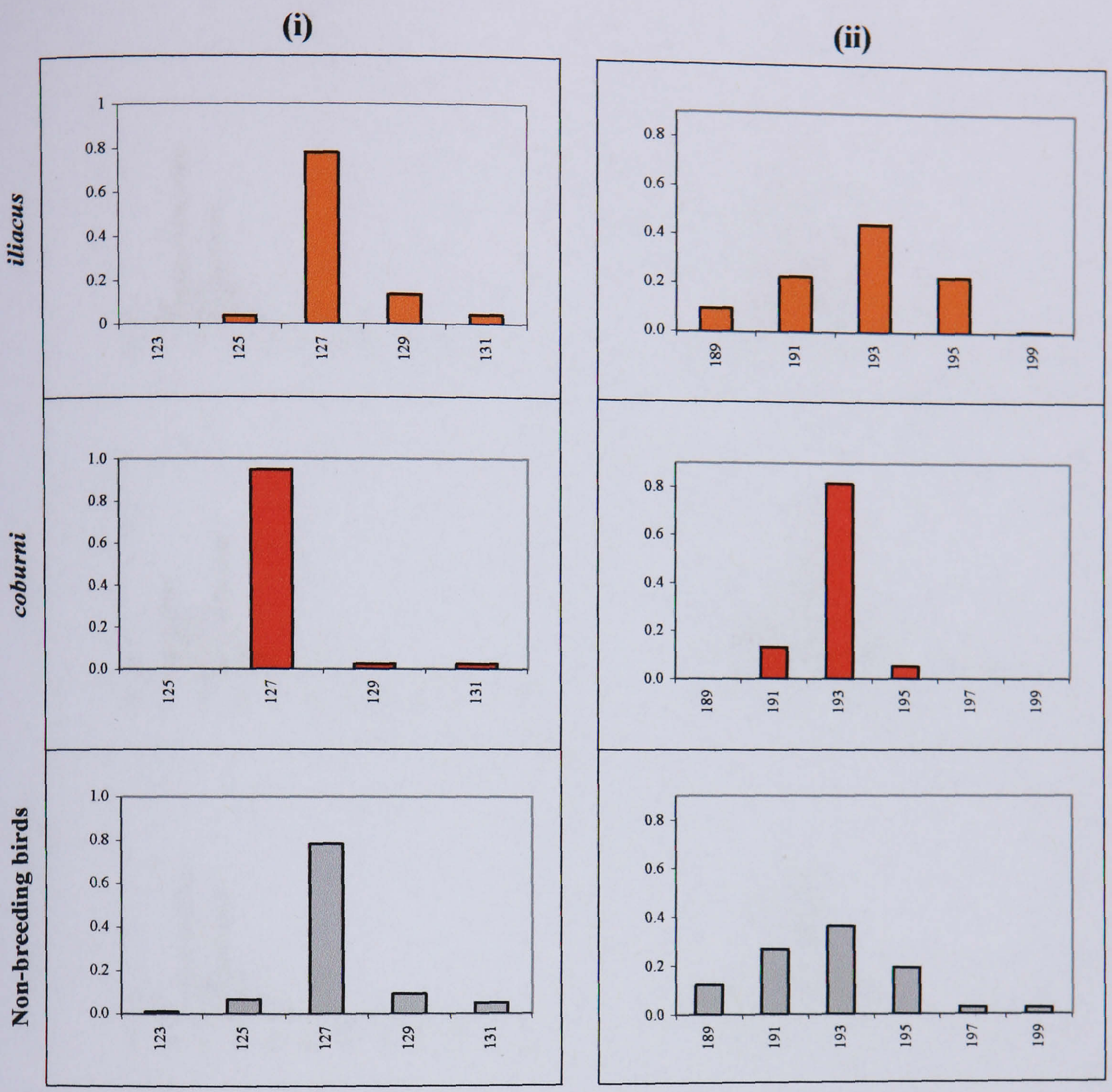


Figure A2.2(a) Allele frequencies at microsatellite loci (i) McyMu and (ii) BB3, in populations of two races of breeding Redwings iliacus *(top row) and* coburni *(centre row), and Redwings caught in Bardsey, UK during the non-breeding season.*

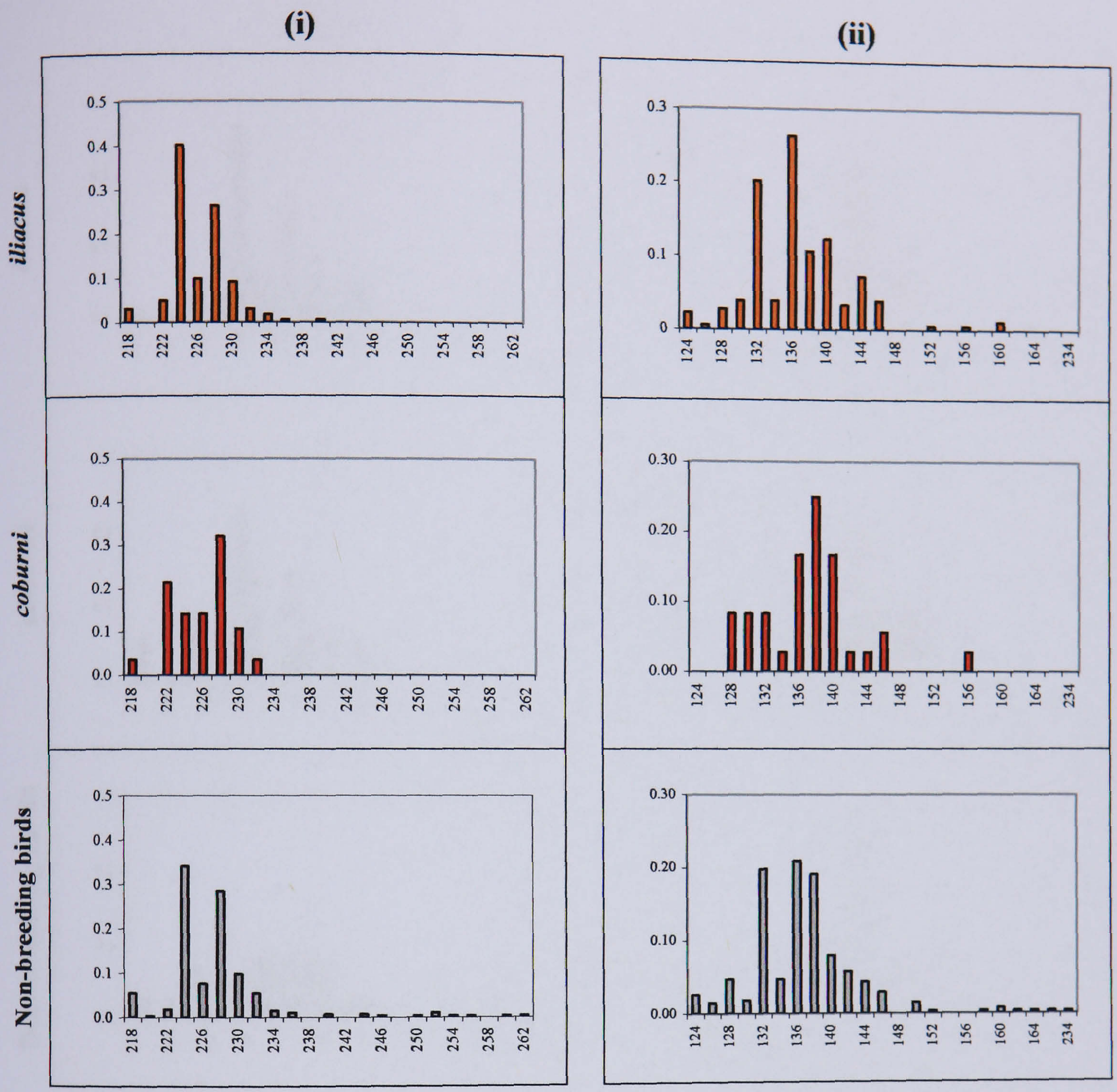


Figure A2.2(b) Allele frequencies at microsatellite loci (i) Ase40 and (ii) PAT43, in populations of two races of breeding Redwings iliacus *(top row) and* coburni *(centre row), and Redwings caught in Bardsey, UK during the non-breeding season.*

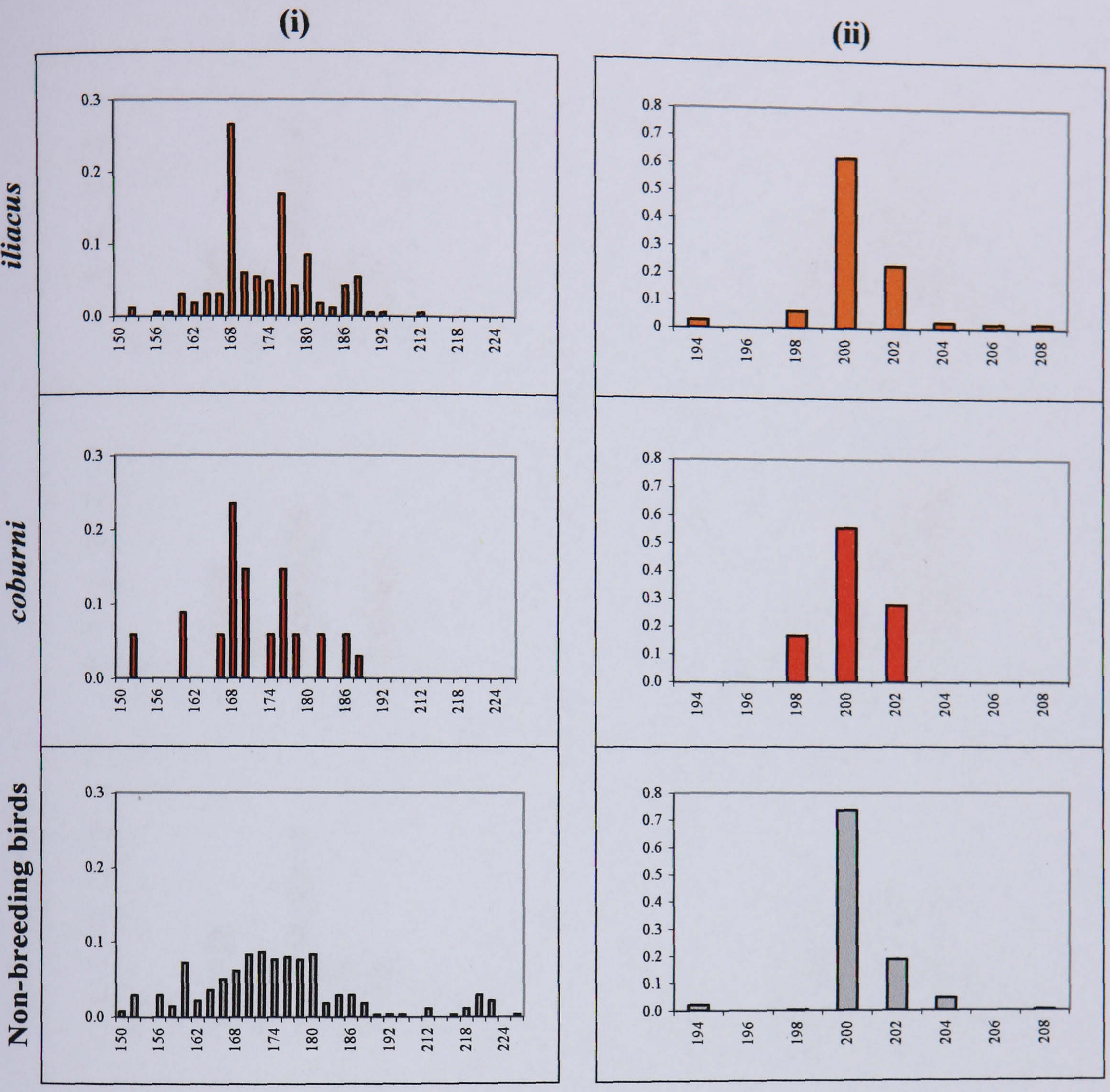


Figure A2.2(c) Allele frequencies at microsatellite loci (i) RW4 and (ii) RW9, in populations of two races of breeding Redwings iliacus *(top row) and* coburni *(centre row), and Redwings caught in Bardsey, UK during the non-breeding season.*

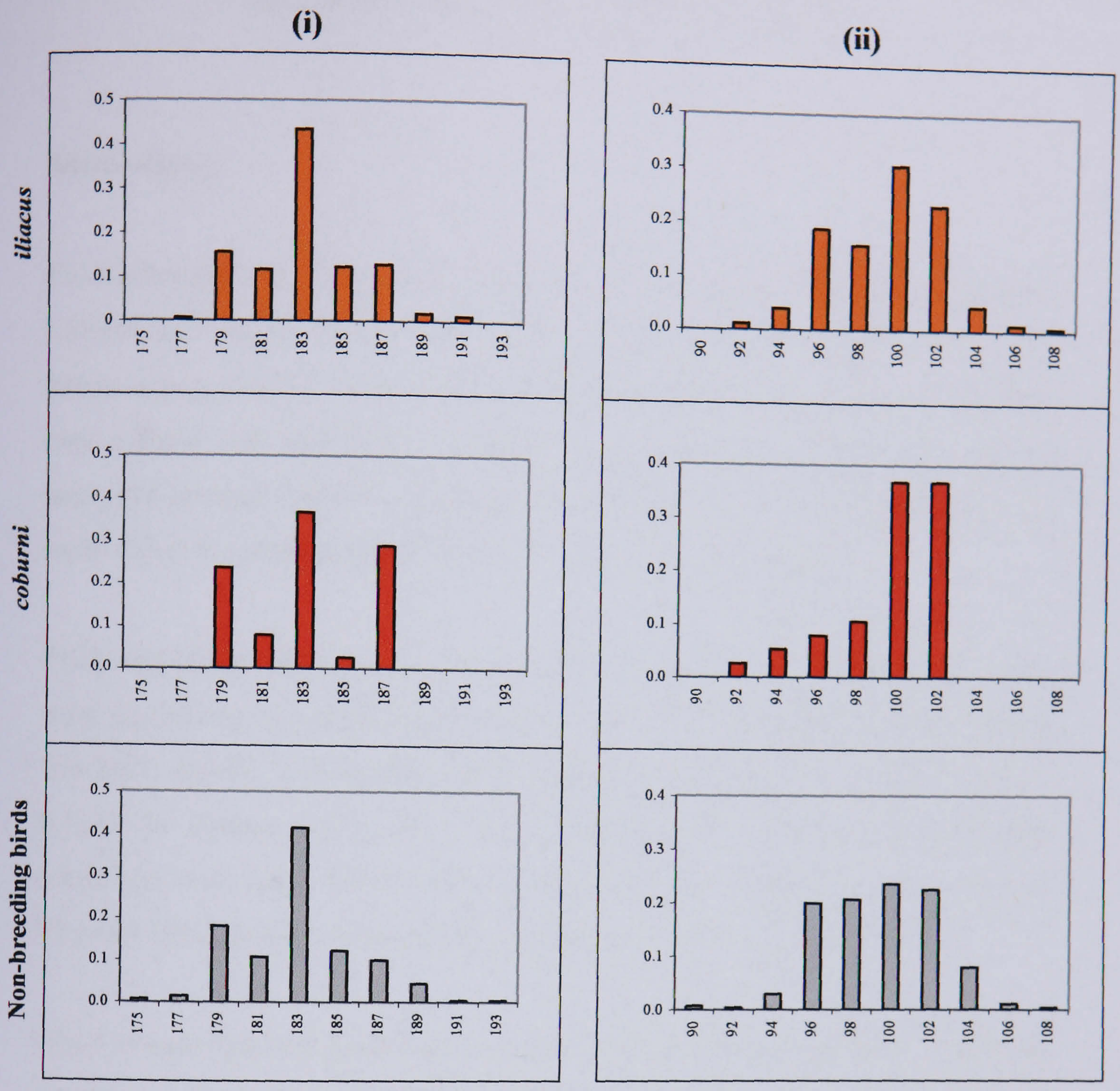


Figure A2.2(b) Allele frequencies at microsatellite loci (i) TGU06 and (ii) TGU07, in populations of two races of breeding Redwings iliacus *(top row) and* coburni *(centre row), and Redwings caught in Bardsey, UK during the non-breeding season.*

APPENDIX 3: Preliminary Investigation Into Population Genetic Structure of MtDNA Control-region Sequences

Methodology

For Redwings, a mtDNA control region segment was amplified using the primers L16743 and H1248 (Tarr, 1995). Five to eight individuals from each of eight breeding populations, seven of the nominate race *iliacus*, and one of the Icelandic race *coburni*. For Blackbirds, a mtDNA control region segment was amplified using the primers L16743 and H417 (Tarr, 1995). Two to four individuals from each of five breeding populations were selected for sequencing.

DNA extraction methodology is described in Chapter 3. PCR amplifications were performed in a total volume of 20µl (1 x *Taq* buffer [$(NH_4)_2$ SO_4, 67mM Tris-HCl, 0.01% Tween-20], 2mM $MgCl_2$, 0.1mM dNTPs, 0.4µM L16743; 0.4µM H Primer and 1.5U *Taq* (Bioline)). The following amplification conditions were used: 94°C for 3min, then 35 cycles of 94°C for 40s, 50°C for 40s, and 72°C for 90s, with a final extension step of 72°C for 4min.

Cycle sequencing was performed in a total volume of 10µl, using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems) with primer H417 for Blackbirds, and primer L16743 for Redwings. Sequencing of Blackbird samples was performed at Newcastle University, under the following conditions: 35 cycles of 96°C for 10s, 50°C for 5s, and 60°C for 4min. The sequencing products were then purified with NaAc and ethanol, and resuspended in 12µl ultrapure H_2O. Blackbird samples were analysed on an ABI Prism®310 automated sequencer.

Sequencing of Redwing samples was performed at the NERC Molecular Genetics Facility at Edinburgh University, under the following conditions: 25 cycles of 95°C for 30s, 50°C for 20s, and 60°C for 4min. Samples were cleaned using Edge Biosystems Performa DTR V3 plates, then transferred to plates, and dried in a vacuum concentrator. 10µl of Hi-Di Formamide was added to each

well, and the plate briefly heated to 95°C for 2mins, then cooled to 4°C. Redwing samples were analysed on an ABI Prism®3730 automated sequencer.

All sequences were aligned and edited using BioEdit (version 7.0.9.0; Hall, 1999).

Results

For Redwings, a section of mtDNA approximately 650bp long was sequenced. However, no clear differences were found, either within or between any of the breeding population sequences, Figure A.2.

For Blackbirds, a section of mtDNA approximately 400bp long was sequenced. Differences were found both within and between breeding population sequences, although a number of nucleotides could not be defined with certainty, making it difficult to quantify levels of within versus between population variation. A 66bp section of the mtDNA sequence (selected to illustrate differences between individuals and populations) in 14 Blackbirds (from five different breeding populations) is illustrated in Table A.2.

TTCAGGACATAACATCCCCC~TACACCCTAG~CACAACTTGCTCTTTTGCGCCACTGGTTCCTATTTCAGGGCCATAACTTGA
TCCATTCCTTCTCTCTTGCTCTTCACAGATACAAGCGGTCGGGATGAATACTCCTCATTCTCTCTCGTAATCGCGGCATTCCG
ACCGTCTCTGCACTTTTCCTTTTCTGGGGGTCTCTTCAATAAGCCCTTCAAGTGCGTAGCAGGTGATCCCTTCCTCTTGACAT
GTCCATCACATGTGCGTCGAACTATCGTCCCCTGAAACCGCATAACTTGTCATGGTTTCATCGTATAACCCGTCGCATACTCG
GATACTGATGCACTTTGACCTCATTCACGAGGGGGAGGTTATTTGCCTCTTAAGTATGCAGATAATGCAATGGTCACCGGAC
ATACTCAATTTATTTCCTCTTTCTAGGATTTTCCATCTAAACTCCCAAAAAACCATCATTTTTTGTTCGTTTATTTTTATCTTGA
CATTTTTGTTTACATTAACTAAAAATTAACCAAATTTTTAACCACATCTCCCTACCTCACACCAAAACATTTACCATCACAGT
ATCAACAAACAACTTTCCTCTATTTTCCCCCTGCCAATCAATCCACAAAAACAAATACCTTCCCTTCCCCCCCCC

Figure A.3 A 657bp section of mtDNA control region segment (L16743; Tarr, 1995) in Redwings (n = 51) from eight different European breeding populations. No clear differences either within or between breeding populations were found.

Table A.3 A 66bp section of mtDNA control region segment (H417; Tarr, 1995) in Blackbirds (n = 14) from five different European breeding populations. The first sequence is displayed in colour; thereafter, only nucleotide differences between individuals are displayed in colour. Nucleotides that could not be defined are designated 'N'.

Population	**MtDNA Sequence**
Titran, NOR	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCCATCTTAAGCGACGCCCGCGGCCTAGGCCAAAGGT
	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCNATCTTAAGCGACGCCCGCGGCCTAGGCCAGAGGT
	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCCATCTTAAGCGACGCCCGCGGCCTGGGCCAGAGGT
	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCCATCTTAAGCGACGCCNGCGGCCTAGGCCAGAGGT
Hanko, FIN	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCGATCTTAAGCGACGCCGGCGGCCTAGGCCAAAGGT
	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCGATCTTAAGCGACGCCGGCGGCCTGGGCCAAGGGT
	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCGATCTTAAGCGACGCCGGCGGCCTANGCCAAAGGT
	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCGATCTTAAGCGACGCCGGCGGCCTAGGCCAAAGGT
Radolfzell, DEU	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCCATCTTAAGCGACGCCCGCGGCCTGGGCCAGAGGT
	GAGTGGATTTTGGGTATTAAGGAGCAGTGTCCATCTTAAGCGACGCCNGCGGCCTGAGCCAAAGGT
Norfolk, GBR	GNGTGGATTTTGGGTATAAAGGAGCAGTGTCANTCTTAAGCGACGCCNGCGGCCTGGGCCAGANGT
	GNGTGGATTTTGGGTATAAAGGAGCAGTGTCANTCTTAAGCGACGCCNGCGGCCTANGCCNGANGT
	GNATGGATTTTGGGTATAAAGGAGCAGTGTCANTCTTAAGCGACGCCNGCGGCCTANGCCAGANGT
	GAATGGATTTTGGGTATAAAGGAGCAGTGTCANTCTTAAGCGACGCCNGCGGCCTGGGCCNGANGT
Eidfjord, NOR	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCGATCTTAAGCGACGCCGGCGGCCTAGGCCAGAGGT
	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCGATCTTAAGCGACGCCGGCGGCCTGGGCCAGAGGT
	GAGTGGATTTTGGGTATAAAGGAGCAGTGTCGATCTTAAGCGACGCCGGCGGCCTAGGCCAAAGGT
	GGATGGATTTTGGGTATAAAGGAGCAGTGTCGATCTTAAGCGACGCCGGCGGCCTAGGCCAGAGGT

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