Spleen size variation during long-distance migration in the garden warbler *Sylvia borin*

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Long-distance migratory passerines show extreme flexibility in body and organ mass in adaptation to the demands of their annual spring and autumn migrations. The extent of observed mass changes commonly entails temporary organ dysfunction. We collected garden warblers *Sylvia borin* at various phases during their spring and autumn migration in order to explore a potential trade-off between investment in migration and immune function by examining spleen size. The avian spleen is the principal organ for resistance to disease and parasitic infection in birds and spleen size is assumed to be reflective of immune activity, especially antibody production. If spleen size indeed reflects spleen function, and thus immune activity, there may be a trade-off between immune function and migration, both during the migratory flight itself when there is no nutritional intake, as well as during the stopover phase when depleted tissues necessary to continue migration are being restored. Spleen mass remained at the low level observed immediately after a flight across a major ecological barrier, in contrast to both body mass and other organ masses, which were readily restored during stopover. Clear benefits of a reduced spleen are most likely to occur if spleen maintenance and spleen function are costly, but these costs are unknown.
Key words: garden warbler, *Sylvia borin*, body composition, phenotypic flexibility, immune function, resource allocation, trade off.

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During migration, many birds alternate between short, but energetically costly, flight phases (2–3 days) and longer refuelling phases of 2–3 weeks (Fransson 1995, Biebach 1998). The visiting of a series of geographical sites during stopover is a crucial aspect of migration from the viewpoint of immune functioning. One may assume that the encounter rate with new, unknown pathogens increases with the number of different sites visited. Overall, migratory species may be exposed to a larger variety of infectious agents and their antigens than resident species, and the former may thus require higher investment in the immune system. Indeed, migratory species have been shown to have larger lymphoid organs involved in the immune system (bursa of Fabricius and spleen) than closely related resident species (Møller & Erritzøe 1998). However, migratory pied flycatchers *Fidecula hypoleuca* caught upon arrival at the breeding grounds had reduced spleen sizes and reduced lymphoid activity (Silverin 1981, Fänge & Silverin 1985). Migration is also an episode in a bird's annual cycle with high rates of energy turnover and the energetically costly processes necessary to complete migration successfully may deprive other processes, such as immune function. In several other situations of endurance exercise a trade-off can occur between energy turnover and some measure of immune defence (due to a secondary sexual character: Saino & Møller 1996; breeding: Deerenberg et al. 1997, Nordling et al. 1998;
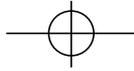
flying: König & Schmidt-Hempel 1995). Such trade-offs are consistent with the proximate resource allocation hypothesis, which assumes a costly immune system and immune functioning (Sheldon & Verhulst 1996). Ultimately, the trade-off may reflect an optimal allocation of resources to maximise fitness (Deerenberg et al. 1997).

Spleen function in birds is far more oriented towards disease resistance than to erythropoiesis and storage of blood as compared to spleen function in mammals. In mature birds, the spleen is the major lymphoid organ, with a pivotal role in humoral and cell-mediated components of immune function (John 1994). The size of lymphoid organs such as the spleen is used in toxicological screening methods as indicators of an immunopathological condition, i.e., a currently activated immune system (e.g. Luster et al. 1998, 1992, 1993, Weeks et al. 1992, Dietert et al. 1996). Recent investigations on cliff swallows *Petrochelidon pyrrhonota* showed that spleen volume varies with parasite load, indicating a close positive correlation between spleen size and ecto-parasitism (Brown & Bomberger Brown 2002). However, spleen size in snow geese *Chen caerulescens caerulescens* sampled during winter and migration was only weakly correlated to helminth load (Shutler et al. 1999). Histological examination of spleens of migratory pied flycatchers (Fänge & Silverin 1985) and non-migratory willow tits *Parus montanus* (Silverin et al. 1999) showed that large spleens had a high diversity of splenic tissue structures containing large volumes of lymphoid components, whereas small spleens had low tissue diversity and all tissue types were poorly developed. Their results strongly suggested that large spleens were immunologically active (production of antibody), in reaction to current antigenic challenges, whereas small spleens (less than 25 % of large spleen weight) were inactive.

In contrast to coastal species inhabiting pathogen-poor environments (Piersma 1997), in migratory 'land' birds there may be a conflicting need for reduction of the immune system to meet high energy demands during migratory flight and stopover and for a functional immune system to avoid risks of infection during stopover phases. The question we address here is: What happens to the spleen of migratory 'land' birds given these conflicting demands on the immune system? Several strategies can be envisioned for migratory birds to deal with this conflict. First, adopting the hypothesis of

costly immune functioning (Lochmiller & Deerenberg 2000), there may be a seasonal shut-off of immune defence mechanisms to allow allocation of resources to other processes, resulting in a reduced spleen size. Second, such a reduction may be temporary, i.e., during migratory flight only. A third strategy emerges from the notion that spleen size probably reflects current immune activity, i.e. reactions to new antigenic challenges. Thus migratory birds may maintain or even enhance spleen size (by analogy to the winter immuno-enhancement hypothesis: Nelson & Demas 1996), because the birds are frequently attacked by new antigenic agents and spleen function is too important for survival to be sacrificed.

In this study we examine the pattern of changes in spleen size of garden warblers *Sylvia borin* during winter residence, spring and autumn migration. The results may shed light on the reasons behind changes in spleen size in other bird species during other phases of the annual cycle.

Methods

Sampling locations

Birds were collected for carcass analysis in their wintering quarters, and during migration prior to and directly after the crossing of a major ecological barrier: the Sahara desert and the Mediterranean sea (Fig. 1). In their wintering quarters in Tanzania, birds were collected near Sanja Juu (3°10' S, 37°05' E) between 16 and 29 March 1997. Some of these birds were still moulting, but variation in mass or fat was not related to moult status. During the spring migration, birds were captured in NE Ethiopia near Jijiga (9°21' N, 43°48' E) between 26 April and 2 May 1998, and on the Mediterranean coast of Egypt at Zaranik (31°08' N, 33°25' E) between 7 and 11 May in 1996, and again between 29 April and 17 May 1998. The Ethiopian birds were probably about to leave for their next migratory flight phase across the Sahara. In Egypt, birds were caught during the early morning hours and had probably just completed a flight lasting several days across the Sahara (Biebach et al. 2000). During autumn migration between 30 August and 5 September 1996, we caught birds at a stopover site in Kargicak, SE Turkey (36°40' N, 33°25' E), close to the Mediterranean coast. The exact status of these
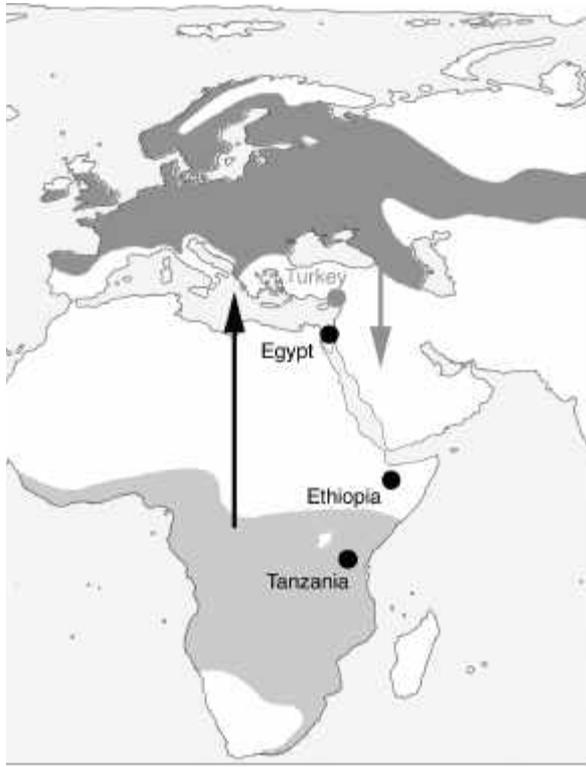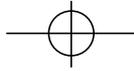
Figure 1. Map of Africa, part of Asia and Europe showing the various catching locations of gardens warblers. Dark grey area in Europe indicates breeding range for garden warblers, light grey area in Africa gives the wintering range. Grey arrow and sampling location refer to autumn migration, black arrow and sampling locations refer to spring migration.

birds – middle or late stopover phase – was less well defined (see also Bauchinger & Biebach 2001 for a detailed description of the sites in Egypt and Turkey). At each location, a sample of the birds was killed: 18 in Tanzania, 9 in Ethiopia, in Egypt 9 birds in 1996 and 10 in 1998 and 12 in Turkey.

Field experiment

In both years in Egypt, an experiment was carried out to simulate a stopover phase. Upon capture ten birds were transferred to cages and kept at natural ambient temperature and photoperiod for several days with food provided at libitum (in 1996 for 7 days with water and mealworms *Tenebrio molitor* and in 1998 for 9 days on a standard diet (Gwinner et al. 1988; see Hume & Bie-

bach 1996 for details). These birds were killed by cervical dislocation on the morning of the seventh or ninth day, respectively, and their bodies were processed as described below (for more details see Bauchinger & Biebach 1998, 2001).

Phenotypic measures

All birds were weighed to the nearest 0.1 g immediately after trapping, and wing length, tarsus length, and fat and breast muscle score were measured. Of the birds that were killed for carcass analysis, the breast (left M. pectoralis major and M. supracoracoideus) and lower leg muscles of one side and the intestinal tract were removed within a few minutes and frozen (muscles) or stored separately in paraformaldehyde for later analysis. The remaining carcass was also stored frozen. Further dissection took place in the laboratory and 'wet' mass was determined, i.e., mass of the organs soaked in paraformaldehyde after excess fluid had been dried off. Apart from the breast and leg muscles, organs of the digestive tract (proventriculus, gizzard, small intestine, colon), and other organs comprising heart, liver and spleen were removed and measured. To assess comparability between fresh organ mass and organ mass after storage in paraformaldehyde, a calibration experiment on hearts and spleens of eight zebra finches *Poephila guttata* was performed. After 400 days of storage in paraformaldehyde, both organs revealed almost identical mass compared to fresh mass measured immediately after dissection. Linear regression analysis of fresh mass (m_f) to mass after storage in paraformaldehyde (m_p) showed a close fit (linear regression, spleen: $m_p = 0.001 + 0.954 m_f$; heart: $m_p = -0.014 + 1.052 m_f$; $R^2 = 0.99$, $P < 0.001$ for both organs; spleen mean $m_f \pm 95 \%$ C.I. = 0.0168 g $\pm$ 0.0071; heart mean $m_f \pm 95 \%$ C.I. = 0.1977 g $\pm$ 0.0203). Results for muscles and intestinal tract have been reported elsewhere (Biebach 1998, Bauchinger & Biebach 1998, Bauchinger & Biebach 2001).

Statistical analysis

Comparison of mass among localities, with Egypt 1996 and 1998 included as two different groups, was analysed by one-way ANOVA, followed by a Tukey-HSD test when group values differed significantly. The differences between the experimental groups in Egypt (the
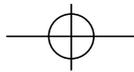
two years were analysed separately) were examined using linear regression with year and experimental effect included as categorial variables. All values are expressed as means ± s.e. All statistical analyses were done using the SPSS statistical package.

Results

Migratory phases

Body mass changed markedly with migratory phase (Fig. 2a). Total body mass in winter (Tanzania) and prior to a migratory flight (Ethiopia) was around 20 g. Immediately after the flight across the Sahara, body mass was reduced to about 16 g ($F_{4, 52} = 10.9, P < 0.001$; Biebach 1998). During autumn stopover (Turkey) body

mass was restored to levels similar to those during winter and prior to migratory flight. Mass changes of other organs such as heart ($F_{4, 53} = 17.5, P < 0.001$) and liver ($F_{4, 53} = 27.3, P < 0.001$) showed a pattern more or less comparable to that of body mass. In contrast, however, spleen mass was not only reduced immediately after a flight phase, but remained low during stopover ($F_{4, 53} = 17.5, P < 0.001$). Spleen mass during autumn stopover was low and indistinguishable from spleen mass immediately after the Sahara crossing during spring migration (Fig. 2b).

Experimental stopover

Several birds caught in Egypt after the spring migratory flight over the Sahara were allowed to recover for 7 (1996) or 9 days (1998) with ad libitum food. The du-
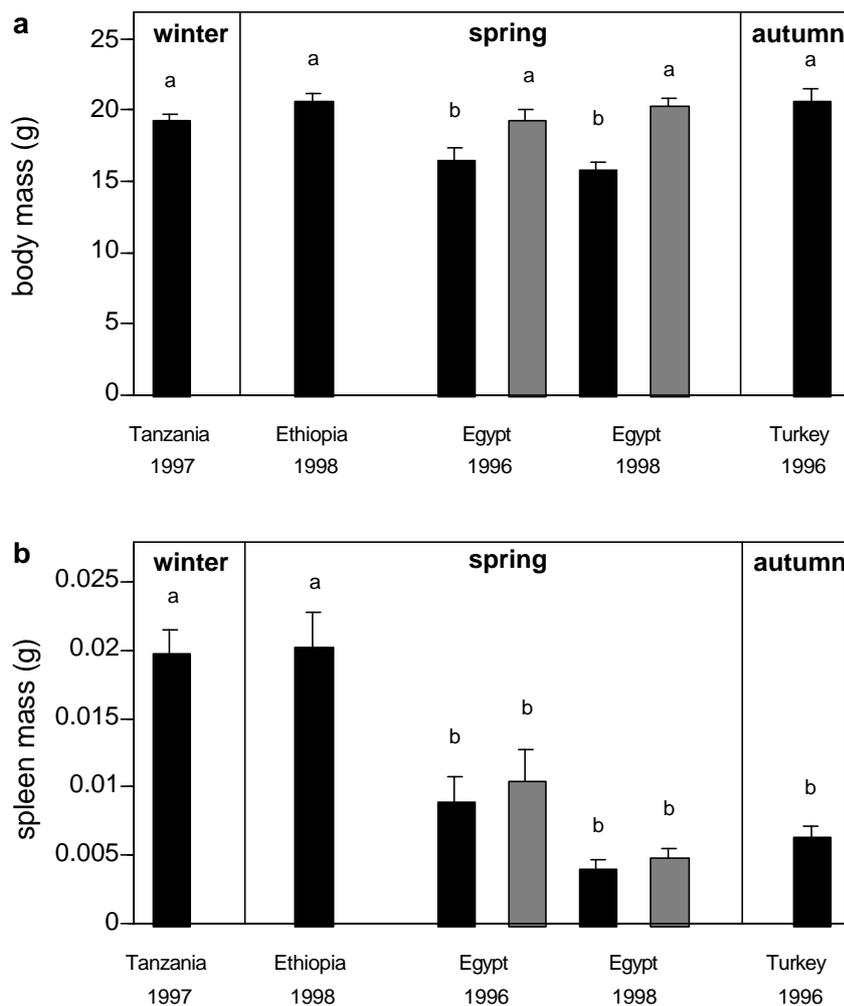
Figure 2. (a) Average body mass and (b) spleen mass of garden warblers in winter and during spring and autumn migration. The Tanzanian site lay within the wintering quarters, in Ethiopia birds were about to cross a major ecological barrier, the Egyptian site was immediately after the barrier and in Turkey birds were caught at a stopover site. Grey histograms indicate birds experimentally forced into stopover by caging them and providing them with ad libitum food; bars indicate 1 s.e.; a and b refer to significantly different means as determined by Tukey-HSD tests.
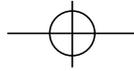
Table 1. Mean ± s.e. of total body mass and organ mass (g) of garden warblers caught in Egypt immediately after crossing the Sahara (post-flight) and of birds that were kept in captivity on ad libitum food for 7 or 9 days to simulate stopover.

| | 1996 | | 1998 | |
	Postflight	Day 7	Postflight	Day 9
Sample size	9		10	
Body	15.97 ± 0.60	20.04 ± 0.55	16.56 ± 0.81	19.16 ± 0.55
Heart	0.112 ± 0.015	0.165 ± 0.015	0.148 ± 0.007	0.179 ± 0.007
Liver	0.231 ± 0.039	0.317 ± 0.025	0.343 ± 0.011	0.479 ± 0.029
Spleen	0.004 ± 0.001	0.005 ± 0.001	0.009 ± 0.002	0.011 ± 0.003

ration of this experimental stopover phase sufficed for the birds to reach stabilised levels of body mass (Bauchinger & Biebach 1998). Body and organ masses upon arrival and at the end of the experimental stopover are listed in Table 1. Upon arrival at the catching location, body masses were similar in 1996 and 1998, but organ masses were higher in 1998 (Table 2). Body mass, heart and liver mass recovered significantly during the experiment (Table 2). The same was true for breast and leg muscles (Biebach 1998, Bauchinger & Biebach 2001).

The spleen was exceptional in that its mass did not change significantly during the experimental recovery phase and remained very small, at half of its winter or pre-flight value (in 1996) or even smaller (in 1998).

Discussion

Our results suggest that spleen mass may not only be reduced during flight phases but that spleens remain

Table 2. Linear regression models of body or organ mass of garden warblers on year and effect of experiment. P-values refer to the contribution to the explained variance of the model. The effect of the first-order interactions of year and experiment on mass were also tested, but proved to be non-significant in all cases.

		(Increase in) deviance	(Increase in) d.f.	P
Body mass	Final model	122.95	38	
	Constant		1	
	Year	0.62	1	n.s. (rejected)
	Experiment	116.61	1	<0.001
Heart mass	Final model	0.050	37	
	Constant		1	
	Year	0.006	1	<0.05
	Experiment	0.017	1	=0.001
Liver mass	Final model	0.267	37	
	Constant		1	
	Year	0.191	1	<0.001
	Experiment	0.122	1	<0.001
Spleen mass	Final model	$8.0 \, E^{-4}$	38	
	Constant		1	
	Year	$3.2 \, E^{-4}$	1	<0.001
	Experiment	$0.2 \, E^{-4}$	1	n.s. (rejected)
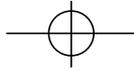
small during most of the migratory period. All the organs examined were atrophied after migratory flight – including those actively used during flight, such as breast muscle and heart (Biebach 1998, Bauchinger & Biebach 2001, Biebach & Bauchinger 2002). However, the latter were reduced to a lesser extent than other organs such as the digestive tract (Biebach 1998, Biebach & Bauchinger 2002), liver and spleen. Body mass and most organ masses are restored to a large extent during stopover (Biebach 1998, Bauchinger & Biebach 1998, this study). In contrast, during stopover spleen mass remained indistinguishable from post-flight values. This conclusion is supported by the data obtained in the field during autumn stopover, and by the measurements obtained during experimentally enforced stopover. Because we had no information about the geographic source of the birds, we cannot rule out the possibility that the observed pattern resulted from population-specific differences in organ mass.

Seasonal variation in mass of lymphoid organs has been described for a number of bird species (review in John 1994). In several migratory species, a reduced spleen has been observed after spring migration. The spleen of white-crowned sparrows *Zonotrichia leucophrys gambelii* was reduced relative to pre-migratory winter values (Oakeson 1953) and the spleen of pied flycatchers increased from low values upon arrival at the breeding grounds to maximum mass during breeding (Silverin 1981). In mallards *Anas platyrhynchos*, spleens of migrants, but also of wintering birds, were smaller than those of birds during both autumn and late-winter moult (Heitmeyer 1988). Thus, these studies are consistent with the present results that spleen mass is reduced immediately after migratory flight. Also, the magnitude of the observed changes (maximum about 50 %) was similar to the size differences observed in our study. Whereas spleens of mallards were already small prior to spring migration, there was no indication of such an anticipatory decrease in spleen mass in passerine migrants (white-crowned sparrow, Oakeson 1953; garden warbler, this study). Our study examined changes in spleen mass during migration, which may help to narrow down the potential factors causing the reduction in spleen size. It remains to be elucidated why spleens do not increase again in size during stopover, contrary to all other organs.

The rapid regrowth of these other organs may simply reflect that they are actively used during stopover, whereas the continued atrophied state of the spleen may indicate that the immune system is either not challenged, or does not respond to a challenge. The increase in spleen size and structural development during reproduction in the pied flycatcher (Fänge & Silverin 1985) can easily be explained by increased immune activity in response to increased pathogen pressure due to increased vector populations and vector activity in temperate zone spring and summer (Atkinson & Van Riper III 1991). Other factors influencing pathogen exposure include contact with conspecifics and variation in pathogen populations.

Whereas the cages in which the birds of the enforced stopover experiment in Egypt were kept may have shielded them from antigenic challenges, the autumn stopover birds in Turkey were caught in their natural environment and therefore we cannot exclude the possibility that in this situation the birds have not been exposed to new antigens. Thus, lack of exposure to new antigens is one possible explanation for the continued small spleen sizes during stopover.

It is also possible that the observed reduction in spleen size during migration is caused by immunosuppressive or immunoredistributing stress hormones (Braude et al. 1999). Although corticosterone levels were not elevated during migratory flight (Schwabl et al. 1991, Gwinner et al 1992), brief periods of stress featuring high levels of corticosterone may suffice to impose enough suppressive force to keep (parts of) the system in a non-reactive state. This may apply to birds arriving at stopover sites with depleted fat stores and emaciated flight muscles that have been shown to have high levels of corticosterone similar to levels during other stressful events (Jenni et al. 2001). However, plasma corticosterone concentrations of post-flight and day 9 birds in the Egypt experiment of 1998 were almost identical (< 2 ng/ml; Bauchinger 2002). It is therefore unlikely that an elevated stress response was the cause of the reduced spleen size during the examined phases of migration.

If we assume that in birds in general a small spleen is indicative of reduced structural diversity and impaired immune function, our data indicate a reduced adaptive immune activity during the whole migratory period. Although we found reduced spleens, there are no experimental data to show that this means reduced immune function. However, the finding that latent *Borrelia* infection in juvenile redwings *Turdus iliacus* are reacti-

vated during migratory restlessness (Gylfe et al. 2000), supports our idea of impaired immune function during migration. To assess whether a reduced spleen size during the migratory period indeed reflects reduced overall immune function one needs to assess the potential of the system to react by describing its state and by challenging the humoral and cell-mediated components. To date no information on this topic is available.
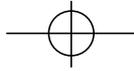
The benefits of continued reduced spleen size during migration may come from economising on the use of resources. As a result of inactive adaptive components of the immune system (in which the avian spleen plays a pivotal role), resources can be allocated to current prime activities, such rebuilding fat stores and organs needed to complete migration, and thereby increasing the rate of recovery and preparation during stopover. Higher fat loads increase flight range (Alerstam & Lindström 1990), and birds in higher body condition may have lower mortality rates during migration (Owen & Black 1989, Cooch et al. 1991, Francis et al. 1992). A shortened stopover time is also important given that migrating passerines follow in general a strategy of time minimisation (review in Alerstam & Lindström 1990), and early arrival at the breeding may enhance reproductive success (Møller 1994).

The magnitude of the benefits of reduced spleen size depends on its physiological, i.e., metabolic costs. A small, atrophied spleen is immunologically inactive (Fänge & Silverin 1985, Silverin et al. 1999) and its maintenance costs are probably low. The larger spleens as found in winter, pre-flight and on the breeding grounds reflect normal activation, a situation in which no overt signs of disease are apparent, but in which the adaptive immune system is active against non-self antigens present in the environment, such as food antigens and commensal microbes. Neither the metabolic costs of an small atrophied spleen, nor those of a large and active, functional spleen are available in the literature.

The costs of reduced spleen size during migration should further be evaluated in terms of risks, e.g., contraction or relapse of a (parasitic) infection, which are widespread among (migratory) birds, especially passerines (Greiner et al. 1975, Bennett et al. 1982, Valkiunas 1993). Studies assessing and quantifying these risks and their metabolic and fitness costs are absent.

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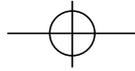
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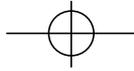
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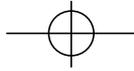
Population densities of forest birds in a heavy metal pollution gradient

Tapio Eeva[1], Vesa Koivunen[2] and Harri Hakkarainen[1]

We censused forest birds along the pollution gradient of a copper smelter in southwest Finland to investigate whether a long-term heavy metal pollution has decreased bird densities in the surroundings of a pollution source. Point counts were performed at nest-box sites for which there are long-term data on the breeding densities of cavity-nesting species. In addition, control points without nest-boxes were chosen to obtain density estimates free from the possible effect of artificial nest-sites. Six habitat variables were measured at each sampling point to separate the pollution effect from habitat variation. Species richness was slightly lower in the polluted area, but the difference could be explained by habitat variables. In general, the bird community structure in the polluted area of Harjavalta corresponds relatively well with that of the background area. Of the 37 species analysed, six showed decreased density in the polluted area that could not be explained by any of the habitat variables. These species can be divided into two categories according to their ecology and feeding habits: ground feeders (robin *Erithacus rubecula*, blackbird *Turdus merula*, song thrush *T. philomelos*) and conifer foliage gleaners (goldcrest *Regulus regulus*, crested tit *Parus cristatus*, willow tit *P. montanus*). Ground feeders are probably affected negatively by pollution-related changes in the soil and ground layer. Foliage gleaners may have suffered from the loss and poor quality of needles in coniferous trees and consequent decrease in abundance of their invertebrate food.
Key words: population density, forest birds, community changes, pollution, heavy metals.

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Birds are considered to be good indicators of environmental changes (Koskimies 1989, Furness & Greenwood, 1993). Environmental pollution has led to some of the most prominent examples of negative human impact on bird populations (e.g. Ratcliffe 1967, Cooke 1973, Newton & Wyllie 1992, Furness 1993). In addition to many well documented cases of detrimental effects on individual species, a few studies have reported pollution-related changes at the level of bird communities (Flousek 1989, Tomek 1992, Gilyazov 1993). Despite an increasing number of pollution impact studies there is still a lack of studies connecting community level changes to environmental pollution. The prob-

able reason for this is that most often the wide geographic scale of pollution makes it difficult to acquire comparable and accurately controlled data on the effects of pollution on bird communities. An exception to this are point sources of pollutants which are surrounded by a heavily polluted zone, across which pollutant levels decrease with distance from the pollution source.

To determine what kind of effects air pollution might have on the population densities of breeding forest birds, we performed point counts at increasing distances from a point source of air pollutants, a copper smelter, which emits large quantities of heavy metals
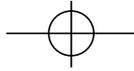
into the surroundings (Jussila & Jormalainen 1991). Point counts were carried out at nest-box sites for which there are long-term data on breeding densities of cavity-nesting species. We can thus compare the results of the point counts to the known densities of cavity-nesting species. In addition, control plots without nest-boxes were chosen to estimate bird densities free from the possible effect of artificial nest-sites. At the same time, we measured six habitat variables from each sampling point to separate the effects of pollution from habitat variation. We aim to identify the species or groups of species that are vulnerable to the long-term effects of heavy metal pollution.

Material and methods

Study area

The study was conducted in the surroundings of the town Harjavalta (61° 20' N, 22° 10' E), SW Finland in the summer of 2001. The main source of local air pollutants is a factory complex producing copper, nickel and fertilisers in the centre of the town. Sulphuric oxides and heavy metals (especially Cu, Zn, Pb and Ni) are common pollutants in the area (Kubin 1990, Jussila 1997). Elevated heavy metal concentrations occur in the polluted area due to current and long-term deposition from the copper smelter (e.g. Jussila 1997, Koricheva & Haukioja 1995, Eeva & Lehikoinen 1996). Heavy metal concentrations decrease exponentially with increasing distance from the smelter and approach normal background levels at sites farther than 5 km from the smelter. Some cavity-nesting birds breeding in the vicinity of the smelter suffer from low breeding success (Eeva & Lehikoinen 1996, Eeva et al. 1997) and reduced survival rates (Eeva & Lehikoinen 1998).

Bird censuses

The data were collected at 14 study sites between 0.8 and 11 km from the copper smelter, each of which had 30–50 nest-boxes (in total 587). Study sites were classified into three categories according to the distance to the pollution source (zone I, <2 km; zone II, 2–7 km; zone III, >7 km), corresponding to heavy, moderate and low levels of pollution (see also Eeva et al. 1997). Relative bird densities of forest birds were estimated by

point counts between May 21 and June 21. Point counts are considered a preferred method in fine-grained forest habitats that are typical of our study area (Bibby et al. 1992). Two points were counted at each nest-box site and, in addition, two control points were chosen outside the nest-box sites to provide density estimates free from the possible effect of artificial nest-sites on bird densities. The average minimum distance between sampling points was 278 m, which should guarantee that same birds are not recorded twice (recommended minimum distance is 250 m; Koskimies & Väisänen 1991). Point counts were performed by the method of Koskimies & Väisänen (1991) for counting breeding land birds. The censuses were carried out between 04.00 and 09.00 hrs, avoiding windy and rainy days. Each point was censused for 5 min. and for each species the number of observed individuals (pairs) was recorded. All censuses were done by the same observer. Each point was counted four times, once a week. The total number of point counts was 224 (14 sites × 4 points per site × 4 weeks). The mean density of four successive counts was calculated for each point and this was used as a dependent variable in analyses.

Habitat variables

The forests in the area are dominated by Scots pine *Pinus sylvestris*, which forms mixed stands with Norway spruce *Picea abies* and birches *Betula* spp. The field layer is dominated by dwarf shrubs *Vaccinium vitis-idaea* and *V. myrtillus*. At sites closest to the factory complex, ground layer vegetation is patchy and poorly developed due to the long-term effect of pollution (Salemaa & Vanha-Majamaa 1993).

Special attention was paid in selecting sampling points so that they would represent a similar habitat type, i.e. relatively barren pine-dominated forests typical of the study area. To account for the remaining variation we measured, at each sampling point, six habitat variables that we considered should describe the major natural habitat differences within our study area. Sampling sites were classified from the most barren to the most luxuriant according the type of ground layer vegetation, following Kalliola (1973): 1 = absent, 2 = *Cladonia* and *Calluna* types, 3 = *Vaccinium vitis-idaea* type, 4 = *Vaccinium myrtillus* type, 5 = *Oxalis acetosella* – *Vaccinium myrtillus* type. The relative proportions of the three dominant tree species were estimated
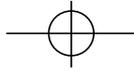
visually as follows: 1 = absent, 2 = sparse, 3 = moderate, 4 = dominant. Timber volume was measured by using a relascope and hypsometer: volume (m^3/ha) = basal area (m^2/ha) $\times$ 0.5 $\times$ tree height (m). Habitat patch size (ha) was estimated from digitised maps with Mapinfo 5.0 by measuring the size of continuous forest area around each point. From the habitat variables, three principal components (PC) were calculated using the PRINCOMP procedure of SAS (SAS Institute Inc. 1989). PC1 explained 37 %, PC2 28 % and PC3 13 % of the variation in data. From zones I to III the percentage of pine decreased from 55 to 32 %, spruce increased from 11 % to 28 % and birch remained relatively constant (from 23 % to 21 %).

Statistics

Bird densities were calculated from the point count data using the formula of Järvinen (1978): D (pairs/km^2) = $3 \times N \times c^2 / \pi$, where N = number of observations per counting point and c = species-specific constant that corrects for the differences in detectability (Järvinen & Väisänen 1983). Density estimates were not calculated for those species for which we had fewer than 10 observations. These species are included, however, in the calculations of species diversity. Shannon-Wiener diversity indices (H = $-3p_i \times \log_2 p_i$, where p_i = the proportion of ith species of the total density) were calculated from the corrected density estimates, not from the original counts.

From the five years of nest-box data (1996–2000) we calculated the nest-box occupancy rate (%) and the density of nests per km^2 at each study site and in each year, using the nearest-neighbour distance method (Krebs 1989): D = n / [$\pi \times 3(r^2)$], where D = population density, n = number of nests and r = distance to nearest nest of the same species.

The relationship between distance from the copper smelter and bird density was analysed by an ANCOVA model where distance (2nd order, km) to smelter, nest-box effect (0 = absent, 1 = present) and habitat variables were used as explaining factors. For the ANCOVA we selected the habitat variables that best correlated with the first three principal components (see above). These were: 1. the proportion of spruce, 2. habitat patch size and 3. timber volume. By including habitat variables as covariates in the models we aimed to explain natural habitat-related variation in our census data. Be-

cause long-term pollution has also affected vegetation, especially the field layer, habitat effects cannot totally be separated from pollution effect. However, this will only make our analysis more conservative with regard to the number of species that show significant pollution-related changes. Log-transformation was made on the distance and habitat patch size before the analyses to normalise distributions. All means are presented with their standard errors ($\pm$ s.e.).

Results

The mean number of species observed at sampling points was slightly lower in zone I (16.4 ± 0.54, n = 20) than in zones II and III (18.8 ± 0.57, n = 24 and 19.4 ± 0.38, n = 12, respectively; Tukey's test, df = 53, P < 0.05). However, the difference was due to the habitat effect: after adding the habitat variables into the model the effect of distance was no longer significant (ANCOVA, for distance $F_{1,49}$ = 0.04, P = 0.83). The proportion of spruce explained the majority of the variation in the data, even though the effect was only marginally significant (ANCOVA, for spruce $F_{2,49}$ = 3.02, P = 0.058). Similarly, the Shannon-Wiener diversity index was slightly lower in zone I (3.2 ± 0.05, n = 20), than in zones II and III (3.5 ± 0.05, n = 24 and 3.5 ± 0.05, n = 12, respectively; Tukey's test, df = 53, P < 0.05), but the difference could be explained by the habitat effect (ANCOVA, for distance $F_{1,49}$ = 0.34, P = 0.56).

Bird densities are shown in Table 1. After taking into account the habitat effects there were six species that showed a positive relationship and two species showing a negative relationship to the distance from the pollution source. Species that were less abundant in the polluted area (decrease in % from background level; Fig. 1) were: crested tit *Parus cristatus* (77 % decrease), willow tit *Parus montanus* (77 %), goldcrest *Regulus regulus* (94 %), robin *Erithacus rubecula* (78 %), blackbird *Turdus merula* (83 %) and song thrush *Turdus philomelos* (87 %). Tree pipits *Anthus trivialis* and yellowhammers *Emberiza citrinella* were more abundant in the moderately polluted area (zone II) than in the polluted or unpolluted areas (Table 1).

The availability of nest-boxes significantly increased the density of three of the most common cavity-nesting birds (increase in % for combined data): pied flycatcher *Ficedula hypoleuca* (355 %), blue tit *Parus caeruleus*
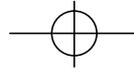
Table 1. Mean (± s.e.) bird densities at three distance zones (I: <2 km, II: 2–7 km, III: >7 km) around the pollution source (Tukey's test: means with the same letter are not significantly different). The effect of distance to pollution source is shown after removing the habitat effects in an ANCOVA model. Significant (P < 0.05) habitat effects shown by '+' or '–'. Separate values for areas with and without nest-boxes (nb) are shown for species in which density was dependent on the availability of nest-boxes.

Species	Acronym	Density (pairs/km²)			Non-habitat-related effect of distance[1]	Habitat			
		Zone I $n = 20$	Zone II $n = 24$	Zone III $n = 12$		Boxes	Spruce	Patch	Volume
Anthus trivialis	ATRI	7.2 ± 2.4a	11.8 ± 1.9a	5.6 ± 1.7a	–			+	
Carduelis chloris	CCHL	6.4 ± 1.4a	6.9 ± 1.4a	1.0 ± 0.50b	0			–	
Carduelis spinus	CSPI	7.8 ± 1.5a	15.6 ± 1.7b	15.3 ± 1.7b	0				
Columba palumbus	CPAL	0.14 ± 0.09a	0.39 ± 0.08a	1.5 ± 0.26b	0		+		
Corvus monedula	CMON	0.35 ± 0.13a	0.11 ± 0.05ab	0.0 ± 0.0b	0			–	
Corvus corone	CNIX	1.6 ± 0.28a	1.0 ± 0.18a	1.1 ± 0.20a	0			–	
Dendrocopos major	DMAJ	2.1 ± 0.67a	2.3 ± 0.72a	1.0 ± 0.68a	0				
Emberiza citrinella	ECIT	17.2 ± 4.4a	22.0 ± 4.1a	11.6 ± 3.5a	– ¦				
Erithacus rubecula	ERUB	6.4 ± 2.1a	14.2 ± 3.3a	29.4 ± 4.8b	+				
Ficedula hypoleuca[2]	FHYP	38.6 ± 11.5a	33.9 ± 10.9a	19.1 ± 18.3a	0	+			
Ficedula hypoleuca[2], nb		115.6 ± 13.8a	116.3 ± 11.5a	111.1 ± 15.4a					
Fringilla coelebs	FCOE	65.4 ± 5.3a	89.8 ± 3.6b	90.6 ± 5.0b	0			+	
Garrulus glandarius	GGLA	0.64 ± 0.64a	2.7 ± 1.1a	4.3 ± 1.8a	0				
Loxia curvirostra	LCUR	0.04 ± 0.04a	0.50 ± 0.19ab	0.65 ± 0.22b	0				
Loxia pytyopsittacus	LPYT	0.48 ± 0.26a	1.32 ± 0.63a	0.53 ± 0.36a	0				
Motacilla alba[2]	MALB	5.4 ± 5.4a	0.0 ± 0.0a	0.0 ± 0.0a	0	+			
Motacilla alba[2], nb		14.5 ± 5.9a	10.6 ± 5.2a	0.0 ± 0.0a				–	
Muscicapa striata	MSTR	11.2 ± 3.2ab	22.2 ± 4.5a	4.7 ± 3.2b	0			+	
Parus ater	PATE	0.0 ± 0.0a	2.2 ± 1.2a	10.3 ± 4.6b	0				
Parus caeruleus[2]	PCAE	70.8 ± 19.0a	23.6 ± 9.0b	5.9 ± 5.9b	0	+	+		
Parus caeruleus[2], nb		106.2 ± 19.0a	44.2 ± 15.2ab	17.7 ± 7.9b					
Parus cristatus	PCRI	3.8 ± 2.1a	11.5 ± 3.7a	16.7 ± 6.4a	+				
Parus major[2]	PMAJ	75.0 ± 8.3a	37.7 ± 3.8b	31.7 ± 2.5b	0	+			

Species	Acronym	Density (pairs/km²)			Non-habitat-related effect of distance[1]	Habitat			
		Zone I n = 20	Zone II n = 24	Zone III n = 12		Boxes	Spruce	Patch	Volume
*Parus major*², nb		96.4 ± 9.5ᵃ	82.3 ± 8.3ᵃ	73.4 ± 7.8ᵃ					
Parus montanus	PMON	2.9 ± 1.6ᵃ	7.1 ± 2.2ᵃᵇ	12.7 ± 5.4ᵇ	+				
Passer domesticus	PDOM	22.3 ± 6.7ᵃ	0.0 ± 0.0ᵇ	0.0 ± 0.0ᵇ	0			–	
Phoenicurus phoenicurus	PPHO	4.0 ± 1.0ᵃ	0.64 ± 0.37ᵇ	0.0 ± 0.0ᵇ	0			–	
*Phylloscopus collybita*²	PCOL	0.56 ± 0.37ᵃ	3.0 ± 1.8ᵃ	10.2 ± 2.1ᵇ	0	–	+		
*Phylloscopus collybita*², nb		0.28 ± 0.28ᵃ	0.70 ± 0.36ᵃ	6.5 ± 1.2ᵇ	0				
Phylloscopus trochilus	PLUS	46.7 ± 3.6ᵃ	56.6 ± 2.9ᵃ	44.8 ± 5.4ᵃ	0				
Phylloscopus sibilatrix	PSIB	1.1 ± 0.51ᵃ	8.9 ± 1.7ᵇ	0.93 ± 0.63ᵃ	0				
Pica pica	PPIC	2.9 ± 0.50ᵃ	1.1 ± 0.32ᵇ	0.66 ± 0.35ᵇ	0			–	
Pyrrhula pyrrhula	PPYR	2.0 ± 0.71ᵃ	2.8 ± 1.1ᵃ	4.5 ± 1.3ᵃ	0				+
Regulus regulus	RREG	1.8 ± 1.2ᵃ	6.7 ± 2.4ᵃ	31.3 ± 6.6ᵇ	+++				
Sylvia borin	SBOR	1.6 ± 0.84ᵃ	2.1 ± 0.9ᵃ	1.2 ± 0.6ᵃ	0				
Sylvia communis	SCOM	1.4 ± 0.8ᵃ	3.1 ± 1.9ᵃ	0.78 ± 0.78ᵃ	0				+
Sylvia curruca	SCUR	7.7 ± 1.9ᵃ	3.6 ± 1.3ᵃ	8.6 ± 2.4ᵃ	0				
Turdus iliacus	TILI	2.6 ± 0.81ᵃ	9.9 ± 2.1ᵇ	3.1 ± 1.4ᵃ	0				
Turdus merula	TMER	3.2 ± 1.3ᵃ	10.0 ± 2.0ᵃ	18.9 ± 2.8ᵇ	+		+		
Turdus philomelos	TPHI	1.3 ± 0.57ᵃ	2.0 ± 0.6ᵃ	9.7 ± 1.8ᵇ	+				
Turdus pilaris	TPIL	14.1 ± 3.3ᵃ	12.2 ± 3.5ᵃ	2.3 ± 1.6ᵃ	0				
Turdus viscivorus	TVIS	0.40 ± 0.22ᵃ	1.8 ± 0.63ᵃ	1.1 ± 0.61ᵃ	0			+	

[1] From ANOVA model where distance, nest-box effect, proportion of spruce, forest patch size and timber volume were used as explaining factors (+++ P < 0.001, ++ P < 0.01, + P < 0.05, 0 = not significant).

² The number of sampling points per zone for I: n = 10, II: n = 12, III: n = 6.
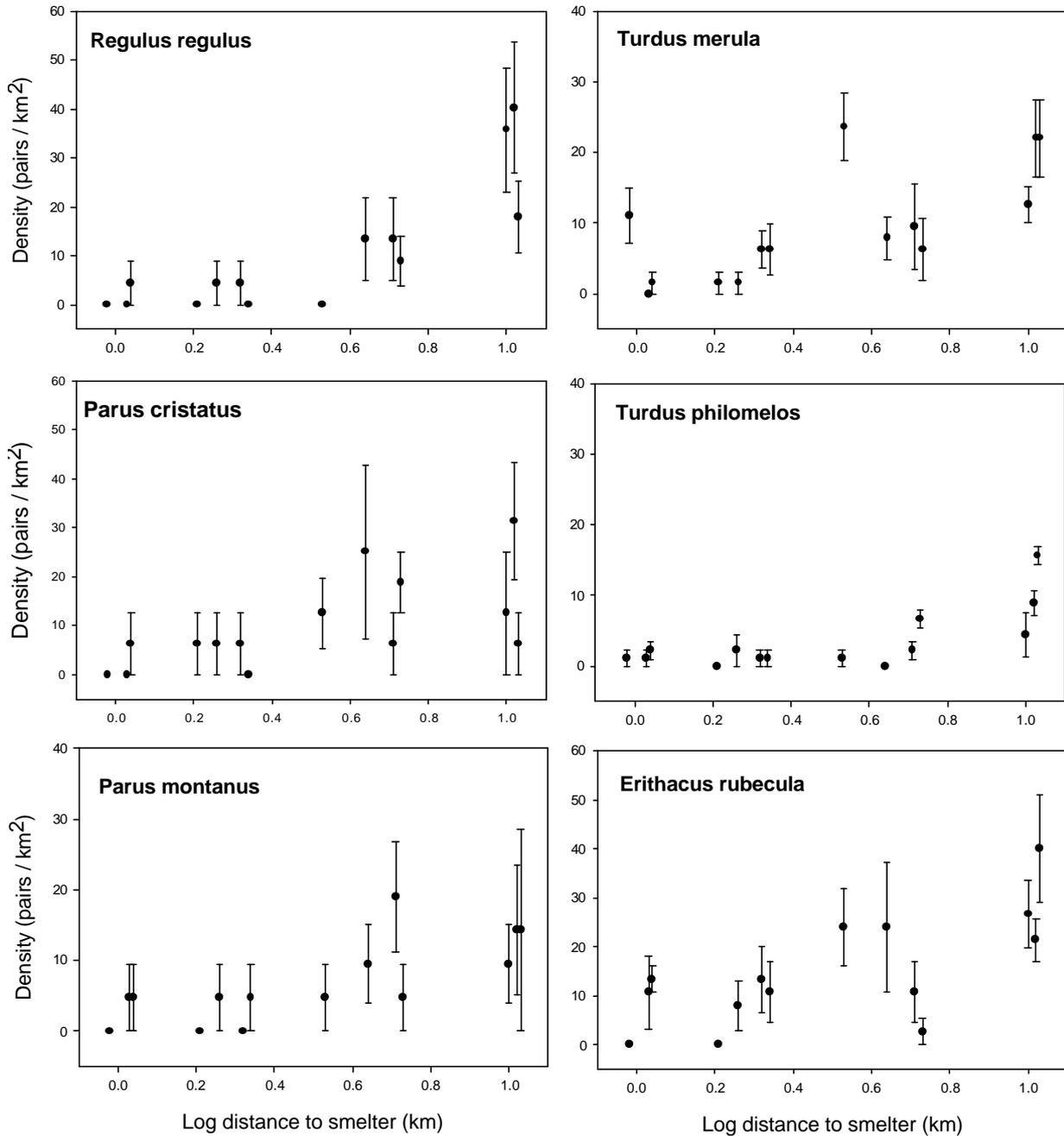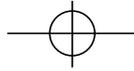
Fig. Mean (± s.e.) bird densities (pairs) at 14 study plots along the pollution gradient for those six species which showed a decreasing trend towards the pollution source that could not be explained by any of the habitat characteristics studied.

(166 %) and great tit *Parus major* (172 %) (Table 1). Pied wagtails *Motacilla alba* also showed higher densities at sites with nest-boxes, whereas chiffchaffs *Phylloscopus collybita* bred at somewhat lower densities at the nest-box sites (Table 1). For cavity-nesting birds, comparison of density estimates with observed breeding densities in our nest-box sites revealed that point counts gave good estimates for the density of pied
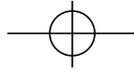
Table 2. Mean (± s.e.) densities of nests and nest-box occupation rate (%) of hole-breeding bird species in three distance zones (I: <2 km, II: 2–7 km, III: >7 km) around the pollution source in 1996–2000. Tukey's test: means with the same letter are not significantly different. The number of study sites where nest-boxes were regularly checked during five years (n = 27, 30, 15 for three zones, respectively). Chi-square test for occupied v. unoccupied nest-boxes. The number of nest-boxes which were regularly checked during five years (n = 2990). Second and replacement nests are not included.

| Species	Density (pairs/km^2) Occupation (%)			Π^2	P
	Zone I	Zone II	Zone III		
Ficedula hypoleuca	108.0 ±10.2[a]	116.7 ± 8.59[a]	121.1 ±13.4[a]		
	36.6	41.9	35.6	5.7	0.059
Parus major	49.1 ± 6.83[a]	47.2 ± 5.11[a]	56.8 ± 9.53[a]		
	15.8	21.5	22.0	13.2	0.0014
Parus caeruleus	7.4 ± 2.13[a]	11.4 ± 3.32[a]	4.6 ± 2.34[a]		
	4.4	4.9	4.6	0.3	0.86
Parus ater	0.0 ± 0.00[a]	0.13 ± 0.07[a]	9.4 ± 5.74[b]		
	0.07	0.18	2.5	44.6	<0.0001
Parus cristatus	0.49 ± 0.44[a]	0.76 ± 0.49[a]	0.76 ± 0.17[a]		
	0.15	0.82	1.7	14.3	0.001
Phoenicurus phoenicurus	3.1 ± 2.12[a]	0.06 ± 0.06[a]	0.0 ± 0.00[a]		
	0.74	0.27	0.0	5.8	0.054

flycatcher, coal tit *Parus ater* and redstart *Phoenicurus phoenicurus*, whereas point counts seemed to overestimate the breeding numbers of great tit and blue tit (Table 2).

Of the six habitat characteristics, the proportion of spruce (1st principal component) explained most of (36 %) the habitat variation in our data. Because several bird species are known to favour spruce dominated forests the proportional effects of the two intercorrelated environmental factors, air pollution and proportion of spruce, on bird densities was further studied using partial correlations (Figure 2). The smaller amount of spruce in the polluted area, which may be partly a consequence of a sensitivity of spruce to air pol-

Figure 2. Partial correlations of bird densities in relation to two environmental factors: air pollution (distance to the pollution source) and habitat (proportion of spruce). Vertical and horizontal lines show the borders of statistically significant correlations at a level <0.05. Acronyms for the species are shown in Table 1.
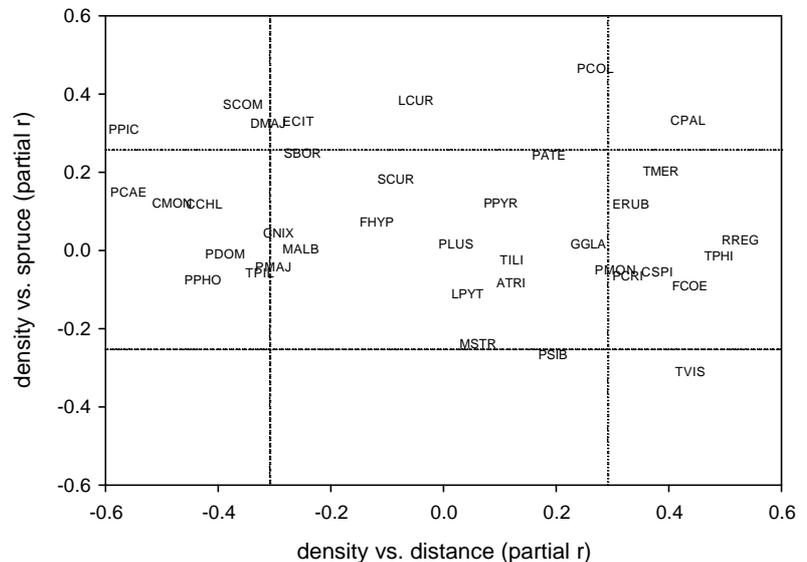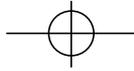
lution, explains the smaller densities of such spruce favouring species as common crossbill *Loxia curvirostra*, chiffchaff and coal tit (Table 1).

Discussion

Six out of 37 bird species showed decreased densities in the polluted area, and they can be divided into two categories according to their ecology and feeding habits: ground feeding Turdidae (robin, blackbird, song thrush) and conifer foliage gleaners of the tit guild (goldcrest, crested tit, willow tit). The result is in agreement with earlier studies on pollution effects on forest bird populations. For example, the three turdid species were also observed to suffer from the effects of pollution in a Polish study near a heavily industrialised area (Tomek 1992). Similarly in the Czech Republic, Flousek (1989) found that especially goldcrest, firecrest *Regulus ignicapillus*, song thrush, wren *Troglodytes troglodytes*, chaffinch *Fringilla coelebs* and coal tit showed decreased densities in spruce forests affected by industrial emissions.

Both direct toxic effects as well as indirect effects (via lowered food availability) have been shown to lower the breeding success of some hole-breeding birds in the vicinity of the pollution source (Eeva & Lehikoinen 1996). There are no data on reproduction for the remaining species and the reasons for their decreased densities may involve both factors. There is no indication, however, of increased adult mortality due to toxicity in our study area for any species. Ground feeders are probably affected negatively by pollution-related changes in soil and the ground layer. Emissions of sulphuric oxides and accumulation of heavy metals in the ground over a timespan of c.50 years have caused clear changes in the ground layer vegetation and invertebrate fauna. Ground layer vegetation is almost absent in the vicinity of the factory complex (Salemaa & Vanha-Majamaa 1993). The species number and biomass of ground living invertebrates are also known to have decreased in the polluted area of Harjavalta (Koponen 1995, Eeva et al. 1997), as well as at other similar sites (Bengtsson & Rundgren 1984). Unfortunately, there is no information available on the number of earthworms (lumbricids) in our study area. Earthworms are an important source of food for ground feeding thrushes (Cramp et al. 1988) and their biomass and species num-

ber decrease around the sources of heavy metals (Tyler 1984, Spurgeon et al. 1994). Earthworms effectively accumulate heavy metals from polluted soils and pass them on to secondary consumers such as shrews (Pankakoski et al. 1994). Accordingly, Beyer & Storm (1995) found that shrews and ground feeding songbirds accumulated high concentrations of lead in the vicinity of a zinc smelter. Another important food source for thrushes are ground living snails, which are known to be sensitive to acidification (Graveland & van der Wal, 1996). Recently, Hames et al. (2002) demonstrated a strong negative effect of acidification on the breeding population of wood thrushes *Hylocichla mustelina* in North America, and suggested calcium depletion and consequent snail loss as a cause of the population decrease.

Foliage gleaners may have suffered from the loss and poor quality of needles in coniferous trees and a consequent decrease in the abundance of their invertebrate food. In the polluted area of Harjavalta, 31 % of pines suffer from severe needle loss (>20 %) compared to 25 % in the background area (Jussila 1997). In the stands nearest to the smelter the trees have only current and one-year old needles, compared to the normal 3–4 age classes in southern Finland (Kukkola et al. 1998). Correspondingly, the mean sulphur content of pine needles are significantly higher in the polluted (over 1000 mg/kg) area than background (mean 930 mg/kg) area (Jussila 1997). For example, canopy-living spiders are sensitive to air pollution and a consequent needle-loss (e.g. Gunnarsson 1988, Sundberg & Funnarsson 1994, Brotons et al. 1998). Reduced numbers of canopy-living spiders and other invertebrates might explain the decreased densities of conifer foliage gleaners in Harjavalta. Reduced needle biomass may also cause other negative effects for foliage gleaners: for example, several *Parus* species foraging in pines with high needle-loss have been shown to spend proportionally more time scanning for predators and less time handling prey than those living in the area with low needle-loss (Hake 1991, Brotons et al. 1998).

The density of cavity-nesting species was increased by 2–3 fold at sites provided with nest-boxes. On the other hand, increased numbers of cavity-nesting species had little effect on abundance of other species. Comparison of the results from point counts with the long term (1996–2000) breeding data on cavity-nesting birds reveals an interesting contradiction regarding to
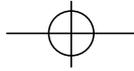
the use of census data for bioindicator purposes. Pied flycatchers and great tits produce, respectively, 30 % and 22 % fewer fledglings in the polluted area of Harjavalta (T. Eeva, unpubl. data). Nevertheless, for these two species, no indication of these detrimental effects could be observed on the basis of point count data. This means that bird censuses alone cannot reveal the species that are potentially in danger in polluted environments. Instead, bird censuses may reveal only those species most sensitive to pollution, whose populations have already collapsed.

Overall, bird densities and bird community structure in Harjavalta resembled relatively well those at the unpolluted control sites (see also Ryösä & Reiniaho 1999). Much more dramatic changes in bird communities have occurred elsewhere, e.g. in the Monchegorsk area of the Kola peninsula, where an 80 % decrease in density of forest species has been reported (Gilyazov 1993). Due to forest decline, species of open habitats now prevail in the most heavily polluted area around the Monchegorsk smelter complex (Gilyazov 1993). We suggests that, in Harjavalta, ground feeders are affected negatively by pollution-related changes in soil and the ground layer. Foliage gleaners may have suffered from the loss of needles in coniferous trees and a consequent decrease in the abundance of their arthropod food. Further studies are needed to reveal the mechanisms behind the changes.

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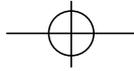
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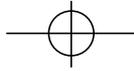
Predicting home range use by golden eagles
Aquila chrysaetos in western Scotland

David R. A. McLeod[1], **D. Philip Whitfield**[2]*, **Alan H. Fielding**[3],
Paul F. Haworth[3] **and Michael J. McGrady**[4]

The conservation and management of golden eagles *Aquila chrysaetos* requires information on home range, which is expensive and time-consuming to collect. We describe modelling techniques for predicting golden eagle ranging behaviour within a Geographic Information System (GIS). The model, called PAT (Predicting Aquila Territory), used data on ranging behaviour and geospatial factors from two areas of western Scotland. A range centre was estimated from the weighted mean nest site location in the past ten years. Range boundaries were estimated from Thiessen polygons, in the presence of neighbouring ranges, and a maximum ranging distance generated from parameters responsive to local range density, in the absence of neighbouring ranges. The model assumed that eagles did not use the sea or freshwater bodies, and avoided areas of human activity and closed canopy forests. The model also assumed that golden eagles preferred areas close to ridges (and other convex terrain features) and close to the centre of the range. The model output, at 50×50 m resolution, was three-dimensional with geographical location as *x* and *y* co-ordinates and use as a percentage of total home range use as the *z* co-ordinate. Comparison of the model's predictions against range use observations suggested that it provided a good fit to observed range use. The PAT model has many potential applications, including the prediction of the likely impact of local developments such as wind-farms and commercial forest expansion on territorial eagles and for providing information useful to managing land use change to the benefit of eagles across large areas.
Keywords: golden eagle, *Aquila chrysaetos*, GIS, home range, modelling.

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Breeding pairs of golden eagles *Aquila chrysaetos* occupy large home ranges, portions of which are actively defended seasonally as an exclusive territory (Watson 1997). Like many large raptors the golden eagle is vulnerable to human influences, such as land-use changes, and rural developments can have a negative impact (Newton 1979, McGrady 1997, Watson 1997, Pedrini & Sergio 2001a, 2002).

Features of the ecology of the golden eagle make it difficult to achieve eagle conservation aims, particularly within human-influenced landscapes. Land man-

agers must sometimes make decisions about the likely effects of land use change on eagles. These decisions must be made within a limited time period or be applied across a large area, precluding the collection of field observations that describe actual range use. In addition, it is time-consuming and expensive to research golden eagle ranging behaviour because the birds mainly occur at low densities in remote mountainous country (Watson 1997). Moreover, agencies responsible for the protection of eagles are often reactive, so the rapid identification of important areas for golden eagles
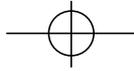
would allow them to be incorporated much earlier into the process of planning developments (McGrady et al. 1997). This would reduce conflict between developers and conservation agencies, lower planning costs, and minimise the possibility of inappropriately placed developments (Brendel et al. 2002, McLeod et al. 2002).

Because it is difficult and expensive to obtain detailed range use data it would be helpful if a model could be developed that used readily available habitat data to predict range usage and, therefore, identify those areas that are important for golden eagles. Here we describe the development of a rule-based model for predicting golden eagle ranging behaviour using a Geographic Information System (GIS) that builds on an earlier simple model (the RIN), and automates the inclusion of factors that appear to affect eagle range use, especially terrain and ridge features (Chalmers 1998). The model is called the PAT (Predicting Aquila Territory). Golden eagles in Scotland are typically birds of open mountainous country, but exploit a wide range of habitats and landscapes and display a wide range of breeding densities that probably affect ranging behaviour (McGrady 1997, Watson 1997). For a model to predict range use successfully it is important that local or regional variation in ranging behaviour is incorporated. Hence, we outline the derivation of PAT model rules by assessing observed ranging behaviour in relation to geospatial factors in two contrasting areas of western Scotland: an inland, mountainous region and a low-lying peninsula that included coastal ranges. Finally we assess how well the model predicts eagle ranging by comparing its performance against observations of range use in the two study areas of western Scotland and a third study area in southwestern Scotland, and we also compare its performance to that of the RIN model. This exercise highlights the PAT model's strengths and weaknesses, thereby pointing to potential improvements and need for further data requirements.

Methods

Modelling eagle range use and the RIN model

When many observations of eagle range use are available, home range can be estimated using a variety of methods, such as minimum convex polygons, or harmonic mean or kernel estimators (e.g. Kenward 2000).

In most situations, however, detailed observations of use are not available and home range must be estimated by other methods. Our objective was to derive a model that could produce an estimate of home range use in golden eagles using only information on eagle nest site location. The model that we developed and describe here, the PAT, is based on an earlier model, known as the RIN (named after the Research and Information Note series in which it was first published; McGrady et al. 1997). It is helpful to understand how the PAT model was developed by briefly describing its precursor's origins.

The simplest method of representing the home range of a golden eagle is to assume it lies within a fixed-radius circle around a nest area, or a range centre, which is the mean location of alternative nest sites (e.g. Watson 1992, Kochert et al. 1999, Pedrini & Sergio 2001a). A more sophisticated method of defining ranges involves Dirichlet tessellation and the production of Thiessen polygons (e.g. Sim et al. 2001). In this method straight lines are drawn mid-way between neighbouring range centres to produce a series of polygons (known as Thiessen polygons) whereby each range contains all the space that is closer to its range centre than to any other. This method has an advantage over simple circles in that it is responsive to differences in nesting density and does not produce any overlap in estimated range use (for more details see Diggle 1983).

As in simpler methods, the RIN model estimates the home ranges of golden eagles in Scotland by first taking the range or territory 'centres' for a group of ranges (described by mean location of recently used nest sites, weighted for use) and drawing up range boundaries equidistant between range centres. For ranges surrounded by near neighbours, a Thiessen polygon results, as in Dirichlet tessellation, but in the absence of near-neighbouring ranges a cut-off of 6 km from the range centre is used to estimate the range boundary (distance based on observations of range use in Argyll, west Scotland). This distance may vary according to the density of breeding eagles and, for example, is likely to be less than 6 km in high density areas such as some of the Hebridean islands (Green 1996). Within the resulting polygon a 'core area', within which 50 % of eagle activity, occurs can be delimited by a circle of 2–3 km radius. Outside of the core area it is assumed that eagles do not use land below an elevation threshold of 150 m a.s.l. although this is likely to be lower in the western Hebri-

dean islands and higher in the eastern Highlands (McGrady et al. 1997).
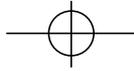
This approach has its advantages, as it is easy to apply in cases when a home range needs to be modelled simply (McGrady et al. 1997, McLeod et al. 2002). However, in all cases so far examined, the RIN model predicts that eagles use larger areas than implied by range use observations (Whitfield et al. 2001, McLeod et al. 2002). The objective of the PAT modelling process was to improve the fit of predicted range use.

Study areas

To derive the rules necessary to generate the PAT model's range use predictions we used range use observations from two study areas in mid and south Argyll in the southwest Highlands and the island of Mull in the Inner Hebrides, respectively (Fig. 1). The mid and south Argyll study area (hereafter called mainland Argyll)
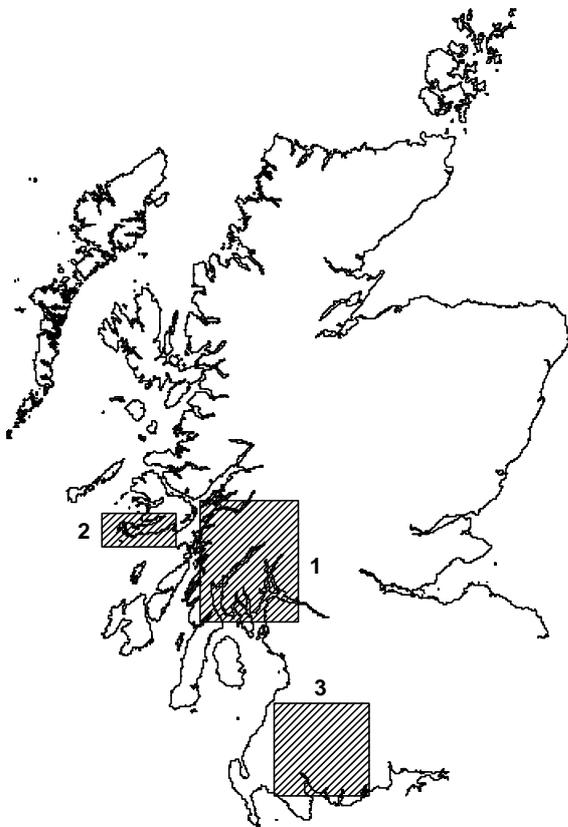
Figure 1. Golden eagle study areas: 1. mainland Argyll, 2. Ross of Mull, 3. Galloway. Individual ranges are not illustrated to retain confidentiality.

was typical of upland sites in the western Highlands of Scotland, consisting of hills, rugged topography and sharp relief in terrain features (< 1100 m, average 230 m a.s.l.; McGrady et al. 1997). The other area was the Ross of Mull, a low altitude (< 400 m, average 100 m a.s.l.) peninsula with gently sloping topography in the southwest of the island of Mull (Fielding & Haworth 1995). Information on nest site use and breeding success of golden eagles was collected in both areas (Green 1996, Whitfield et al. 2001).

Range use observations

Range use data were obtained from two independent studies and combined for the development of the PAT model. We included all observations of range use, collected year-round, to incorporate variations in range use according to season and the breeding status of eagle pairs (Marzluff et al. 1997, P. Haworth & M. McGrady unpubl. data). We also used these data to test the fit of the model's predictions. Because these studies initially addressed different objectives there were methodological differences between studies in the data collection methods. The differences in data collection required different methods of data analysis but reduced the risk that our conclusions and the predictions of the PAT model resulted from a methodological bias.

In mainland Argyll, from July 1991 to April 1996, nine adult golden eagles were captured and radio-tracked in six home ranges (details in McGrady & Grant 1996, McGrady et al 1997, Grant & McGrady 1999). In most cases radios allowed two field observers to locate eagles visually and map their movements using a 1 : 25 000 scale Ordnance Survey map. Tracking usually occurred on one home range per day, but this could change to avoid conflicts with hunters and farmers. The main objective of a day's tracking was to get at least one high quality location of a tagged eagle (a high quality location was the visual confirmation of a location at < 100 m accuracy). On most days more than one high quality record was collected and records were later sorted to promote independence of locations (McGrady et al. 1997, McLeod et al. 2002). Eagles were tracked throughout the year, at all times of the day and in all types of weather. If both birds in a pair were tagged the records were pooled (see also Marzluff et al. 1997) so that there was a set of records from each of the six ranges. Strict random sampling protocols could not be
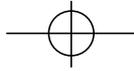
followed because access to the home ranges was limited, but our subjective assessment was that because of the large number of observations that were collected this had little effect on estimated range use.

On Mull, observations of golden eagle location and behaviour were obtained as part of a larger long-term study of the Ross of Mull raptor and scavenging bird assemblage (Fielding & Haworth 1995). Because this study was concerned with many individual birds in a raptor community, radio tracking of individuals was considered impractical. Observational records (n = 1895) of eagles were gathered in an area encompassing five ranges, by two experienced field workers who were on occasion supported by volunteers, between August 1994 and December 1998 inclusive. Random sampling was not possible because of access and safety constraints. Sampling effort was greatest, and approximately constant, throughout daylight hours, between July and October. Observations were collected using binoculars and spotting telescopes and mapped onto 1 : 25 000 maps of the study area. Birds were aged (Tjernberg 1988) and, if possible, sexed on size. Only records of territory holding adults were used in analysis (n = 1382), although we could not identify adult intruders. As in mainland Argyll, records for both sexes were combined for each range. If an observation could not be unambiguously assigned to a particular range it was assigned to the range whose nest was closest to the location of the record. Because eagles on Mull were not fitted with radio tags serial dependence of records was not a potential problem. For this reason, and because we did not wish to compromise sample sizes (Reynolds & Laundre 1990, De Solla et al. 1999, Otis & White 1999, Seaman et al. 1999), all observations from this study area were used in the range modelling procedure.

The lack of random sampling within the Ross of Mull study area meant that the observations were potentially biased and not representative of actual range use. We addressed this by expressing golden eagle records in an area relative to the records of other raptor and scavenging species, as follows.

The 1382 observations of adult golden eagles comprised 7.2 % of all sightings of raptors and scavengers (n = 19291 observations on 14 species). Within the GIS a simple kernel estimator (using a circular buffer, 250 m radius) was applied to each 50×50 m pixel in the Ross of Mull study area (Bailey & Gatrell 1995). In each buffer the proportion of adult golden eagle

sightings was calculated, with 95 % confidence limits (Agresti & Coull 1998), as a proportion of all raptor sightings within the buffer. If the lower confidence limit was greater than 0.072 (the overall proportion of sightings that were adult golden eagles) this was taken to indicate excess usage, while an upper confidence limit less than 0.072 was indicative of under-use. A small number of buffers had no sightings, either because none of the 14 species had been seen or the location was rarely visited. In all other locations usage did not differ from the expected proportion. Although this method probably did not wholly resolve the problem of potential sampling bias, it was an improvement as the number of raptor sightings was large and it was probably best for identification of those areas seldom used by eagles. The method did not depend on the correct allocation of golden eagle sightings to ranges. It also dealt with inequalities in sampling effort because rarely visited locations had wide confidence intervals. We could be confident about under-used locations because buffers typically had many observations from other species. The output from these analyses was an indicative 'preference' map in which each 50×50 m pixel was assigned to one of four possible values: over-used, under-used, proportional (observed = expected) use, no data.

Predictive models are only of value if their predictions are tested on independent data, i.e. data that were not used during the model's development (Fielding & Bell 1997). Fortunately, we were able to obtain range use observations from two golden eagle ranges in Galloway, southwest Scotland, collected under a third independent study; hence the method of data collection differed from the studies on Argyll and Mull. These ranges are isolated from the main golden eagle Scottish breeding area in the Highlands (Fig. 1). Marquiss et al. (1985) and Watson (1997) have provided descriptions of the area and study ranges. The area within 10 km of eyries was divided into 1×1 km grid squares according to the Ordnance Survey national grid. Several experienced volunteer observers visited the area between 1987 and 1991 in the course of keeping the eagles under protective surveillance and to estimate the use of the region by eagles. The number of observations of eagles in each grid square was recorded along with the number of times each grid square was visited by an observer. The relative use of each grid square was expressed as the proportion of visits when an eagle was seen. The relatively coarse scale of analysis was chosen to ac-

commodate the relatively coarse scale at which many observations were made. Visits were not timed but approximately equal times were spent in each grid square. While emphasis was on collecting observations of adult birds, the age of eagles was not recorded in every case and this may have biased records, especially on the periphery of ranges (Watson 1997). If an observation could not be unambiguously assigned to a particular range it was assigned to the range whose nest was closest to the location of the record. The isolation of the ranges, however, meant that such observations were few.
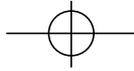
Golden eagle habitat requirements: model assumptions

Our first assumption was that eagles used exclusive ranges with no overlap between neighbours. Although it is apparent that there can be overlap in range use between neighbours (Marzluff et al. 1997, this study), there is good evidence for active defence of an area at least at some times of the year (reviewed by McGrady 1997, Watson 1997). It is also simpler to model exclusive ranges.

Eagles appear to use areas around their nest sites more frequently than other parts of their range. To a degree this is probably because eagles are central place foragers during the breeding season, but the preference is also apparent when eagles are not breeding (McGrady et al. 1997, Marzluff et al. 1997, this study). As nest site locations are often used to define the 'range centre' (e.g. Watson 1992, Kochert et al. 1999, Pedrini & Sergio 2001a), an area around the range centre so defined should be a preferred area.

Golden eagle morphology is adapted for soaring flight (McGrady 1997, Watson 1997), and so features of the terrain that aid soaring flight may affect range use. In the cool climate of Scotland wind deflected upward off terrain features is probably an important aid for flight. In keeping with this suggestion, Chalmers (1998) found a statistically significant association between eagle activity and ridge features (see also Orloff & Flannery 1996, Erickson et al. 1999, Strickland et al. 2000). We therefore assumed that ridges and similar terrain features would be preferred by eagles.

Golden eagles are sensitive to disturbance by humans and tend to avoid areas of human activity, such as settlements and roads (e.g. Anderson et al. 1990, Watson

1997, Petty 1998). There is little quantified information, however, on the distances at which disturbance can occur or which activities are most affected. Throughout their global range, eagle tolerance of human activities varies, and in recent years eagles in an expanding population have moved into areas more disturbed by humans to establish new breeding ranges (Haller 1996). As such a situation is not apparent in Scotland (Watson & Dennis 1992) we assumed that areas of human activity would be avoided by eagles.

Water bodies and the sea provide few air currents that golden eagles can exploit, and provide few prey sources (Watson 1997); they were therefore treated as areas that golden eagles did not use. Golden eagles in Scotland also seldom exploit post-thicket (closed canopy) forests (Marquiss et al. 1985, Watson et al. 1987, Watson 1992, McGrady et al. 1997, 2001, Whitfield et al. 2001). Hence, we assumed that golden eagles did not use forests more than twelve years old, since field observations determined that this was the age when forests became unavailable to them (Whitfield et al. 2001). Golden eagles appear to use particular vegetation types more than others (Marzluff et al. 1997, McGrady et al. 2001). We did not attempt to incorporate any vegetation preferences within the PAT model, however, because across Scotland prey selection differs (Watson 1997) and so vegetation preferences probably also differ, making their incorporation in a generic model difficult.

The GIS, model rule-base and model development

All modelling was undertaken in a raster digital GIS using ArcView® (ESRI). The principal source of terrain data was the Ordnance Survey's (OS) 1 : 50000 raster digital elevation model. As the OS digital elevation data has a pixel size of 50×50 m, terrain features of less than this area could not therefore be identified. Nevertheless, we deemed prediction to this scale as appropriate both to the accuracy of range use observations and to how eagles may select areas within their range. Road and human settlement data came from OS. Water bodies (not including rivers and streams) were derived from the Land Cover of Scotland 1988 (LCS88) dataset (MLURI 1993). Whitfield et al. (2001) described the methods by which post-thicket forests were mapped in the GIS.

As a first step in the model we estimated the range centre as the mean position of used nest sites, up to a
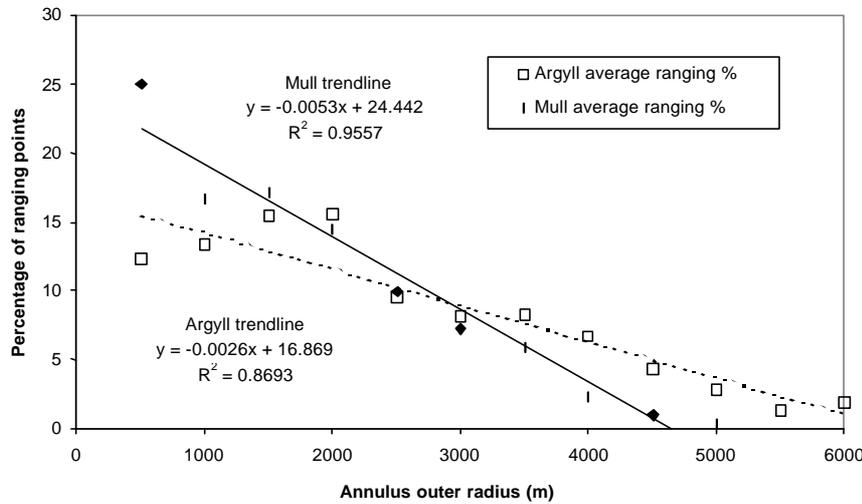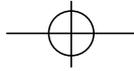
Figure 2. Relationship between distance from the range centre and mean percentage ranging observations for golden eagle ranges on Mull (n = 5) and mainland Argyll (n = 6).

maximum of the previous ten years' usage (see also McGrady et al. 1997, Kochert et al. 1999, McLeod et al. 2002). If a minimum of five years information on nest use was not available, then we used the mean position of known alternate nests. Next, range boundaries were estimated by drawing lines at the equi-distant points between neighbouring range centres (Dirichlet tessellation) to produce Thiessen polygons. In the absence of neighbours or unsuitable habitat we set the boundary of a range at 6 km (see McGrady et al. 1997, McLeod et al. 2002), but we took this as a preliminary measure only, because it was inflexible (see later). Thus far, the model was the same as earlier approaches, including the RIN, and had incorporated the assumption that ranges were exclusive.

The next step was to incorporate a preference for use of areas around nest sites (i.e. the range centre). Concentric annuli, in 500 m width increments, were drawn around the centre of each range (i.e. annulus 1 = 500 m radius, annulus 2 = 1000 m radius, etc.) to form concentric distance bands (i.e. band 1 = 0–500 m, band 2 = 500–1000 m, etc.). Our approach was to assume that eagles' use of areas would be greater within annuli closer to the range centre, and we used the range use observations from mainland Argyll and Mull to derive the rules for how much 'use' should be assigned to (= predicted to occur within) each distance band. We took the complete use of an eagle range to be 100 %, so that each pixel within a range had a use value (the percentage of total range use the model predicted for that 50×50 m tile) and the sum of all pixel use values was 100.

Ranging observations, measured for Euclidean distance to the range centre and assigned to the appropriate distance band, were aggregated for all ranges within each study area. For each study area we plotted the percentage frequency of ranging observations against distance class (Fig. 2). This confirmed that eagles preferred areas close to the range centre. But it was clear that since ranges were markedly different in size, it was necessary to know the maximum ranging distance (as a measure of range size) in order to assign use values to the different 500 m distance bands. In other words, if we could estimate the maximum ranging distance (the x intercept) then from the relationship we could predict the slope (how range use changed with distance from the centre).

The best estimator for maximum ranging distance in a range was found to be the area of the Thiessen polygon for that range (Fig. 3). The reason why a measure of area was a good surrogate for a measure of distance (Fig. 3) was probably that both were influenced by the same features (breeding density and the presence of unsuitable habitat constraining range use). Estimation of maximum ranging distance in turn allowed, for any range, the estimation of the slope of the relationship between percentage ranging observations and distance (Fig. 2), and thus the percentage of range use per distance band (the use value assigned to each 500 m distance band). We also assumed, therefore, a linear decrease in range use occurred between 500 m distance bands with distance from the range centre, as determined by the empirical relationship (Fig. 2).
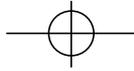
As noted earlier, although we had set the maximum ranging distance at 6 km we had taken this as a preliminary measure only, since it was apparent that in areas of high density (such as on Mull) golden eagles typically did not range as far as 6 km from the range centre. We therefore needed to set maximum ranging distance by a means that was responsive to local breeding density. Deriving a surrogate for maximum ranging distance (using the relationship in Fig. 3) allowed us to estimate the limit for range boundaries in areas where neighbouring ranges were absent. Dirichlet tessellation and Thiessen polygons delineated range boundaries in the presence of near neighbours, as described earlier, but in the absence of near neighbours we assumed that the boundary of an eagle range occurred at the maximum ranging distance from the range centre, estimated according to the relationship in Figure 3. Thus boundaries unconstrained by near neighbours were described by a distance responsive to local breeding eagle density in the PAT model rather than by a fixed 6 km distance as in the RIN model (McGrady et al. 1997). This reflected the observation that eagles whose near neighbours were closer also ranged shorter maximum distances in parts of the range unconstrained by neighbours.

The next stage of the model's development was to incorporate eagles' preference for ridge features. This first required us to use a method for recognising ridge and cliff/plateau edges (i.e. convex terrain features). None of the facilities within ArcView (e.g. watershed or curvature functions) was found to be suitable for our purposes. Instead, using a custom script (a small program written in Avenue, Arcview's programming language), terrain features were automatically detected by comparing the altitude of each pixel with those of its neighbours (McLeod et al. 2002).

The altitude of each pixel was compared to the mean elevation of four opposing pairs of radial arms, each arm five pixels in length, which were orientated NE-SW, N-S, SE-NW, and E-W (the method is illustrated by McLeod et al. 2002). From the elevation values we calculated the angle formed by each pair of opposing radial arms about the 'focal' pixel. If the angle between any pair of radial arms was less than 168° we deemed the focal pixel to be a 'convex terrain feature'. The threshold value for the angle was largely dictated by pixel size but was chosen subjectively because it provided the best fit to features we thought likely to be used by eagles. Although we did not distinguish between cliff/plateau edges and ridges, ridges may be distinguished by this method if all pixels on a pair of opposing radial arms were lower than the focal pixel. This process was carried out on every pixel with-
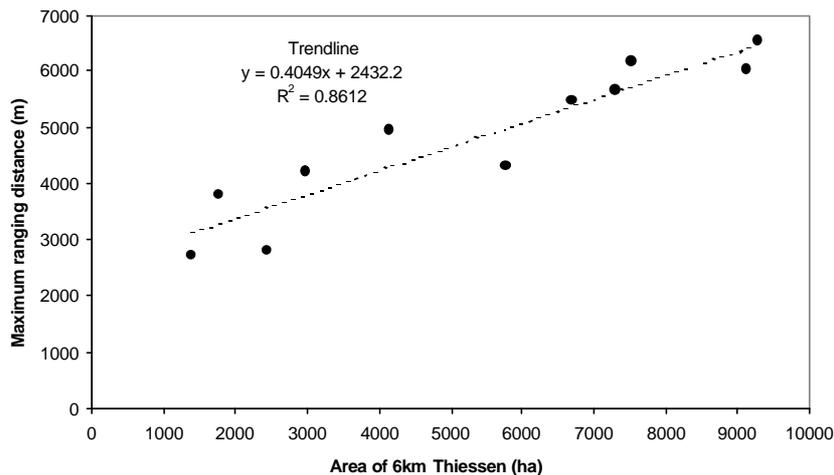
Figure 3. Relationship between maximum ranging distance and the area of a Thiessen polygon, limited to a maximum of 6 km from the range centre in the absence of neighbouring ranges, for the 11 golden eagle study ranges in mainland Argyll and Mull. The peripheral area of a home range where maximum ranging distances were recorded had the fewest empirical data (a general finding: see Seaman et al. 1999) and this may lead to errors in the measurement of home range area and maximum ranging distance. Consequently, values for maximum ranging distance in each study home range were estimated by linear extrapolations from 90 % values taken from a plot of percentage ranging observations against distance from the range centre.
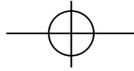
in the maximum ranging distance from the range centre.

To obtain the rule for how use values should be distributed according to terrain features, we drew up incremental 100 m wide distance bands from all convex terrain features in the two study areas up to a maximum of 1200 m distance, as almost every point in a home range was within 1200 m of a convex terrain feature. We then measured all range use observations from both study areas for Euclidean distance to a convex terrain feature, and assigned each observation to the appropriate distance band. Ranging locations of eagles were more frequent within 200 m of a convex terrain feature than would be expected if they were evenly distributed within a 1200 m distance from convex terrain features, confirming that convex terrain features were preferred by eagles (Fig. 4). This relationship gave us the rule for assigning use to each pixel according to distance from convex terrain features.

This rule was incorporated in the model so that within each 500 m wide annulus from the centre, we assumed that range use was distributed between 100 m terrain distance bands according to the observed distribution relative to convex terrain features in Figure 4 (i.e. use was greater closer to convex terrain features). So, for example, if the use value assigned to an annulus around the centre was 30 (%) then the relationship in Figure 4 and the number of pixels in the annulus dictated how the 30 'percentage points' were distributed between pixels across terrain distance bands.

The next set of rules, for eagles' avoidance of areas with human activity and unsuitable habitat, were easier to implement, because the relevant features were simply excluded. In the absence of specific information, buffer zones around human settlements, within which eagles were assumed not to range, were created as follows: single building 250 m, cluster of buildings 400 m, village 600 m, and town 800 m. These distances were based on limited observations of ranging behaviour within the study areas and experience of golden eagles elsewhere in Scotland. Roads are more difficult to buffer, as it is unlikely that traffic volume can be reliably related to road category (especially in the Scottish Highlands). It is not the presence or proximity of the road per se that may affect eagle ranging, but its visibility and traffic volume (Andrew & Mosher 1982, Gonzalez et al. 1992). However, as a simplistic representation, we assumed that eagles did not use areas within 300 m of single carriageway roads and within 500 m of dual carriageway roads.

We also assumed that eagles did not use freshwater bodies (i.e. pixels overlying freshwater had no use value), with no minimum size for exclusion. The sea was excluded by assuming pixels seaward of the coastline in the OS data had no use value. Pixels that overlaid woodland that was over 12 years old in the forest layer of the GIS were also taken to have no use value, because we assumed eagles also avoided this habitat.
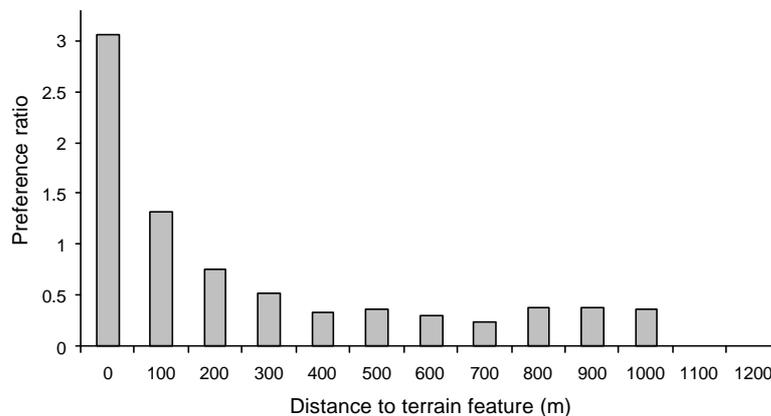
Figure 4. The relationship between distance from a terrain feature (ridge or convex feature) within 100 m wide distance bands and the 'preference ratio' of golden eagle range use (observed range use/expected range use). Expected range use was the availability of land within each distance band. The zero distance class represented ranging over a terrain feature. Eagles were observed more often close to terrain features than expected from the availability of land (11 distance bands, Kolmogorov-Smirnov two-sample test, D = 0.64, P = 0.02, two-tailed).

1. Use nest site locations to derive range centres.

2. Use range centres to derive Thiessen polygons to estimate range boundaries.

3. Draw concentric 500 m wide annuli around the range centre. Assign a % use value to each distance band or annulus according to relationship given by maximum ranging distance of the range. Sum of values for all annuli = 100. Annuli close to range centre have highest use values.

4. Identify ridges and plateau/cliff edges (terrain features) within range, and draw 100 m wide 'terrain distance' bands from each terrain feature.

5. Within each 500 m annulus, distribute the assigned use value among pixels according to distance of pixel from a terrain feature, within the 100 m wide terrain distance bands. Distance bands close to terrain feature have highest use values.

6. Assume open freshwater, the sea, closed canopy forest and areas close to roads and human habitation are not used (i.e. the relevant pixels have no use value).

7. Null use values of some pixels due to step 6 creates an excess of use value in 500m annuli with unsuitable habitat (e.g. forest). Excess use value thereby created is therefore redistributed among 500 m annuli. Step 5 is then repeated.

8. PAT model prediction complete. Each pixel which is suitable habitat has a use value according to its distance from the range centre and distance from a terrain feature. Pixels close to centre and terrain features have highest use values.

Figure 5. Flow chart summarising the steps in the prediction of range use by golden eagles using the PAT model.

Accounting for shifts in range use and final model output

Creating pixels with no use value due to unsuitable habitat (water, closed canopy forest, road buffers, human settlement buffers) was akin to introducing habitat loss to a range. If eagles are prevented from using an area then their proportional use of other areas is altered (Kochert et al. 1999, Whitfield et al. 2001). Hence this shift in range use needed to be incorporated within the PAT model.

We do not know exactly how eagles respond to habitat loss and so to account for such alterations we used a subjective rule. The foundation of this rule was that
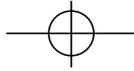
any reduction in the area within a 500 m distance band caused by an exclusion area, resulted in a proportional over prediction of range use in that distance band. This means that, for example, if a distance band has 500 pixels and has been assigned a use value of 16 % then each pixel represents, on average, $16/500 = 0.032$ % of predicted range use. If, however, 250 of the pixels are closed canopy forest then each pixel represents, on average, $16/250 = 0.064$ % of range use, so that simply because much of the distance band is unsuitable habitat this inflates the predicted proportional use for the remaining pixels of suitable habitat in that distance band. It seems very unlikely that eagles would compensate for the loss of habitat only by increasing their use of areas immediately surrounding the lost area, so there was therefore a need to spread the excess of predicted range use within and between the 500 m wide distance bands.

The subjective rule underpinning this redistribution was that 25 % of the excess was retained in the source distance band (where the unsuitable habitat or exclusion area was located), 25 % was shifted towards the range centre in the adjoining distance band, 25 % was shifted away from the centre by one distance band, and 25 % was shifted away from the centre by two distance bands. For an exclusion area in the central distance band or in the outer distance band, excess predicted range use was redistributed equally throughout all other distance bands. In addition, each distance band was restricted to a maximum predicted range use per unit area (based on the observed maximum for eagle ranges in the region): any excess was redistributed using the 25 % rule. Thus for distance band x, expected to contain 16 % of the total predicted range use, but which had its area reduced by 0.5 through the presence of an exclusion area, 2 % (25 % of (0.5×16)) of the predicted range use was shifted to the neighbouring distance band $x - 1$ (i.e. towards the centre), 10 $(8 + 2)$ % was retained in band x, 2 % was shifted out to band $x + 1$, and 2 % was shifted out to band $x + 2$. Having redistributed predicted range use between distance bands because of excluded features, it was then redistributed within distance bands based on the separate preference rules for being near or over terrain features, as described earlier.

This was the last stage in the modelling process (Fig. 5). Following incorporation of all variables, the PAT model output was a raster representation of predicted range use, each 50×50 m pixel having a predicted 'use value'. For each range, the sum of these use values was 100, with the value of each pixel being a percentage of the sum of the values of all pixels. Pixels with higher use values were located near the range centre and around terrain features, and pixels with the lowest values were further away from the centre and terrain features (Fig. 6).

Model evaluation

As noted earlier, due to differences between study areas in data collection methods we had to employ different methods of analysis. For each study range on mainland
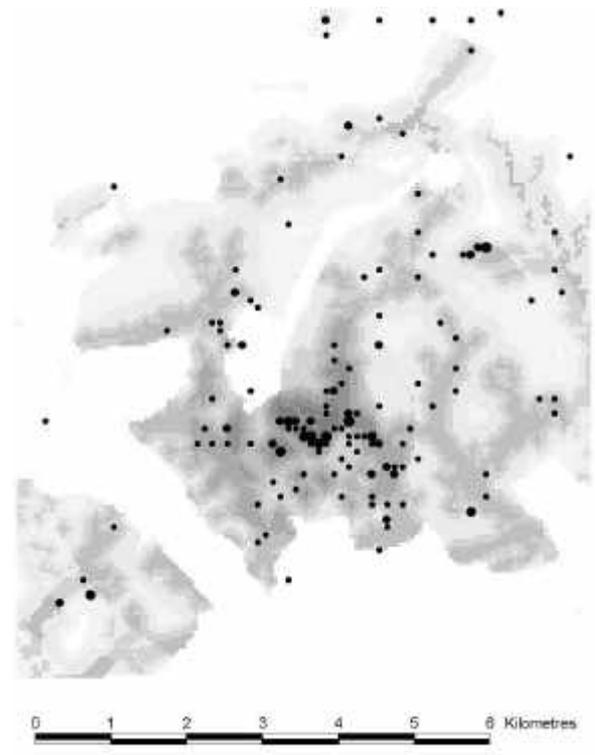
Figure 6. Two dimensional representation of predicted range use for a golden eagle territory according to the PAT model (shaded areas) and observations of actual range use (solid circles – size proportional to number of observations) for a study range in mainland Argyll. For the PAT predictions different intensities of shading represent different classes of predicted range use with darker shading representing greater predicted use of an area (number of classes kept low in this example for clarity of presentation). Note also that range use observations are not necessary to generate the PAT predictions, but in this case the PAT model was run for a study range to give an indication of 'observed' use.
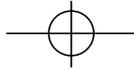
Argyll and Mull the fit of the observed data to the PAT predictions was examined first using quantile-quantile or Q-Q plots. Q-Q plots compare observed and expected quantile values drawn from frequency distributions. If the observed data fit the expected data perfectly the points form a straight line (see Sokal & Rohlf 1995 for details). Expected quantiles were taken from the PAT model's frequency distribution of predicted range use relative to the range centre and the observed quantiles were taken from the frequency distribution of actual range use observations with distance from the range centre. The number of data points varied between ranges because ties were removed. The fit of the observed data to the PAT model was also tested using a Kolmogorov-Smirnov goodness of fit test ($\alpha = 0.05$): data were assigned to 20 classes (5 % increments) of distance from the range centre.

The predicted utilisation surface from the PAT model for the Ross of Mull was overlaid on the preference map within a GIS. To facilitate the overlay operations and comparison of layers, PAT-predicted usage values in each pixel were classified into 8 bins with equal increments and tabulated against the corresponding preference class. Only pixels predicted as having a usage value by the PAT model were included in the analyses. Frequency distributions and mean values of the PAT usage predictions were compared for three of the four preference classes (the no-data class was excluded).

We also tested the model against range use observations collected on two ranges in Galloway. The PAT model was run for the two Galloway ranges values, based on a range centre calculated from the nest sites used over the observation period, and we summed the predicted use for all pixels within each 1 km OS grid square that was visited by observers. We then entered these values in a linear regression against the observed values of use (proportion of visits by an observer to the grid square when an eagle was seen) for the same grid squares. Each range was analysed separately, and any grid squares with no visits were excluded because these grid squares were always some distance from the range centre and would be predicted to be little used by the PAT model. Inclusion of such squares would have introduced a spurious positive relationship between no 'observed use' and low predicted use.

To test whether the PAT model was more 'efficient' than the RIN model we examined whether, in the 11 mainland Argyll and Mull home ranges, it predicted smaller areas for the same percentage of observed range use points. In each range for each model we calculated the area that was predicted in order to encompass the same 50 % of the observed range use points. The RIN model was run with an assumed 2.5 km 'core area' and with a 6 km cut-off from the range centre in the absence of a neighbouring range.

Results

Model output

The PAT produced an output with three dimensions: the geographic location as x and y co-ordinates and predicted range use as a percentage of the total use as a z co-ordinate. Resolution was to 50×50 m (equivalent) pixels, each pixel with a predicted percent use value (Fig. 6).

We generated a 'use surface' by ranking the pixels in descending order according to their individual percentage use value, and then cumulatively summing the use values until notional percentages of total range use were reached. Isolines could then be drawn around all pixels that contributed to each notional percentage of total range use. For example, the 80 % isoline encompassed all of the pixels with the highest use values that summed to 80 %, and represented the geographic area required to encompass 80 % of the predicted ranging of an eagle pair. Other methods, such as a kernel estimator can only derive such a 'density surface' when there are observations of eagle range use. The PAT model generates a density surface using only information on nest site locations.

Model evaluation

The Q-Q plots indicated that there was no consistent bias in the PAT model's predictions of range use for the 11 ranges on mainland Argyll and Mull (Fig. 7). Under-prediction occurred when the PAT predicted that the eagles used areas closer to the range centre more frequently than was observed. Under-prediction may have occurred on some ranges because they were more successful for breeding and so parents spent more time around the nest (range centre). On Mull three range use distributions were under-predicted by the PAT, and two range use distributions were over-predicted. Qualitati-

a)

b)
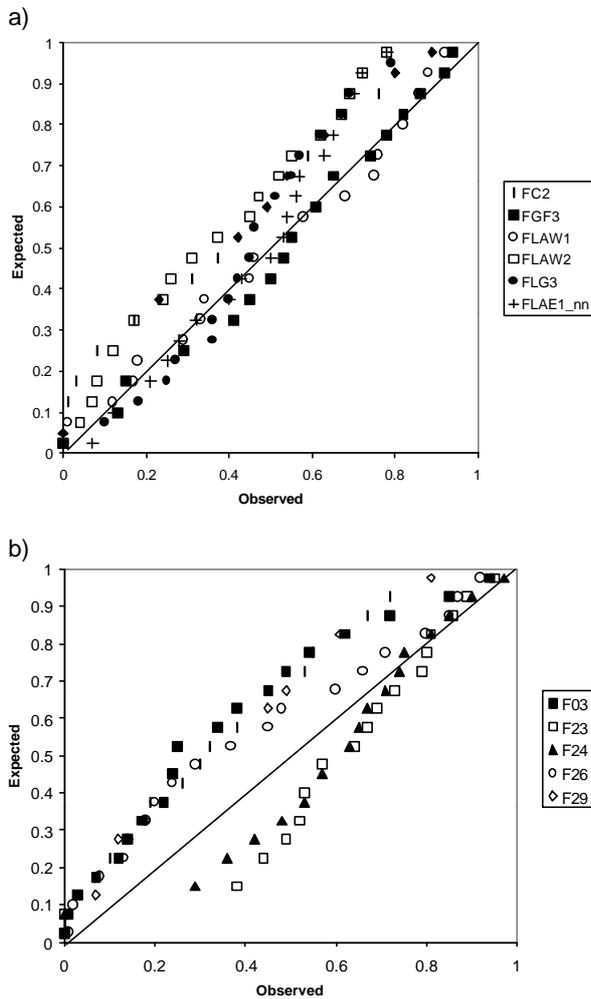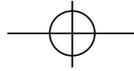
Figure 7. The observed and expected (= predicted by the PAT model) distributions of ranging points with distance from range centre plotted as Q-Q plots for a) six golden eagle home ranges on mainland Argyll and b) five home ranges on Mull. Deviations from the line represent deviations from a perfect relationship between observed and expected. The legend identifies codes for individual ranges.

vely, predictions of the PAT for the mainland Argyll ranges appeared to show a better fit, with a tendency to over-predict. Two of the three under-predicted ranges on Mull were neighbours and had nest sites on high sea cliffs and one of these was highlighted for a lack of fit using the Kolmogorov-Smirnov tests (Table 1). There was no significant difference, however, between PAT-predicted and observed distributions of range use in any of the other ten ranges, suggesting that overall the PAT provided good estimations of observed range use. The

range on Mull with one of the poorest fits of predicted use to observed use had two alternative nest sites 2 km apart that were both used in the study period. Historically (30 years ago) these two alternatives were two ranges but in the last 10 years we are certain that the area has been used by only one pair. A better fit would probably have been obtained if the range had been considered as having two range centres (for split range centres see also McGrady et al. 1997). Prior to analysis, however, we could not justify this split, despite the range's history, because other ranges on mainland Argyll had alternative nest sites as far apart as this Mull range.

One-way ANOVAs indicated that for each of the five Mull ranges the predictions of use by the PAT and the usage preference classes were strongly associated (Table 2). This was a further confirmation that the PAT predictions of range usage were a good fit to the empirically derived estimates of range usage.

For the two ranges in Galloway there was a significant positive relationship between the observed use and PAT-predicted use (range DG1: $n = 130$ grid squares, $t = 7.47$, R^2 (adj) = 0.30, $P < 0.001$; range DG3: $n = 129$ grid squares, $t = 7.85$, R^2 (adj) = 0.31, $P < 0.001$). Removal of one strong outlier in the DG1 regression improved the relationship markedly ($t = 11.54$, R^2 (adj) = 0.51, $P < 0.001$). Weighting the observed values to account for differences in the number of visits between squares had little effect on either the slope or R^2-value of the relationships.

For all 11 ranges in mainland Argyll and Mull the RIN model predicted a larger area than the PAT model to encompass the same 50 % of range use observations (median & range, ha: RIN, 950 & 100–1830; PAT, 730 & 75–1080; Wilcoxon test, $Z = 2.93$, $P = 0.003$). As the percentage of range use observations increased, the area predicted by each model to encompass the observations also increased, but the difference between the models in the predicted area increased too. This was expected given that the RIN is very coarse when including areas as part of a predicted home range, whereas the PAT is much more specific and, therefore, efficient.

Discussion

Kolmogorov-Smirnov tests are especially sensitive to small differences in distributions (Syrjala 1996), so
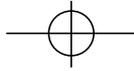
Table 1. Results of Kolmogorov-Smirnov two-sample tests of differences in frequency distributions of golden eagle range use relative to range centre for PAT predictions and field observations for 11 home ranges on the Ross of Mull and mainland Argyll. The term D is the largest unsigned difference. * P < 0.05, two-tailed.

Home range	D
ML03	0.28
ML23	0.30*
ML24	0.20
ML26	0.20
ML29	0.17
C2	0.20
LG3	0.13
GF3	0.17
LAE1A	0.16
LAW1	0.14
LAW2	0.21
All ranges	0.05

the lack of a significant difference between observed and PAT-predicted distributions for most of the study ranges suggests the model was robust. The Q-Q plots indicated that there were no systematic biases in the model predictions. Testing the model against the same data used in its derivation, nevertheless, was not the best test of the model (e.g. Wiens 1989). The better tests of the PAT, however, for two ranges in Galloway that were not used in the model's derivation, also pointed to a reasonably robust predictive capability. Despite the coarseness of the scale of the observations relative to the PAT's predictions and the isolation of Galloway golden eagles, which may have produced unusual range use behaviour, there was a good agreement between observed and predicted use patterns.

Although the PAT appears to produce robust predictions, improvements can be made, possibly including the alteration to range use where the centre is close to a boundary (e.g. when pairs nest on sea cliffs), accounting for split range centres, and accounting for the possibility that golden eagles use terrain features as territorial boundaries. To make such improvements will require further observations of golden eagle ranging. Observations of golden eagle range use elsewhere would also test the utility of the PAT in environments different to western Scotland. The modelling approach may also be worth considering for other raptor species that have similar ecology to golden eagles.

The PAT is a novel approach to modelling golden eagle range use but most features it incorporates have been described by previous studies (see Methods). Relatively few vegetation features were used to predict range use, although golden eagles can exhibit preferences for particular vegetation types that are associated with preferred prey (Marzluff et al. 1997, Pedrini & Sergio 2002), or avoid habitats that are structurally unsuitable for foraging (e.g. Watson 1992, Pedrini & Sergio 2001a, Whitfield et al. 2001). Incorporation of vegetation characteristics would probably improve the predictive ability of the PAT, especially if prey is strongly tied to vegetation that is distributed heterogeneously. Prey in the two study areas was markedly different (Watson 1997), however, so it is perhaps surprising that the model has at least reasonable predictive success when terrain is the principal 'habitat' parameter.

While there may be a link between vegetation classes and terrain features, terrain probably mainly acts as a surrogate for wind and air currents. In essence, the predictive success of the PAT strongly suggests that in Scotland wind and air currents are a resource that ea-

Table 2. Results of ANOVAs for the relationship between eight incremental classes of predicted use by the PAT model and three classes of indicative preference (no data class excluded) as observed on five golden eagle ranges on the Ross of Mull. Sample size was the number of pixels (50 × 50 m equivalent) in each range.

Range	Source	df	Sum of squares	F	P	Source	df	Sum of squares
ML24	Preference	2	86806	42.65	<0.001	Error	4814	4899411
ML29	Preference	2	286313	186.70	<0.001	Error	3403	2609339
ML03	Preference	2	419719	426.92	<0.001	Error	8922	4385766
ML23	Preference	2	1239564	393.64	<0.001	Error	4466	7031742
ML26	Preference	2	296038	143.34	<0.001	Error	6985	7212802
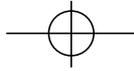
gles select and should be considered as a major contributor to 'habitat suitability'. Rugged terrain probably allows eagles to hunt over a larger area of land per unit time of flight, and may also allow prey to be surprised more easily. In many mountainous regions more rugged areas are also associated with low human disturbance and a low level of development, which are probably favoured by eagles (Watson 1997). Areas associated with thermals, such as patches of scree or rock outcrops, may be important components of habitat selection, especially in warmer environments. The importance of air currents to large raptors when on migration is well known (e.g. Smith 1985), so their influence on more local movements should not be unexpected, even if rarely considered previously (Bögel & Eberhardt 1997). Future studies of habitat selection for some raptors should move beyond a consideration of habitat only in terms of vegetation. This applies not just to species adapted to soaring flight (Strickland et al. 2000) but to species that predominately use other flight behaviours (Jiménez & Jaksic 1993).

Particular activities, including hunting and territory defence might be associated with certain portions of the range. These might be incorporated into estimates of range use if appropriate data were collected, although for golden eagles it may be difficult to distinguish active hunting from simply flying from one part of a range to another (Watson 1997). Some areas may be important only as a connection between areas important for other reasons (Fielding et al. in press). McGrady et al. (1997) pointed out an example of this where a large block of forest may have discouraged use of areas beyond the block's boundaries.

The PAT essentially represents an extension of the RIN, incorporating many features identified by McGrady et al. (1997) as important but not formally included in the RIN. While the PAT probably provides a more accurate prediction of range use (McLeod et al. 2002), the simplicity of the RIN model's implementation should retain its usefulness in many situations. The greater efficiency of the PAT makes it preferable when range use predictions need to be more precise, but its application requires access to supporting software such as digital terrain data and other aspects of a GIS, in contrast to the RIN. On the other hand, ongoing work to improve the RIN should aim to incorporate formally the regional variation in range dimensions that this study has illustrated, especially when considering that regional varia-

tion in density, probably related to food availability, is a common feature of golden eagle populations (e.g. McGrady 1997, Watson 1997, Pedrini & Sergio 2001b, 2002). Both the RIN and the PAT require a priori knowledge of the location of nest sites, and in some poorly studied areas this knowledge will not exist. Nevertheless, the effort to locate nest sites is far less than the effort required to document actual range use.

The impact of developments such as wind-farms and forestry can be assessed according to their proposed location in relation to predicted range use. The models can be used to identify boundaries of protection areas for golden eagles and identify locations where management to improve ranges would be most effective. Connection of the range modelling software with a national database on the distribution of eagle nest sites (Green 1996) provides landscape-scale information on areas predicted to be important for breeding eagles (McLeod et al. 2002), and allows for more strategic conservation planning. Marzluff et al. (1997) emphasised the need for managers to take account of variation in golden eagle range use if conservation strategies are to be effective, while Pedrini & Sergio (2002) have highlighted how regional gradients in density, diet composition and productivity may be used to set priorities for golden eagle conservation at the landscape scale.

use modelling. Scottish Natural Heritage funded the development of the PAT model.

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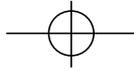
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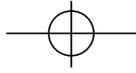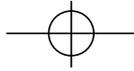
An indicator of maternal stress is correlated with nestling growth in pied flycatchers *Ficedula hypoleuca*

Juan Moreno[1], Santiago Merino, Juan J. Sanz and Elena Arriero

Reproductive success is frequently associated with factors such as hatching date, presence of ectoparasites or maternal age. Seldom is the stress of parents considered as an important determinant of offspring size and condition. In a population of pied flycatchers *Ficedula hypoleuca* breeding in a montane area of central Spain, we estimated fledging success as well as measured offspring size and condition in order to evaluate crucial determinants of reproductive success. We also measured the cell-mediated immunocompetence of 12-day old nestlings with the phytohemagglutinin injection assay. Maternal stress was estimated through the heterophil/lymphocyte ratio (H/L ratio) obtained from leukocyte counts in blood smears. Infection by *Haemoproteus balmorali* and *Trypanosoma* spp. was also obtained from blood smears. The prevalence and intensity of infestation of nests by mites *Dermanyssus gallinoides* and fly larvae *Protocalliphora azurae* were used as estimates of ectoparasitism. Maternal H/L was not significantly related to the presence of mites or infection by haematozoa, while it was positively associated with provisioning rates at the nest and negatively with presence of fly larvae. We examined fledging success and measures of offspring fitness in relation to hatching date, brood size, number of *Protocalliphora* larvae, presence of mites and maternal age and stress. Fledgling mass and wing length were significantly negatively correlated with maternal H/L ratio and presence of mites, while tarsus length was significantly negatively associated with mite prevalence. The other factors had no significant effects. The models explained 33–40 % of variation in fledgling morphological traits. Fledging success and nestling immunocompetence were not associated with any variable. Females that, according to their H/L ratio, were stressed produced smaller and lighter chicks. Parental stress which may be due to health status should be taken into account in future studies of reproductive success.

Key-words: Heterophil/lymphocyte ratio, reproductive success, fledging size, ectoparasitism, maternal stress, pied flycatcher.

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Parents subjected to stresses affecting their general well-being may be unable or unwilling to invest fully in reproduction. In natural situations, stresses may be due to pathogen attacks, intense predation risk, an adverse social environment or other ambient factors. Gustafsson et al. (1994) were among the first to propose that the health status of parents could directly affect reproductive performance. Thus, the infection status of parents by helminths or haematozoa has also been shown to affect breeding phenology and clutch size negatively (reviewed by Møller 1997). The only experimental study in the field manipulating parental infection status by haematozoa has shown that natural levels of infection may impair breeding performance
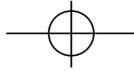
(Merino et al. 2000). However, most of these studies focused on particular infectious agents, which may or may not have been important in the population studied (Møller 1997). A better way to check the effects of health on performance is to evaluate general haematological indices of health, which may offer a compounded view of the effects of all pathogens impinging on individual hosts (Campbell 1995, Coles 1997). In the collared flycatcher *Ficedula albicollis*, breeding success apparently correlates negatively with indicators of prior infection such as white blood cell count, blood sedimentation rate and the presence of immunoglobulins in blood (Gustafsson et al. 1994). In the chinstrap penguin *Pygoscelis antarctica*, late breeders which normally experience low breeding success, showed a higher white blood cell count and a lower cell-mediated immune response (Moreno et al. 1998). Female Magellanic penguins *Spheniscus magellanicus* with more leucocytes in peripheral blood laid smaller eggs and raised fewer chicks to fledging (Moreno et al. 2002). The evidence available suggests that parental health may be a crucial determinant of reproductive success in birds as diverse as penguins and small passerines. There may be other stressful factors besides pathogens in the environment affecting parental well-being. We will focus here on general stress indicators rather than on infection status, assuming that pathogens probably represent the main stressors in natural conditions.

However, there is a need to check for associations between parental stress and breeding success while controlling for other possible factors such as parental age, presence of ectoparasites on chicks, breeding date and brood size. The independent effect of parental stress on performance, while taking into account the complex web of interacting variables in an ecological context, has not been shown previously. While experimental manipulation of parental well-being would be the optimal way forward, this is only feasible for specific stressors, as has been shown for haematozoa by Merino et al. (2000). In the present study we have tried an observational approach in an intensively studied population of pied flycatchers *Ficedula hypoleuca* where most potential correlates of breeding success are known. We have focussed on females, given their stronger impact on offspring size, condition (Moreno et al. 1997) and health (Merino et al. 1996).

As indicators of breeding success we used fledging success (proportion of hatched young that fledge) and

three measures of offspring fitness normally used in avian studies: body mass, tarsus length and wing length. Tarsus length before fledging is significantly correlated with offspring survival in this species (Alatalo & Lundberg 1986, Alatalo et al. 1990). Offspring mass has been repeatedly shown to be positively correlated with survival in passerines (e.g. Tinbergen & Boerlijst 1990, Lindén et al. 1992). Wing length is another measure related to offspring fitness presumably through its effect on flight capacity at fledging (Nilsson & Gårdmark 2001). Avian nestling immunocompetence (IC) has been proposed as a better predictor of offspring survival than fledging mass or condition (Christe et al. 1998, 2001). We have thus used nestling cell-mediated IC as measured by the standard phytohemagglutinin skin testing technique (Lochmiller et al. 1993) as another measure of reproductive success.

As a measure of parental well-being we have used the heterophil/lymphocyte ratio (H/L), which is a widely used stress estimator in poultry (Gross & Siegel 1983, Maxwell 1993). H/L is known to increase in response to various stressors, including infectious diseases, starvation and psychological disturbance, and has been shown to have a small measurement error (Ots et al. 1998). Hõrak et al. (1998) showed higher H/L values in female great tits *Parus major* caring for experimentally enlarged broods, suggesting that a higher reproductive effort induced a higher stress. However, in a non-experimental situation, a higher stress may affect negatively parental effort and consequently breeding performance. The question we want to answer is whether maternal stress is a significant predictor of reproductive success.

Material and methods

Study area and species

The study was conducted in 2000 in a deciduous forest of Pyrenean oak *Quercus pyrenaica* at 1200 m a.s.l. in the vicinity of La Granja, Segovia province, central Spain (40°48'N, 4°01'W). A study of a population breeding in nestboxes in this area has been conducted since 1991 (Sanz 1995). Nestboxes (125×117 mm bottom area) are cleaned every year after the breeding season. Every year, the nestboxes have been checked for occupation by pied flycatchers, and the dates of clutch
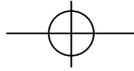
initiation, clutch sizes and number of fledged young were recorded.

Females feeding at the nest were captured with nest-box traps on nestling day 13 and aged as yearling or older (Svensson 1984). Some females could be aged exactly or a minimum estimated age could be established (Sanz & Moreno 2000). Their tarsus length was measured to the nearest 0.01 mm with digital callipers following Svensson (1984), and their mass recorded with a Pesola spring balance to the nearest 0.1 g. Nestling tarsus length and body mass were measured similarly on the same day after recording the wing web swelling (see below). Nestling wing length was measured with a ruler to the nearest 1 mm following Svensson (1984). Data for tarsus length are not comparable to those in other studies which used the distance between bending points (e.g. studies cited in Lundberg & Alatalo 1992 and Merino et al. 1996, Moreno et al. 1997).

Provisioning rates

On the day before nestlings were immunised, the entrance of the nestboxes was filmed for 1 h with a video camera placed 5–10 m away from the nestbox in order to count the number of feeding trips performed by both mates. However, all sessions in which one pair member did not turn up at the nest, although known to be present in the study area, have been excluded from analyses, as its absence could have been due to the birds being disturbed by the camera or other unknown factors. There was no effect on provisioning rates of time of day when films were made as all films were obtained between 0930 and 1445 (correlations for males and females, $P > 0.10$). In total, 49 nests were filmed.

Nestling immunocompetence

The PHA skin test is considered as a useful method to evaluate thymus-dependent function (Goto et al. 1978), and has been routinely used as an assay of T-lymphocyte cell-mediated immune function in studies of poultry (Lochmiller et al. 1993). It is being increasingly used also in field studies, given its benign character compared with other methods used to evaluate immunocompetence (Merino et al. 1999). The main cellular response observed 6 to 12 hours after injection consists of a prominent perivascular accumulation of

T-lymphocytes followed by macrophage infiltration (Goto et al. 1978). The PHA stimulated inflammation disappears normally 48 hours post-injection.

On day 12, nestlings were injected with 0.02 ml of a solution of phytohemagglutinin (PHA) in PBS (10 mg of PHA in 10 ml of PBS) in the left wing web after measuring its thickness at the point of injection. The same amount of PBS was injected in the right wing web in the same manner. Three measures of each web were taken with a digital spessimeter with constant pressure (Mitutoyo 7/547, Tokyo, Japan) to the nearest 0.01 mm to calculate the repeatability of wing web measurements. After 24 h, three new measurements of the thickness of each wing web at the point of injection were taken. The three measurements were averaged as wing web thickness has shown a high repeatability in an earlier study (Moreno et al. 1999a). Cell-mediated immunocompetence (IC) was estimated as the difference between the differences between initial and final measurements of the left minus the right wing web. Only average values for broods have been analysed to avoid pseudoreplication.

Ectoparasite loads

The main ectoparasites of pied flycatcher nestlings are the mite *Dermanyssus gallinoides* and larvae of the parasitic fly *Protocalliphora azurea* (Merino & Potti 1995, 1996). Two measures of mite parasitism are used: simple prevalence/presence/absence, and a semiquantitative infestation score. Infestation was scored as: (0) no mites detected either on nest-box, nest material or nestlings when handled on day 13, (1) nests with scattered mites detected when handling chicks, (2) nests with many mites running over the nestlings when handled (hundreds of mites), and (3) nests with many mites at the nest-entrance, running over the nestbox and nestlings when handled and crawling over the hands of the researcher (thousands of mites). The numbers of pupae of the parasitic fly were counted after fledging by extracting them from the nest material.

Parental haematology

Leucocytes form the basis of the immune system, and their main function is protection against foreign pathogens. Lymphocytes and heterophils are the most abundant types of leucocytes in avian blood (Campbell
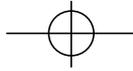
1995). Heterophils are bactericidal phagocytes that enter tissues during the inflammatory response (Maxwell & Robertson 1998). They are non-specific immune cells, in contrast to the highly specific response of lymphocytes (Jurd 1994). Davison et al. (1983) and Gross & Siegel (1983) described a ratio calculated from the proportions of heterophils and lymphocytes present in the circulation of domestic fowl as a measure of stress. The H/L ratio has now become widely accepted as a reliable and accurate physiological indicator of the stress response (Maxwell & Robertson 1998). In poultry, stressors like infections, starvation and disturbance have been associated with increased H/L values. In the wild, the most plausible stressors are pathogens. H/L will be interpreted in the following as a measure of general health as proposed by Ots et al. (1998).

To estimate H/L, a blood smear was obtained from the brachial vein of each female on capture (see above). A drop of blood was smeared on individually marked microscope slides, air-dried, fixed in absolute ethanol and stained with Giemsa. Slides were examined under 1000x magnification with oil immersion to estimate the proportions of different types of leucocytes. Examination stopped when 100 leucocytes other than thrombocytes had been found (thrombocytes normally present an irregular, aggregated distribution). Fields with similar densities of erythrocytes were scanned for all individuals (323 ± 52 erythrocytes per field, n = 53 individuals). H/L was estimated from the numbers of heterophils and lymphocytes per 100 leucocytes obtained in these counts.

Blood parasite score

The same smears used for haematology were scanned for haematozoa following methods described in Merino et al (1997). *Haemoproteus balmorali* and *Trypanosoma* spp. were the only blood parasites found in the study population. Prevalences were 13 % for *Haemoproteus* and 43 % for *Trypanosoma*. Only presence/absence will be used in analyses.

Statistical analyses

The numbers of pupae, hatching date and maternal H/L were not normally distributed, and so were log-transformed before performing parametric statistical analyses. Fledging success was subjected to square-root
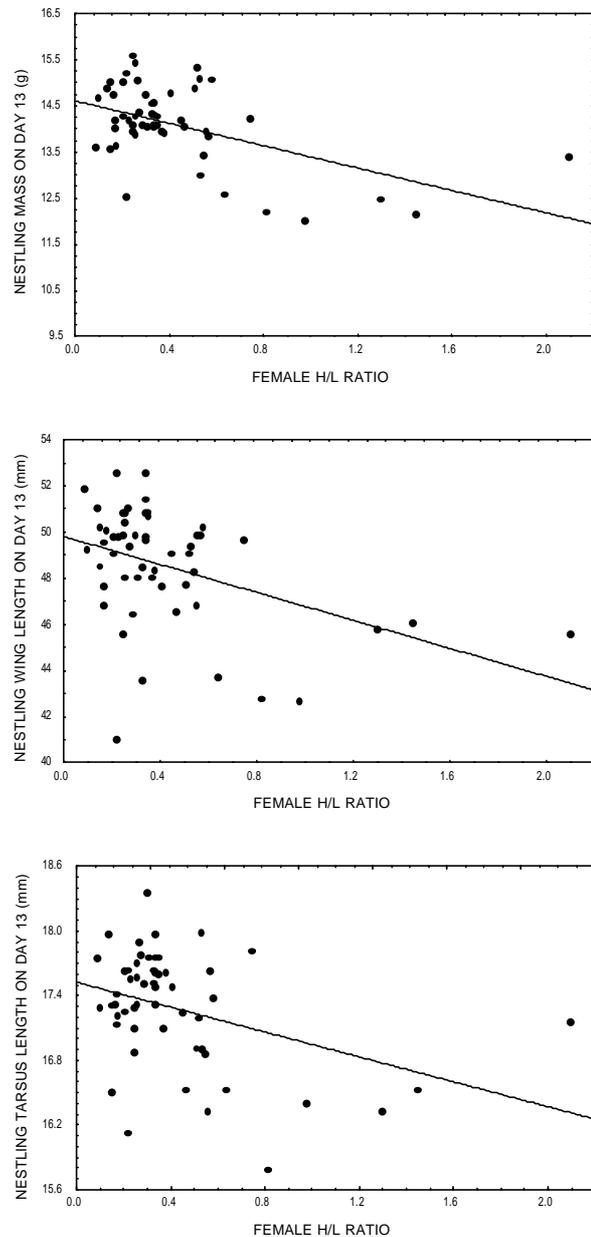
Figure 1. Significant negative associations between size measures of nestling pied flycatchers at 13 days old and maternal H/L ratio (see text). Regression analyses given in the text were performed on logarithmically transformed H/L data. Lines presented are derived from linear regression and are illustrative.

arcsin transformation before analysis. Brood means have been used in all analyses. Maternal H/L was related to different continuous or discrete variables with the GLM module from the STATISTICA package. The
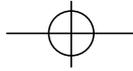
same module was used to relate offspring fitness to potential determining variables. GLM analyses were run separately on the five breeding success variables: fledging success, nestling mass, tarsus length and wing length and nestling IC. The following independent variables were included in the analyses of reproductive performance: hatching date, brood size on day 13, presence/absence of mites, number of fly pupae, female provisioning rate, female age and female H/L. These variables were included because they have been shown to be associated with breeding success in studies of avian breeding biology. When analysing fledging success, clutch size was used instead of final brood size, given that final brood size is included in the dependent variable. Nestling morphology may be partly determined by inheritance (Alatalo et al. 1990, Potti & Merino 1994). To control for possible resemblance effects between parents and offspring, we ran the models including the midparent values. The number of variables included was a compromise between trying to include most potentially affecting variables and keeping the analyses manageable. Only probabilities below 5 % are considered significant. Effect sizes (% of variance explained) are presented in all cases when probabilities were below 10 %.

Results

Mean maternal H/L was 0.42 ± 0.35 (n = 56; range 0.09–2.10). Pathogens and parental effort have been previously linked with stress in breeding birds. Thus, we performed a GLM with presence/absence of mites, infection by *Haemoproteus* and *Trypanosoma*, number of fly pupae and female provisioning rates. Only the number of fly pupae and provisioning rates had significant associations with H/L (fly pupae: $F_{1,42} = 4.7$, P = 0.04; provisioning rate: $F_{1,42} = 8.1$, P = 0.007), the model explaining 30 % of variation in this stress indicator ($F_{5,42} = 5.0$, P = 0.001). Surprisingly, the number of pupae showed a negative association with female H/L (slope = -0.26 ± 0.12 (s.e.)), while provisioning rates were, as expected, positively associated with H/L (slope = 0.36 ± 0.12 (s.e.)).

Fledging success was not significantly associated with hatching date, clutch size, ectoparasites, female age, H/L or provisioning rate in a GLM analysis (P > 0.10 in all cases). Barely 3 % of the variation in fledg-
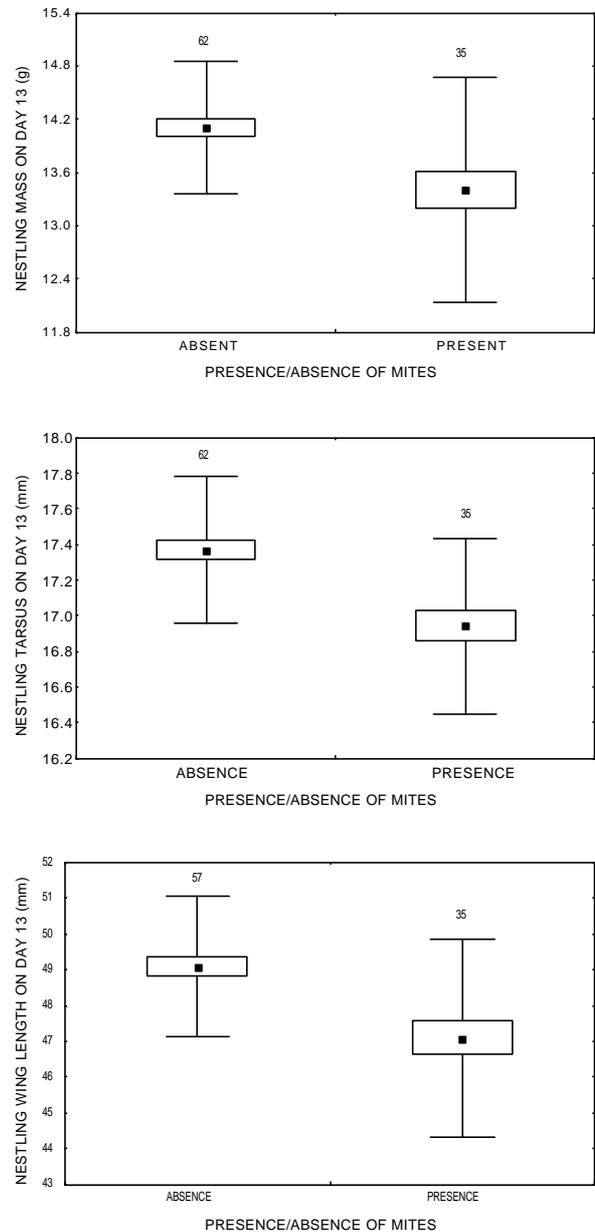
Figure 2. Comparison of size measures of nestling pied flycatchers at 13 days old in nests with or without mites *Dermanyssus gallinoides*. Boxes represent 1 s.e., bars are 1 s.d. and figures are the numbers of broods analysed.

ing success was explained by the model. Nestling mass was significantly negatively associated with female H/L ($F_{1,48} = 8.7$, P = 0.005, effect size = 11 %, Fig. 1) and negatively with the presence of mites ($F_{1,48} = 5.6$, P = 0.023, effect size = 7 %, Fig. 2). The whole model
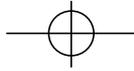
explained 40 % of the variation in nestling mass and was highly significant (P < 0.001). Tarsus length was negatively associated with the presence of mites ($F_{1,48}$ = 8.0, P = 0.007, effect size = 11 %, Fig. 2), while maternal H/L approached significance ($F_{1,48}$ = 3.9, P = 0.056, effect size = 5.4 %, Fig. 1). The whole model explained 33 % of the variation and was highly significant (P = 0.002). Both maternal H/L ($F_{1,48}$ = 4.5, P = 0.041, effect size = 5.3 %, Fig. 1) and mites ($F_{1,48}$ = 4.8, P = 0.034, effect size = 5.7 %, Fig. 2) showed negative associations with wing length, the model explaining 43 % of the variation in wing length (P < 0.001. Nestling mass and wing length showed weak, but significant, correlations with midparent values ($F_{1,58}$ = 4.7, P = 0.035, R^2 = 0.06 and $F_{1,59}$ = 4.3, P = 0.042, R^2 = 0.05). Tarsus length was not significantly correlated with the midparent value ($F_{1,61}$ = 1.4, P = 0.24). Including midparent values in GLM models rendered them nonsignificant, the only significant variable remaining being female H/L. Nestling immunocompetence was not significantly associated with any of the variables included in the GLM analysis (P > 0.10). Barely 3 % of the variation in nestling IC was explained by the model.

Discussion

Our results suggest that nestling growth of pied flycatchers was more strongly associated with maternal well-being in our study year than with other factors such as breeding date, competition in the nest among siblings or direct measures of parental effort such as provisioning rates. The genetic component of nestling morphology as expressed by midparent values appears unimportant in the present study, being apparently hidden by strong environmental effects (Alatalo et al. 1990, Potti & Merino 1994, Moreno et al. 1997, Moreno et al. in press). Females with H/L values above 0.5, which is the level considered optimal for poultry by Gross & Siegel (1993), raised small chicks. Between 5 and 10 % of the variation in nestling morphological traits before fledging was explained by this factor alone. Only the presence of mites showed as strong an association with nestling growth as maternal health status. Mites have earlier been shown to have detrimental effects on offspring fitness in Mediterranean pied flycatcher populations (Merino & Potti 1995, Moreno et al. 1999b, Potti et al. 1999). The association with ma-

ternal stress is more surprising and has previously been overlooked in most correlative studies of avian reproductive success (but see Moreno et al. 2002).

A striking result of this study is that stressed females provisioned their broods at higher rates but raised smaller chicks. This leads to the question of whether maternal stress is the cause or the consequence of brood undernourishment, given the experimental evidence that a higher provisioning effort leads to stress (Hõrak et al. 1998). Also, it has been shown that brood demand through begging increases in undernourished broods (Smith & Montgomerie 1991, Price & Ydenberg 1995), which in some cases results in negative associations between offspring size and provisioning rates (Tinbergen 1981, Nur 1984). However, mites also induce intense begging by chicks but their presence in this study did not affect maternal stress and did not explain the associations between maternal stress and nestling growth. Moreover, mite-infested broods have been shown not to elicit increased maternal energy expenditure in this species (Moreno et al. 1999b). Such evidence suggests that there are no simple direct effects of offspring undernourishment on maternal effort.

Studies of avian reproductive performance normally try to detect effects of variables such as breeding date, brood size, parental age, provisioning rates or ectoparasite load. Parental well-being is rarely considered, and then mostly in relation to the impact of specific parasites. Here we show that in our study year, maternal stress as measured by the H/L ratio was one of the most important factors explaining variation in offspring mass, tarsus and wing length. This study, together with a few others (Gustafsson et al. 1994, Moreno et al. 1998, Moreno et al. 2002), suggests that the omission of measures of parental health and stress in studies of avian reproductive success may impede a complete understanding of individual differences in parental performance.
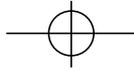
ringing of birds in the study area. This paper is a con-
tribution from the field station 'El Ventorrillo'.

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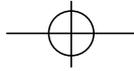
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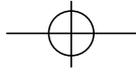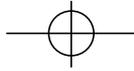
No evidence of genetic differentiation among lesser *Carduelis flammea cabaret* and common redpolls *Carduelis f. flammea*

Richard Ottvall[1], Staffan Bensch[1], Göran Walinder[2] and Jan T. Lifjeld[3]

The remarkable variation in plumage and morphological characters in the redpoll complex *Carduelis flammea-hornemanni* has puzzled taxonomists for several decades. In contrast, molecular studies have not revealed any genetic differentiation among the phenotypic redpoll forms. This could either be a result of high present-day gene flow or due to morphological differentiation following a rapid and recent population expansion. We sequenced a major portion (960 bp) of the mitochondrial control region in individuals of the two taxa *Carduelis flammea flammea* and *C. f. cabaret*. Birds were sampled on autumn migration in southern Sweden (*n* = 30) and on breeding areas in southern Norway (*n* = 11). We found 22 variable sites defining 26 different haplotypes, of which most (22/26) were singletons. The level of haplotype and nucleotide diversity was low in the two taxa and we found no evidence of genetic differentiation. A mismatch distribution was very similar to that expected from a sudden population expansion model. Our estimates suggest that the redpoll population expanded during the last glaciation episode from a small population to a long-term effective population size of 230 000 females. The findings in our study suggest that the morphological differentiation between the two taxa occurred rather recently but after the population expansion.
Key words: mtDNA, control region, population expansion, effective population size, phylogeography.

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The redpoll finch complex *Carduelis flammea-hornemanni* has received much attention from taxonomists due to its remarkable variation in plumage and morphology. Some authors have suggested one highly variable species, *flammea*, while others have suggested seven species (see Knox 1988 for review). The most widely held view recognises two species, the common redpoll (including *flammea*, *islandica*, *rostrata* and *cabaret*) and the arctic redpoll (including *hornemanni* and *exilipes*). The distribution range of the common and the arctic redpolls includes the higher latitudes of the Holarctic, where the two forms breed sympatrically over large areas (Knox 1988). Observations of intermediate birds have been put forward as evidence of hy-

bridisation, but due to improved knowledge of plumage variation, most 'intermediate' redpolls can now be unambiguously assigned to a species identity (Molau 1985, Knox 1988). The most distinctive redpoll form, the lesser redpoll *C. flammea cabaret* was until recently restricted to Britain, Ireland and the Alps. This form has recently expanded its breeding range towards Scandinavia and is now breeding sympatrically with *flammea* in southern Norway in years when *flammea* is breeding in the lowlands (Lifjeld & Bjerke 1996). Assortative mating has been found in one area in Norway and the latest recommendation was therefore to split *cabaret* as a distinct species from *flammea* (British Ornithologists' Union 2001, Knox et al. 2001). While dif-

ferences in plumage and morphology are quite distinctive (Lindström et al. 1984, Knox 1988, Herremans 1990, Knox et al. 2001), we know little about the genetic relationship between *cabaret* and *flammea*.
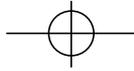
Seutin et al. (1995) examined mtDNA variation in the redpoll complex by using restriction enzymes of mtDNA extracts from specimens of five taxa from both North America and Europe. They found no evidence of genetic structure related to either geographic origin or taxonomic identity. This contrasts with the clear morphological differences between the taxa, suggesting that the species complex had diverged relatively recently and that large and relatively stable population sizes had prevented mtDNA differentiation (Seutin et al. 1995). However, the number of individuals examined in that study was small and the European sample consisted of only two *flammea* and three *cabaret*. We therefore decided to investigate a larger sample of *flammea* and *cabaret* for sequence variation in the mitochondrial control region, the most variable part of the mitochondrial genome and thus the most appropriate choice for intraspecific studies (Edwards 1993, Wenink et al. 1993, Bensch & Hasselquist 1999). We make two predictions based on the result of Seutin et al. (1995).

First, we expect *flammea* and *cabaret* to be undifferentiated in their mtDNA control region sequences. Second, if the populations have been stable as earlier suggested (Seutin et al. 1995), the distribution of pairwise difference between haplotypes should follow expectation at mutation-drift balance (Rogers & Harpending 1992).

Methods

Data collection

Blood samples of redpolls were collected on autumn migration in 1997 and 1999 at Falsterbo Bird Observatory in southern Sweden (55° 23' N, 12° 50' E; 17 *cabaret* and 13 *flammea*) and on breeding grounds in southern Norway at Eidsberg, Østfold county (59° 31' N, 11° 14' E; 5 *cabaret* and 4 *flammea*) and Øvre Heimdalen, Øystre Slidre county (61° 25' N, 8° 52' E; 2 *flammea*) in 1993–1994 (Fig. 1). Individuals were identified to subspecies from plumage and morphological characters (Lindström et al. 1984, Svensson 1992) and for this study we only considered taxon-diagnostic specimens.
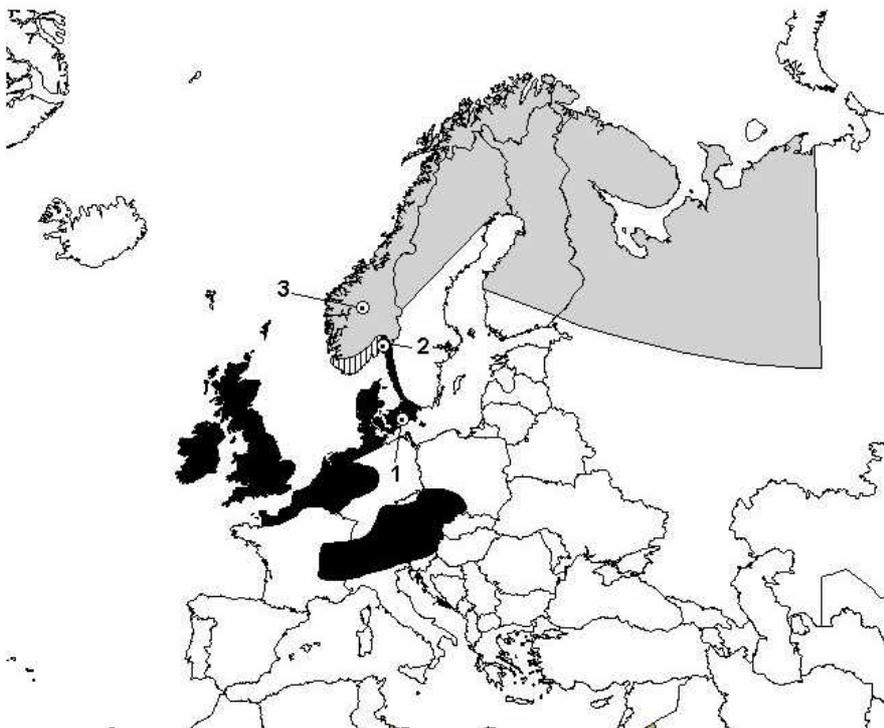
Figure 1. Map showing the European breeding range of the redpoll subspecies *Carduelis f. flammea* (grey) and *C. f. cabaret* (black) and the three sites for sample collection: 1) Falsterbo, 2) Eidsberg and 3) Øvre Heimdalen. In 'irruption' years, the southern limit of the distribution of *flammea* in Norway reach the distribution of *cabaret* (stippled).
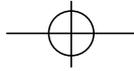
Molecular analyses

Genomic DNA was extracted from blood samples using a standard proteinase k phenol/chloroform procedure. We used the primers L16743 and H1248 (Tarr 1995) to amplify the control region. Polymerase chain reaction (PCR) was performed in volumes of 25 µl and included 25 ng of total genomic DNA, 0.125 mM of each nucleotide, 1.5 mM MgCl$_2$, 1 X PCR buffer (Perkin Elmer), 0.6 µM of each primer and 0.5 units of Taq DNA polymerase. The PCRs were run using the following conditions: 30 s at 94 °C, 30 s at 53 °C, 45 s at 72 °C (35 cycles). Before the cyclic reactions the samples were incubated at 94 °C for 3 min, and after completion at 72 °C for 10 min. Fragments were sequenced directly from both ends and with an internal primer L437 (Tarr 1995), using dye terminator cyclic sequencing (big dye) and loaded on an ABI PRISM™ 310 (Perkin Elmer). A total length of 960 bp covering domain II and III was sequenced in each of 41 individuals (Haplotype #1 in GenBank AF416737).

Descriptive statistics and data analyses

We tested for structuring among the four redpoll samples (*flammea* and *cabaret* from Sweden and Norway, respectively) by calculating ϕ_{ST} statistics using the program Arlequin 2.0 (Excoffier et al. 1992). Significance of variance components between populations was tested with a randomisation procedure provided in the program. We used MEGA (Kumar et al. 1993) to calculate the relationship between haplotypes and visualised these in an un-rooted neighbour-joining tree using Jukes and Cantor's distance.

Nucleotide diversity (π), D statistics (Tajima 1989) and the parameters used to estimate changes in effective population size (θ and τ) were calculated using the program DnaSP 2.52 (Rozas & Rozas 1997). The rate of molecular evolution (s) for the control region is not known, but for various species of birds has been suggested to be between 2 % and 20 % per million year (Baker & Marshall 1997). Because its rate relative the cytochrome *b* gene is variable and taxon specific (Ruokonen & Kvist 2002) and perhaps much higher than inferred from indirect phylogenetic evidence (Lambert et al. 2002), we report the result calculated for low (2 %), intermediate (10 %) and high (20 %) rates. As in Seu-

tin et al. (1995) we assumed the generation time (g) for redpolls to be 2 years.

At equilibrium, θ equals $2u$N where θ is the mean number of pairwise differences, u the mutation rate of the sequence (total number of mutations per sequence per generation) and N is the female effective population size. According to Watterson (1975), a population of constant size is expected to have a mismatch distribution following

$$F_i = \theta^i / [(\theta + 1)^{i+1}]$$

where F_i is the proportion of haplotypes differing by i substitutions and θ is estimated from the data as the observed mean of pairwise differences (Rogers & Harpending 1992).

The pairwise distribution of differences between haplotypes may reveal historical changes in the effective population size (Rogers 1995). The simple situation for which Rogers & Harpending (1992) developed their calculations assumes that an initial population of size N_0 is at equilibrium and suddenly changes to a new population size of N_1, and that this is observed from sequence data t generations later. The time since population size change can be derived assuming that $\tau = 2ut$ and $u = \mu L$ (μ is the mutation rate per nucleotide and generation, L is the length of the studied DNA sequence).

From the estimates of θ and τ we used DnaSP 2.52 (Rozas & Rozas 1997) to calculate the expected distribution of pairwise differences under the hypothesis of a sudden population expansion that happened t generations ago. We estimated the population size before expansion assuming that $\theta_0 = 2uN_0$ and that $\theta_0 = (v - m)^{1/2}$, where v is the variance and m is the mean of the observed pairwise number of differences (Rogers & Harpending 1992). An estimate of the present population size can be obtained using the fact that the vertical intercept (F_0) of the observed distribution of pairwise differences roughly equals $1 / (1 + \theta_1)$ (Rogers & Harpending 1992).

Results

A total of 26 different haplotypes were detected in the 41 sequenced individuals (Table 1). Most haplotypes (22/26) were detected in single individuals only. There were 22 variable sites of which only one involved a
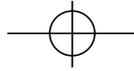
Table 1. Variable sites in the control region among redpolls from Sweden and Norway. The sites are numbered relative to positions in haplotype #1 (GenBank AF416737).

Haplotype	Position			*cabaret*		*flammea*		Total
	1 2 2 2 2 2 3 3 3 7 9 0 2 6 7 9 0 3 7 4 8 7 7 2 9 0 3	3 3 4 4 5 5 5 6 6 8 8 8 3 7 1 8 9 3 6 9 3 5 4 0 4 0 7 2 8 4	9 9 4 4 8 9	Fbo	Norway	Fbo	Norway	
#1	GACCAACCCA	ACTATCATTA	TT	4	0	1	1	6
#2	. . . .	. . . . C T	. .	0	1	0	0	1
#3	. . . .	. . . . C . . C . .	. .	0	0	0	1	1
#4	. . . .	. . . . C . .	. .	1	0	0	0	1
#5	. . . . T .	. . . . C . .	. .	1	0	0	0	1
#6	. . . T	. . . . C . .	. .	0	0	1	0	1
#7	. . T	. . . . C . .	. .	0	0	1	0	1
#8	. . T	. . . . C . . C . G	. .	0	1	0	0	1
#9	. . T . . G	. . . . C . . C . G	. .	0	0	1	0	1
#10	. . . .	. . . . C C .	. .	0	1	0	0	1
#11	. . . .	. . . . C C G	. .	0	0	1	0	1
#12	A	. . . . C . G	. .	1	0	0	0	1
#13	A . . . . -	. . . . C . .	. .	0	0	1	0	1
#14	. . . .	. . . . G	. .	0	0	1	0	1
#15	. . . .	. . . . C . G	. .	3	1	1	1	6
#16	. . . . . . T . . .	. . . . C . G	. .	1	0	0	0	1
#17	. . . . T . .	. . . G . . . C . G	. .	0	0	1	0	1
#18	. . . . G	. . . . C . G	. .	0	0	1	0	1
#19	. . . .	. T . . . . . C . G	. .	0	0	1	0	1
#20	. G	. . . . . T G C . .	. .	0	0	1	0	1
#21	A G	. . . . . T . C . G	C C	1	0	0	0	1
#22	. G	. . . . . T . C . G	. .	2	1	1	0	4
#23	. G	. . C . . T . C . G	. .	3	0	0	0	3
#24	. . T	. . . . C . G	. .	0	0	0	1	1
#25	. . . .	G . . . . . . C . G	. .	0	0	0	1	1
#26	. . . .	. . . . . A . C . G	. .	0	0	0	1	1
Total				17	5	13	6	41

transversion, the remaining being transitions. The number of haplotypes was similar in the two subspecies (17 in *flammea* and 12 in *cabaret*) as was the level of nucleotide diversity (*flammea* π = 0.00303; *cabaret* π = 0.00311). The overall nucleotide diversity was 0.00317 (s.d. = 0.00023). The estimates of Tajimas's (1989) D statistic were negative for both *flammea* (–1.61) and *cabaret* (–0.31) but not significantly different from the neutral mutation hypothesis (P > 0.05).

We found no evidence of genetic structuring when comparing the samples from southern Sweden and Norway (AMOVA; *flammea* ϕ_{ST} = –0.08, n.s.; *cabaret* ϕ_{ST} = –0.01, n.s.). Moreover, there was no sign of differentiation between the two subspecies (ϕ_{ST} = 0.01, n.s.). The absence of genetic structure between either sampling regions or subspecies is obvious from the neighbour joining tree (Fig. 2) in which individuals from two subspecies appear equally scattered. Because we did not find any evidence of mtDNA differentiation between *flammea* and *cabaret*, we pooled the data for the following analyses.

The mismatch distribution (Fig. 3) was very similar to expectation from the sudden population expansion model (χ^2_8 = 3.34, n.s.) and significantly different from a constant population size model (χ^2_8 = 32.3, P < 0.001). Using a divergence rate of 10 % per million years for the control region, our estimates of τ (2.78), θ_0 (0) and θ_1 (20.0) suggested that the population expanded 63 000 years ago from a very small population to a long-term effective population size of 230 000 females. A
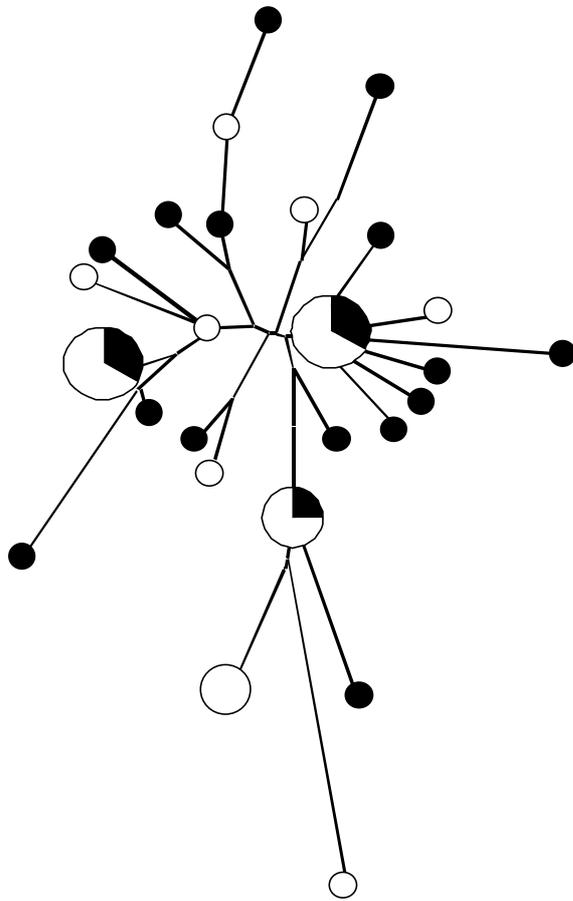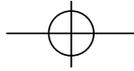
Figure 2. Unrooted neighbour-joining phylogram of red-poll mtDNA haplotypes (*n* = 26) based on control region sequences (960 bp). Sizes of circles correspond to number of observed individuals (1–6) per haplotype. Filled = *flammea*, open = *cabaret*.

lower divergence rate (2 %) would put the expansion time back to 310 000 years BP and a higher divergence rate (20 %) as recently as 32 000 years BP. Hence, the population expansion appears to have occurred within the period of the last glaciation cycle, although there is considerable uncertainty in the estimate because of our poor knowledge of molecular evolution in control region sequences.

Discussion

Variation in mitochondrial control region sequences suggests that the two redpoll taxa *flammea* and *cabaret* are panmictic. A potential limitation of our study is that

samples were collected at only three localities, all situated within a limited part of the *flammea*/*cabaret* breeding range. Our results agree, however, with the finding of Seutin et al. (1995), where redpoll individuals from almost the whole breeding range in the Holarctic were included. Seutin et al. (1995) estimated the mean divergence (π) between lineages to be 0.295 %, very similar to our estimate of 0.317 %. Moreover, we could not find any genetic structuring between the samples from the breeding area in Norway and from the migration locality at Falsterbo. It is therefore likely that our results would hold, had we used samples collected from a larger area of the species' breeding range.

Strong differentiation in morphological traits combined with the lack of genetic differentiation, parallels many other studies of mtDNA variation among bird populations (Zink & Dittmann 1993, Greenberg et al. 1998). Such a pattern could be a result either of high levels of current gene flow and strong selection on adaptive traits to maintain differences, or alternatively, adaptive evolutionary change in morphology occurring too recently for neutral mtDNA to diverge. These two mechanisms may also act in concert to an uncertain degree.

Present-day gene flow

The traditional view is that subspecies and recently differentiated sister species of birds have evolved in allo-
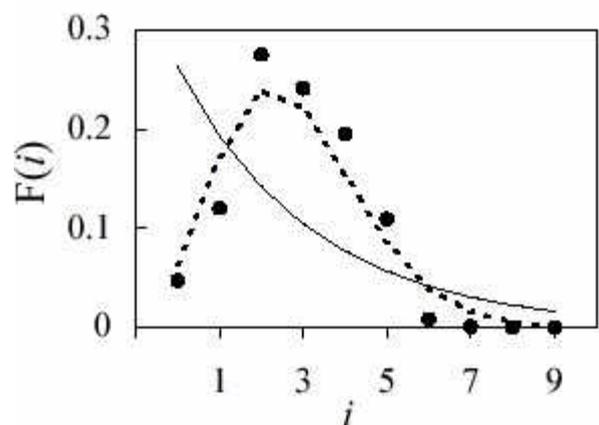
Figure 3. Frequency distribution of pairwise nucleotide differences between mtDNA haplotypes (mismatch distribution). The solid line indicates the expected distribution under a constant population size model, and the stippled line the expected distribution under a population increase model.
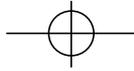
patric habitat refugia during Pleistocene glaciation cycles (e.g. Avise & Walker 1998, Klicka & Zink 1999). For example, *cabaret* might have been confined to the Alps of Central Europe and *flammea* in a different habitat pocket somewhere else in Eurasia or in North America. If this was the case, however, the subspecies would have accumulated at least some differences in mtDNA haplotype frequencies. The question is therefore; could post-glacial gene flow have eliminated differences in haplotype frequencies to the level of apparent panmixis presently observed between *cabaret* and *flammea*?

There is no evidence of present gene flow between *flammea* and *cabaret* but, before the northward expansion of *cabaret* into Scandinavia, gene flow was probably very restricted. This is because the breeding ranges of *flammea* and *cabaret* were separated by >300 km (Hagemeijer & Blair 1997) . After years of irruptive migration, *flammea* has occasionally been found to breed south of its normal breeding range (Cramp & Perrins 1994) when gene flow might have occurred. Within *flammea* Seutin et al. (1995) found no geographic structure between Europe and North America, suggesting a large panmictic population including both continents. Although redpolls are agile birds with a nomadic dispersal pattern, a substantial present-day gene flow between North America and Eurasia seems rather unlikely. Hence, the similarity between *cabaret* and *flammea* in Europe, and between *flammea* in Europe and North America, calls for a common explanation that does not depend on high levels of present-day gene flow.

Population size change

An alternative explanation to the incongruent pattern of molecular and morphological variation in *flammea* and *cabaret*, is that adaptive evolutionary change in morphology occurred too recently for neutral mtDNA to diverge. Such a pattern is particularly likely to arise following a rapid population increase and range expansion from a bottlenecked population, a scenario that redpolls and many other species of higher latitudes have experienced after the retreat of the last ice age (Rogers 1995, Hewitt 1996, Merilä et al. 1997, Zink 1997). This is because neutral alleles are less likely to get lost due to genetic drift in growing populations (Otto & Whitlock 1997) whereas adaptive traits rapidly evolve in the

newly colonised habitats (Schluter & Nagel 1995). In support of this, we rejected the population 'equilibrium' model as pairwise sequence differences gave a good fit to expectations from the 'sudden expansion' model (Rogers & Harpending 1992). The marked peak in the distribution of pairwise differences at a distance of two mutations is consistent with a model of a small population ($\theta_0 = 0$) that has experienced a large population expansion (Marjoram & Donnelly 1994). According to Harpending (1994), a large and stationary population should generate a ragged distribution of pairwise sequence differences. The smooth curve observed in our data set from redpolls suggests that the population has been expanding since the bottleneck.

The population bottleneck, followed by the population increase, implies that the redpoll habitats were greatly affected at some point during the Pleistocene. The common redpoll prefers subalpine birch forests and bushy tundra (Cramp & Perrins 1994), habitats that may have increased in range during glaciated periods, and one can speculate whether this habitat increase promoted the population expansion. Seutin et al. (1995) concluded that the different redpoll taxa radiated in large and stable populations. In contrast, our data on haplotype and nucleotide diversity in redpolls are not consistent with a population showing long-term demographic stability. However, the present analyses cannot establish whether the population expansion preceded the differentiation of the two taxa. The similar haplotype frequencies strongly suggests, however, that they differentiated after the population expansion, and hence in relatively large populations towards the second half of the last glaciation.

Species status

Based on the variation in mtDNA there is no reason to rank the different redpoll taxa as distinct species. The lack of genetic differentiation, however, does not exclude the presence of reproductive barriers between the two taxa. Hybridisation has not yet been conclusively established but such observations are difficult in secretive birds such as redpolls. Assortative mating between *flammea* and *cabaret* found by Lifjeld & Bjerke (1996) supports their species status. On the other hand, more birds with intermediate plumage and morphology have been captured at Falsterbo Bird Observatory in recent years (observations by GW). Of the redpolls caught in
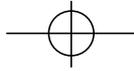
autumn 1999, about 10 % of individuals could not be identified to taxon. These unidentified individuals showed a *cabaret*-like plumage but a morphology similar to *flammea* or vice versa. We believe that these intermediate birds are likely to have been of hybrid origin because *cabaret* is the most distinctive form of the redpolls and should not cause any identification problems (Herremans 1990, Knox et al. 2001). Alternatively, the variation in plumage and morphology within *flammea* is more extensive than presently appreciated (Svensson 1992, Knox et al. 2001) and includes both small individuals and phenotypes with plumage colouration resembling *cabaret*. To our knowledge, this has never been suggested previously and we find it very unlikely as an explanation for the intermediate phenotypes captured at Falsterbo. While we are waiting for more detailed studies on sympatrically breeding *flammea* and *cabaret*, we recommend that the two taxa should be treated as subspecies.

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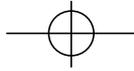
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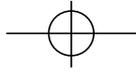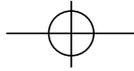
Niche partitioning between immigrant Palearctic willow warblers *Phylloscopus trochilus* and resident Afrotropical warblers in three woodland habitats in Zimbabwe

Volker Salewski[1,2], Peter Jones[2,1] and Juliet Vickery[3]

Palearctic migrants are thought to coexist with resident species in sub-Saharan Africa by foraging in more open habitats and by being more flexible and opportunistic in their foraging behaviours and utilisation of resources than their Afrotropical counterparts. We assessed how migrant willow warblers *Phylloscopus trochilus* partitioned resources with two ecologically similar resident warblers, the burnt-necked and green-capped eremomelas *Eremomela usticollis* and *E. scotops,* in acacia, miombo and mopane woodlands in Zimbabwe during the northern winter of 1999–2000. We examined whether foraging willow warblers differed from residents in microhabitat selection and food intake rates, and whether they used a wider range of foraging tactics. Unlike either eremomela species, willow warblers occurred in all three habitat types. In acacia they showed a significantly greater diversity of feeding techniques, perhaps reflecting a greater diversity of prey taken, compared to the burnt-necked eremomela. In miombo, however, willow warblers did not differ significantly in feeding repertoire from the green-capped eremomela, but did show a significant difference in feeding microhabitat within the vegetation. In mopane woodland, where eremomelas hardly occurred, willow warblers showed the highest diversity of feeding techniques and may be able to exploit resources there that eremomelas cannot. Although there may be some microhabitat displacement and niche partitioning between willow warblers and eremomelas, we do not know which mechanism facilitates this separation, when it exists at all, because direct interspecific interactions were virtually nonexistent. We cannot yet assess the extent to which such interactions, together with the willow warbler's apparently greater behavioural flexibility, might favour its coexistence with the local community of ecologically similar arboreal insectivores.
Key words: palearctic migrants, willow warbler, eremomela, niche partitioning, foraging behaviour.

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The nature of the mechanisms that may structure animal communities is much debated and the role of interspecific competition in particular is difficult to detect in the field (Huston 1994). In many situations it is only possible to monitor putative mechanisms of niche partitioning that might enable species to coexist. The role of the interspecific interactions that occur when large numbers of migrant birds join resident communities in their winter quarters has been reviewed by Greenberg (1986) and Leisler (1992), who suggested that compe-

tition might result in spatial separation and niche shifts, but direct evidence is weak.
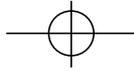
Food is probably the most important resource affecting migrants in their winter quarters (Sherry & Holmes 1996). There is no intra- or inter-specific competition for mates or breeding sites and potential niche partitioning should involve mainly habitat and microhabitat selection and foraging ecology. Previous studies of niche partitioning and the mechanisms to maintain coexistence between Palearctic migrants and resident species in sub-Saharan Africa have led to some general assumptions: Palearctic migrants were assumed to forage higher and in more peripheral parts in the vegetation and, in general, in more open habitats (Lack 1990, Leisler 1992). They were also assumed to be more flexible and opportunistic in their utilisation of resources, use a wider range of foraging tactics, and have a higher foraging speed than their Afrotropical counterparts in their respective guilds (Lövei 1989, Leisler 1992, 1993). Although some of these assumptions were recently confirmed by Baumann (2001) for orioles, other authors found contradicting results. In Kenya, Palearctic migrants were more specialised in food selection than residents (Lack 1986), and in Ivory Coast, Palearctic migrants did not forage in more open microhabitat or in more peripheral parts of the vegetation than resident members of their guilds in general (Salewski et al. in press).

Here we present observations of the feeding ecology in winter quarters of the willow warbler *Phylloscopus trochilus*, a leaf-gleaning Palearctic migrant that winters almost throughout sub-Saharan Africa in a variety of climatic and vegetation zones (Urban et al. 1997). Throughout its large geographical range it occurs sympatrically with several Afrotropical members of the same family and guild with which it might interact.

Comparative ecological studies of willow warblers and residents have been performed in Kenya (Rabøl 1987, 1993) and Ivory Coast (Salewski 1999, Salewski et al. in press). These studies revealed that willow warblers foraged more within the vegetation and used their wings more often for foraging than the ecologically similar green-backed eremomela *Eremomela pusilla* (Salewski et al. in press). In Kenya, Rabøl (1987) suggested that the arrival of willow warblers led to microhabitat displacements among Afrotropical species but he later rejected his earlier conclusions and stated that

interspecific competition played a lesser role than he previously thought (Rabøl 1993).

The present study attempts to assess how willow warblers partition resources with ecologically similar resident species in Zimbabwe. We examine whether foraging willow warblers differ from residents in microhabitat selection, whether they differ from residents in food intake rates and search area, and whether they are more flexible in their foraging behaviour, as stated in earlier reviews (Lövei 1989, Leisler 1992). Habitat selection and population densities of willow warblers in different woodland types in Zimbabwe will be addressed elsewhere (P. Jones et al., unpubl. data).

Methods

Study Area

The Sengwa Wildlife Research Area (373 km²) is situated in western Zimbabwe (18°10' S, 28°14' E) at an altitude of 800–950 m a.s.l. The area is drained by three rivers, the Sengwa, Lutope and Manyoni. Cumming (1975, cited in Guy et al. 1979) recognised 16 different vegetation types, the most widespread of which are mopane woodland dominated by *Colophospermum mopane*; miombo woodland, formerly dominated by *Brachystegia boehmii, B. spiciformis* and *Julbernardia globiflora*, though *B. boehmii* has greatly decreased because of elephant browsing; and the Sengwa/Lutope riverine woodland in which *Acacia tortilis* is dominant, though *Combretum imberbe* and *Diospyros sinensis* are also common.

There are three seasons: the hot wet (November–April), the cool dry (May–July) and the hot dry (August–October). Palearctic migrants begin to arrive in Sengwa at the end of the dry season and remain throughout the ensuing rains.

Target species

Several species of ecologically-similar arboreal insectivores, mainly warblers, are widespread in the Sengwa Wildlife Research Area (Jacobson 1979, Salewski et al. 2001). Apart from willow warblers, however, only burnt-necked and green-capped eremomelas *Eremomela usticollis* and *E. scotops* were sufficiently abundant to provide enough foraging data to be analysed here.
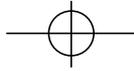
Foraging observations

Fieldwork was carried out between 7 December 1999 and 29 February 2000. Over this period we made observations of the feeding behaviour of willow warblers, Burnt-necked and green-capped eremomelas in the three principal woodland habitats – acacia, mopane, and miombo. We tried to apportion our time equally between habitats and evenly over the study period, but this was not done to a fixed timetable and the decision where to go was also influenced by the need to collect an adequate number of observations for each species in each habitat. Overall we collected foraging data on 25 days in acacia woodland, 26 days in mopane and 29 days in miombo; sample sizes are given in the text as necessary. Observations were made mostly in the early morning starting at sunrise and lasting about four hours, or in the late afternoon over the 2–3 hours before sunset. Occasional observations were carried out for one or two hours around noon.

We searched for the target species opportunistically in each habitat. We did not restrict our observations to birds only after they had changed trees, which reduces any bias towards more actively foraging and therefore more readily detectable individuals (Lens 1996), because in most cases the vegetation was so open that any discovery bias was negligible. When a bird was found we noted whether it was alone or in a flock and, if the latter, we noted flock size and species composition. We then followed the individual through binoculars for as long as possible. The following observations were recorded onto a dictaphone: numbers and kind of movements the bird performed (hop, run, jump, jump-fly, fly), distance moved (m), and numbers of feeding attempts (visible pecks at presumed prey) and feeding techniques used. Feeding techniques were: standing (taking the food item with both feet on the substrate and wings closed); jumping (jumping towards an item with closed wings); jump-flight (jumping towards the item with the aid of the wings but only to maintain balance); flight (flying towards the item and taking whilst in the air); and hover (hovering in front of the substrate from which an item is taken). Although it was normally easy to see when a feeding attempt had been made, it was not possible to tell whether it was successful.

We also noted the tree species in which the bird was foraging and several microhabitat parameters. These were: height of the tree, height of the bird, position in the tree where the bird was foraging (centre or edge of the crown) and percentage cover above the foraging bird within in a circle of 1 m radius around it. The duration of the observation was measured with a stop watch. When the bird was lost we started to look for another individual. In cases where we repeatedly lost and re-located the same individual (for example, due to being temporarily obscured by foliage or branches) this was noted and later treated as one observation. When the focal bird was foraging in a flock we tried to observe another individual when the first was lost. Nevertheless, we cannot rule out the inclusion of some non-independent data due to multiple observations of the same birds. We assume that this is unlikely because of the high number of observations over a long period in different locations even when within the same habitat. We could not determine the age or sex of the foraging birds.

For further analysis of foraging rates we used only observations that lasted longer than 20 s. We calculated distance travelled per minute, position changes (movements) per minute, number of feeding attempts per minute and number of feeding attempts per metre travelled for each individual. We used the mean values of each parameter for comparisons between species or within species in different habitats. We also calculated the percentage of total feeding attempts in which each feeding technique was used by an individual during each observation sequence; we used the means of these percentages for further analysis. This was not possible to avoid because the alternative method, using the absolute number of all techniques, may yield biased, non-independent results because many more feeding attempts were recorded from some individuals than others.

A foraging height index was calculated from the microhabitat parameters by dividing the height of the foraging bird by the height of the tree (Nyström 1991). An index of '1' means that the bird foraged at the top of the tree and an index of almost '0' that it foraged near the ground.

Diversity of feeding techniques was calculated using the Shannon index and differences between diversity indices were compared by t-test (Magurran 1988). The index is 'moderately sensitive' to sample size. Because sample sizes were much greater for willow warbler compared to the resident species, we took a randomly selected subsample of only 32 willow warbler observa-
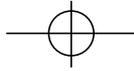
Table 1. Microhabitat parameters of foraging willow warblers, burnt-necked and green-capped eremomelas in three woodland habitats. Parameters defined in text. Values are given as mean ± s.e. Significant differences in parameter values between species in the same habitat are given in bold; * = P < 0.05.

Species	Habitat	n	Cover (%)	Tree height (m)	Height of bird (m)	Height index
Willow warbler	Acacia	208	24.8 ± 1.5	13.1 ± 0.2	**9.6 ± 0.2***	0.73 ± 0.01
	Mopane	51	27.0 ± 3.1	10.1 ± 0.8	7.4 ± 0.7	0.66 ± 0.03
	Miombo	90	31.7 ± 2.8	7.3 ± 0.5	5.2 ± 0.4	0.67 ± 0.03
Green-capped eremomela	Miombo	30	31.7 ± 4.6	9.2 ± 0.6	6.8 ± 0.5	0.74 ± 0.03
Burnt-necked eremomela	Acacia	44	25.1 ± 3.8	13.5 ± 0.4	**10.8 ± 0.4***	0.80 ± 0.02

tions to calculate the Shannon index for interspecific comparisons.

There was no significant observer bias in our dataset.

Microsoft SPSS for Windows 6.0.1 was used for statistical tests (Norusis 1993). Means are given ± s.e. For significance tests $\alpha = 0.05$ was accepted but, where multiple comparisons were made within a dataset, a sequential Bonferroni correction was applied using the Dunn-Šidak method (Sokal & Rohlf 1995). Probability levels are given where these remained significant after such correction. All percentages were arcsin transformed prior to analyses, except for those used in the calculation of Shannon indices.

Results

Willow warblers occurred most commonly in acacia woodland (P. Jones et al., unpubl. data), where we observed 241 individual birds during a total of 217 minutes of focal-animal sampling; they were less common in miombo (122 birds, 73 minutes) and mopane (67 birds, 52 minutes). Burnt-necked eremomelas occurred only in acacia (50 birds, 53 minutes). Green-capped eremomelas occurred more commonly in miombo (41 birds, 27 min) than mopane (7 birds, 4 min) and were absent from acacia. Sample sizes used in statistical tests are given in the text and may be less than those given above where data were missing.

Microhabitat

A stepwise discriminant analysis of microhabitat parameters (Table 1) of willow warblers revealed signifi-

cant differences between habitats in the heights of the trees where birds foraged and the height index (Wilks $\lambda = 0.72$ and 0.70, respectively, both P < 0.0001). Willow warblers used higher trees in acacia habitat (13.1 ± 0.2 m) than in mopane (10.1 ± 0.8 m) and miombo (7.3 ± 0.5 m). They foraged relatively higher in the trees in acacia (height index 0.73 ± 0.01) than in mopane (0.66 ± 0.03) or miombo (0.67 ± 0.03). Comparisons between species were therefore carried out only within habitats, excluding mopane where too few Afrotropical warblers were observed foraging.

Acacia

The dominant tree species in acacia habitat was *Acacia tortilis* and almost all observations of foraging willow warblers (95 %, n = 234) and burnt-necked eremomelas (98 %, n = 43) were in this species. A stepwise discriminant analysis of microhabitat parameters showed that burnt-necked eremomelas foraged significantly higher up in trees (10.8 ± 0.4 m) than willow warblers (9.6 ± 0.2 m; Wilks $\lambda = 0.98$, P < 0.027) but that there was no difference in height index or in the amount of cover above them (Table 1). The low eigenvalue (0.02) of the canonical discriminant function, however, shows that the model does not discriminate well between the two groups and only 68 % of willow warblers and 48 % of burnt-necked eremomelas were correctly classified.

When burnt-necked eremomelas foraging in monospecific flocks were compared with birds foraging in mixed-species flocks with willow warblers, a stepwise discriminant analysis revealed no differences in the parameters analysed. Burnt-necked eremomelas foraging in monospecific flocks were found more often (n = 12) in the centre of the crown than at the edge (n = 3), whereas in the presence of willow warblers they fed equally
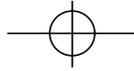
often in the centre (n = 11) and at the edge (n = 11) but the difference only approached significance ($\chi^2 = 3.41$, df = 1, P = 0.06), probably because of the low number of observations. Similarly, no difference was found in microhabitat parameters between willow warblers foraging in monospecific flocks (n = 155) and in mixed specific flocks with burnt-necked eremomelas (n = 19).

Miombo

A stepwise discriminant analysis of microhabitat parameters of willow warblers and green-capped eremomelas foraging in miombo habitat revealed an apparent difference in the heights of trees used (Wilks $\lambda = 0.96$, P = 0.028) but this just failed to achieve significance after Bonferroni correction. The two species foraged in the same density of cover. However, willow warblers foraged significantly more often in the centre of the crown than green-capped eremomelas. Willow warblers were observed on 74.5 % of occasions in the centre and 25.5 % at the edge (n = 94), whereas green-capped eremomelas were seen equally in the centre and at the edge (n = 36; $\chi^2 = 7.1$, df = 1, P = 0.008). Green-caped eremomelas showed no differences in any of the microhabitat parameters whether they foraged in monospecific flocks or in mixed species flocks with willow warblers. For willow warblers the number of observations was not high enough for analysis.

Foraging behaviour

A stepwise discriminant analysis was carried out on the four foraging parameters (distance/min, position changes/min, feeding attempts/min and feeding attempts/metre) of willow warblers in different habitats

(Table 2). There were no significant differences between habitats in the distance moved per minute or foraging attempts per metre but willow warblers moved more often while foraging in miombo (25.1 ± 1.0 position changes.min^{-1}) than in acacia (21.6 ± 0.6) or mopane (19.0 ± 1.3; Wilks $\lambda = 0.95$, P = 0.0002). They also had a higher feeding rate in miombo (3.8 ± 0.3 items.min^{-1}) than in acacia (3.1 ± 0.2 items.min^{-1}) or mopane (2.0 ± 0.4 items.min^{-1}; Wilks $\lambda = 0.91$, P < 0.0001). Further interspecific comparisons were carried out only within habitats.

In acacia habitat a stepwise discriminant analysis between willow warblers and burnt-necked eremomelas revealed a significant difference only in foraging rate (Table 2; Wilks $\lambda = 0.97$, P = 0.02). Thus, willow warblers had a higher feeding rate (3.1 ± 0.2 items.min^{-1}) than burnt-necked eremomelas (1.9 ± 0.2 items.min^{-1}) but did not move significantly farther or more often when foraging. However, only 36 % of willow warblers and 78 % of burnt-necked eremomelas were correctly classified by this behaviour. There was no difference in either species whether they foraged in monospecific flocks or in mixed flocks with each other.

In miombo habitat a stepwise discriminant analysis between willow warblers and green-capped eremomelas showed that there was a significant difference only in the distance moved when foraging (Wilks $\lambda = 0.86$, P < 0.0001). Green-capped eremomelas moved farther (8.7 ± 1.1 m.min^{-1}) during foraging than willow warblers (5.0 ± 0.3 m.min^{-1}) but there were no differences in the number of changes in position per minute, or in the number of feeding attempts per minute or per metre travelled. 75 % of willow warblers and 61 % of green-capped eremomelas were correctly classified, indicat-

Table 2. Rates of feeding attempts, changes of position, distance travelled during foraging by willow warblers, burnt-necked and green-capped eremomelas in three woodland habitats. Values are given as mean ± s.e. (n). Significant differences in parameter values between species in the same habitat are given in bold; * = P < 0.05, *** = P < 0.001.

Species	Habitat	n	Feeding attempts (items.min^{-1})	Feeding attempts (items.metre^{-1})	Position changes (n.min^{-1})	Rate of travel (metres.min^{-1})
Willow warbler	Acacia	187	**3.1 ± 0.2***	1.2 ± 0.1	21.6 ± 0.6	3.9 ± 0.2
	Miombo	81	3.8 ± 0.3	1.1 ± 0.2	25.1 ± 1.0	**5.0 ± 0.3***
	Mopane	52	2.0 ± 0.4	0.6 ± 0.1	19.0 ± 1.3	4.4 ± 0.5
Burnt-necked eremomela	Acacia	40	**1.9 ± 0.2***	1.0 ± 0.2	22.7 ± 1.0	3.4 ± 0.4
Green-capped eremomela	Miombo	31	3.7 ± 0.5	0.6 ± 0.1	26.6 ± 1.7	**8.7 ± 1.1***
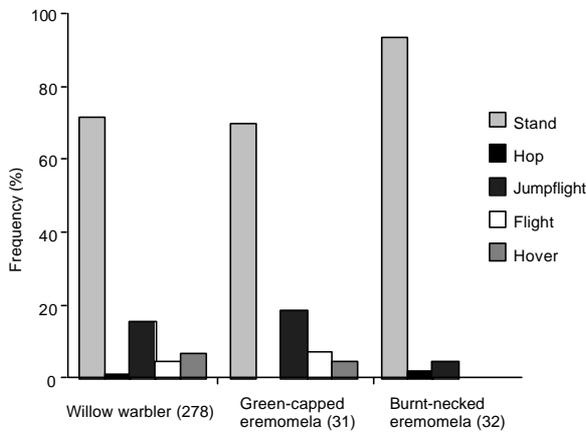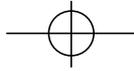
Figure 1. Frequencies of feeding techniques in willow warblers (all three woodland habitats combined), green-capped eremomelas (mopane and miombo combined), and burnt-necked eremomelas (acacia only).

ing that there is a high interspecific overlap. Green-capped eremomelas showed no differences in any of these parameters whether they foraged in monospecific flocks or in mixed species flocks with willow warblers. For willow warblers the number of observations was not high enough for analysis.

Foraging techniques

Willow warblers used five techniques to catch food items, green-capped eremomelas used four, and burnt-necked eremomelas used only three techniques (Fig. 1). The most common technique was standing when taking food items from the substrate, which was mostly leaves or twigs. This technique was used to the greatest extent by burnt-necked eremomelas (93.2 % ± 3.7), whereas it was less common in willow warblers (71.4 % ± 2.3 %) and green-capped eremomelas (69.7 % ± 6.8 %). Other techniques used by burnt-necked eremomelas were hopping to the item (2.1 % ± 1.6 %) or, the only technique in which this species used its wings, jump-flights towards the food (4.7 % ± 3.5 %). Willow warblers and green-capped eremomelas used their wings much more, including jump-flights (willow warbler: 15.6 % ± 1.8 %, green-capped eremomela: 18.5 % ± 6.1 %), hovering (willow warbler: 6.7 % ± 1.2 %, green-capped eremomela: 4.7 % ± 3.4 %) and direct flights (willow warbler: 4.8 % ± 1.1 %, green-capped eremomela: 7.0 % ± 2.7 %). Jumping towards a food item was used in a small number of feeding attempts by willow warblers (1.1 % ± 0.6 %) but was never observed in green-capped eremomelas.

Across all habitats willow warblers and green-capped eremomelas showed a similar high diversity in the use of different feeding techniques (Shannon index = 1.02 (n = 32 out of 277) and 0.90 (n = 32), respectively), whereas burnt-necked eremomelas showed a markedly lower diversity than the other two species (0.30, n = 31). The difference between willow warbler and burnt-necked eremomela (1.03, n = 32 out of 162 v. 0.30, respectively, in acacia woodland only) was significant (t-test: t = 5.74, df = 218, P < 0.001). The diversity indices for willow warblers and green-capped eremomelas feeding in miombo were almost the same (0.89, n = 32 out of 75 and 0.85, n = 28, respectively). When willow warblers feeding in different habitats were compared, they showed the highest diversity of feeding techniques in mopane (1.12).

Interspecific interactions

All three target species were frequently observed together in mixed species flocks with each other (Table 3) but interspecific interactions were never observed between willow warblers and burnt-necked eremomelas. Only once, in miombo woodland, was a willow warbler supplanted by a green-capped eremomela. Apart from aggression between target species, interspecific aggression was observed in the willow warbler only during an outbreak of the noctuid caterpillar *Eutelia polychorda* in mopane woodland. A single willow warbler successfully defended a mopane tree against two grey-headed sparrows *Passer griseus* and soon afterwards against an amethyst starling *Cinnyricinclus leucogaster*. Another willow warbler twice attacked a spotted flycatcher *Muscicapa striata*.

Discussion

We could detect only a limited degree of microhabitat partitioning between willow warblers and Afrotropical residents in this study. In acacia woodland willow warblers foraged in relatively lower parts of the trees than burnt-necked eremomelas, but there was considerable overlap. There was some suggestion that burnt-necked
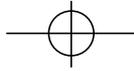
eremomelas fed less in the interior of the tree crowns when willow warblers were present. In other studies in Europe the interior parts of trees have been shown to be preferred by the dominant members of monospecific as well as mixed species flocks (Alatalo & Suhonen 1990, Lens & Dhondt 1992) but we cannot assess whether our observations resulted from active displacement of one species by the other. In Kenya, however, Rabøl (1993, correcting Rabøl 1987) found no displacement of residents by willow warblers or vice versa. In miombo woodland we found no differences in microhabitat parameters between willow warblers and green-capped eremomelas, except that the latter foraged to a greater extent in the outer parts of the vegetation. In miombo as well as in mopane willow warblers sometimes foraged low in the vegetation and occasionally even on the ground. This behaviour was never observed among willow warblers foraging in acacia or in either of the eremomelas.

Our results contrast with earlier observations that Palearctic migrants, if they occur in the same habitat as residents, forage higher and more often in the periphery of the vegetation (Leisler 1992), which was confirmed for willow warblers by Lack (1985) and Rabøl (1993), but they are in line with observations from Ivory Coast (Salewski et al. in press). Our observations also show that willow warblers do not forage in less dense cover than the two resident species.

The rate of movement while foraging, distance travelled and rate of feeding attempts did not differ greatly between willow warblers and residents, contrary to Leisler (1992), although in acacia woodland willow warblers made a greater number of feeding attempts per minute than burnt-necked eremomelas. Rabøl (1993) found a similar difference between willow warblers and resident species in acacia woodland in Kenya. We can-

not assess the feeding success of each species, however, because it was impossible to observe whether a potential food item was actually ingested, though Lovette & Holmes (1995) showed that the number of feeding attempts is positively correlated with the number of potential prey encountered. We assume, therefore, that in acacia woodland willow warblers had access to more food items per unit time than did burnt-necked eremomelas, even though they foraged in almost identical microhabitats. In miombo woodland foraging willow warblers made the same number of movements and had the same rate of food intake as green-capped eremomelas, but did not travel as far. Green-capped eremomelas used more short flights that are more energy demanding than short hops (Tatner & Bryant 1986). However, the adaptive advantage of the observed behavioural differences are not clear as we do not know what food was taken or the energy requirements of the birds.

Willow warblers showed a significantly higher diversity in foraging techniques than burnt-necked eremomelas, due to a greater use of the wings whilst foraging. Lovette & Holmes (1995) showed that differences in feeding techniques reflect different prey taken. We infer, therefore, that willow warblers took a wider range of prey than burnt-necked eremomelas, which might also explain their higher feeding rate if it reflects an ability to catch food that burnt-necked eremomelas cannot reach. In this case, our study confirms the assumption that migrants are more flexible and opportunistic in utilisation of prey, take more food on the wing and use more foraging tactics than resident species (Leisler 1992). In miombo woodland, however, the situation was different: willow warblers showed no differences in foraging techniques from green-capped eremomelas. In mopane, where eremomelas hardly occurred, willow warblers used their wings more often than in other habi-

Table 3. Numbers of observations of willow warblers, burnt-necked and green-capped eremomelas as singletons or in monospecific and mixed-species flocks in three habitats.

Species	Habitat	n	Single birds		Monospecific flock		Mixed-species flock	
Willow warbler	Acacia	235	46	(20 %)	158	(67 %)	30	(13 %)
	Mopane	67	16	(24 %)	39	(58 %)	11	(16 %)
	Miombo	102	64	(52 %)	51	(42 %)	7	(6 %)
Green-capped eremomela	all	46	1	(2 %)	24	(52 %)	21	(46 %)
Burnt-necked eremomela	Acacia	49	3	(6 %)	10	(20 %)	36	(74 %)
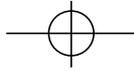
tats. By doing so, they might be able to use resources in that habitat which eremomelas cannot.

This study has shown that there may be some micro-habitat displacement and niche partitioning between willow warblers and whichever of the resident eremo-mela species is the most abundant in the habitat under consideration. However, we do not know which mecha-nism facilitates this separation, when it exists at all, be-cause direct interspecific interactions were virtually nonexistent. We cannot yet assess the extent to which such interactions, together with the willow warbler's apparently greater behavioural flexibility, might favour its coexistence with the local community of similar arboreal insectivores, which the willow warbler often outnumbers (P. Jones et al., unpubl. data) because other factors not investigated in this study (e.g. variation in vegetation structure, prey diversity; Greenberg 1986) probably influence the species' ecology too. A multi-factorial approach is therefore necessary to understand the mutual influence of the species concerned and the simple assumption that niche shifts found in resident communities are only influenced by the presence/ab-sence of migrants (Rabøl 1987) might be misleading.

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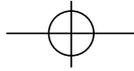
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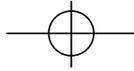
Haematological parameters, mass and moult status in dunlins *Calidris alpina* preparing for spring migration

Simon Verhulst, Kees Oosterbeek and Leo W. Bruinzeel

Haematocrit and buffy coat, the proportion of blood volume taken by red and white blood cells respectively, are commonly used as health indicators in humans. We investigated haematocrit and buffy coat in relation to the development of breeding plumage (proportion of feathers replaced), mass and capture date in dunlins *Calidris alpina* prior to spring migration. In particular, we tested the hypothesis that dunlins with relatively more advanced breeding plumage were in better condition. When comparing between capture sessions, buffy coat decreased over time. Within capture sessions buffy coat was not correlated with development of breeding plumage, but in each capture session birds with high mass had lower buffy coat, suggesting that birds with high mass were in better health. When comparing between capture sessions, haematocrit increased before departure, which is presumably an adaptation to migration because it increases oxygen transport capacity. Within capture sessions haematocrit was positively correlated with breeding plumage score, but neither was correlated with mass. However, the haematocrit of a bird with a given plumage score was independent of the date at which it attained that score, and thus there was no evidence that birds that started moult earlier and had a more advanced breeding plumage were in better condition. Keywords: dunlin, *Calidris alpina*, waders, leucocytes, erythrocytes, PCV.

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Migratory birds are generally thought to benefit from early arrival on the breeding grounds, because reproductive success is usually higher in early breeding birds (Perrins 1970, Møller 1994, Verhulst et al. 1995, Both & Visser 2001, Tulp & Schekkerman 2001). Increasing migration speed in order to advance the arrival date will occur at the expense of nutritional stores at arrival, because birds will have refuelled less along the way, or departed earlier with lower nutritional stores. Consequently migratory birds face a trade-off between arrival time and arrival condition (Alerstam & Lindström 1990, Ens et al. 1994). In addition to the build up of nutritional stores, preparation for migration and breeding includes numerous other physiological processes (Gwinner 1990), e.g. the development of a breeding plumage. Such changes may take place at the ex-

pense of the build up of nutritional stores, and consequently their development may depend on condition. In this paper we explore whether in dunlins *Calidris alpina* preparation for migration includes changes in two haematological parameters which may indicate condition. In particular we investigated whether individuals differing in the degree to which they moulted into breeding plumage also differed in condition.

The dunlin is a small migratory wader without extravagant morphological secondary sexual characters, but both sexes develop a similar breeding plumage. Moult into the breeding plumage takes place during spring migration and takes 23–25 days (Ferns & Green 1979). In the population we studied there is striking variation in the development of breeding plumage shortly before departure (see below). Given the duration of moult, and
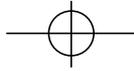
that the earliest laying dunlins in northern Scandinavia start laying approximately two weeks after these birds depart from the Wadden Sea (Stiefel & Scheufler 1989), it seems likely that either there is still variation in development of breeding plumage during territory settlement and pair formation, or birds with less developed summer plumage delay their departure. If the development of the breeding plumage acts as a signal in intra- or inter-sexual selection on the breeding grounds, it seems likely that birds with a more developed breeding plumage will breed earlier, or on better territories.

As measures of condition we used body mass, haematocrit and the buffy coat. Body mass increases before departure in our study population (Goede et al. 1990a), and for instance bar-tailed godwits *Limosa lapponica* on spring migration with more advanced moult have higher mass (Piersma & Jukema 1993). Haematocrit (proportion of blood volume taken by erythrocytes) determines the oxygen transport capacity (Hammond et al. 2000), and there are several indications that haematocrit is higher when birds are in good condition. Haematocrit is typically lower in birds that suffer food shortage (Svensson & Merilä 1996, Piersma et al. 2000, own unpublished observations on oystercatchers *Haematopus ostralegus*, and starlings *Sturnus vulgaris*). Furthermore, more ornamented individuals in two other migrating bird species had higher haematocrit (Piersma et al. 1996, Saino et al. 1997). An elevated buffy coat (proportion of blood volume taken by white blood cells; Wardlaw & Levine 1983) indicates acute or chronic infections (Harrison & Harrison 1986, Gustafsson et al. 1994). The buffy coat can therefore be expected to be higher in birds with low condition, but unfortunately there are very few studies that have investigated this in free-living birds. Collared flycatchers *Ficedula hypoleuca* rearing experimentally enlarged broods had more blood parasites and a larger buffy coat (Gustafsson et al. 1994). Chinstrap penguins *Pygoscelis antarctica* with a high buffy coat had lower reproductive success (Moreno et al. 1998). When the workload required to earn food was experimentally increased in captive starlings this resulted in an increase in buffy coat (S. Verhulst et al. unpubl. data). These results suggest that high buffy coat indicates low condition.

Methods

Dunlins were caught with mist nets on Schiermonnikoog (53° 28.6' N, 6° 13.8' E) in the Dutch Wadden Sea in three capture sessions: I mid March (11–14 March 2002), II mid April (14–15 April 2002) and III late April (24–26 April 2001). Different dunlin populations use the Wadden Sea as spring staging area at different times. The number of dunlins in the Wadden Sea starts to increase at the end of February. Dunlins present in April have spent the winter in the Wadden Sea, the British Isles, in France or in Tunisia (Meltofte et al. 1994). These birds, of subspecies *Calidris a. alpina*, breed in northern Scandinavia and north-west Siberia, and leave the Wadden Sea at the beginning of May, when they are replaced by other populations of dunlins that breed further east in Siberia (Goede et al. 1990b). Thus capture session III (24–26 April) took place shortly before the expected departure date of the 'April' population.

Captured birds were processed immediately at the nearby field station of the University of Groningen. Birds were ringed with a numbered metal ring, weighed and biometric measurements (tarsus, wing length and width, bill length, length of middle toe) were taken. All biometrics were measured by the same person (K.O.). To reduce the number of variables for body size we used one parameter: the first principal component based on all five size parameters (Hair et al. 1998). We refer to this new parameter as 'body size'. It explained 64 % of the variance, and loading was approximately equal for all body size measures (r between –0.4 and –0.5). Mass can vary due to differences in nutritional stores, but also due to differences in structural body size. We therefore calculated residual mass, which in this study is the residual of a regression of mass on body size ($r^2 = 0.43$, $n = 91$, $P < 0.001$; mass $= 50.0 + 1.60 *$ body size). The extent to which birds had developed their breeding plumage was scored separately for belly and back on a five point scale (0–4 corresponding with 0 %, 25 %, 50 %, 75 %, 100 % breeding plumage respectively). Plumage score of belly and back were strongly correlated (capture session I: all plumage scores 0; capture session II: $r = 0.87$, $n = 22$, $P < 0.001$; capture sessions III: $r = 0.83$, $n = 40$, $P < 0.001$), and so we calculated breeding plumage score as the mean of plumage scores of belly and back. Plumage score reflects the proportion of feathers replaced, regardless of whether the new
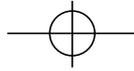
feathers had completed their growth. Therefore we also scored whether birds were still growing feathers on back and belly on a four point scale (moult score 0 = no growing feathers, 1 = some growing feathers, 2 = intermediate proportion of growing feathers, 3 = many growing feathers). Moult score of belly and back were correlated (capture session II: r = 0.79, n = 22, P < 0.001; capture session III: r = 0.44, n = 40, P < 0.005), and we calculated the moult score as the mean of moult scores of belly and back. Unfortunately, dunlins cannot be reliably sexed on the basis of biometric information. Birds were classified as yearlings on the basis of presence of buff fringes on the inner medium coverts and the condition of the primaries (Prater et al. 1987).

Blood samples (one heparinised capillary, approximately 65 ηl) were taken from the brachial vein, after puncture with a 0.5 mm needle. Capillaries were sealed with wax and centrifuged for 10 min at 12,000 rpm (relative centrifugation force 12,477 g) within 2 h after they were collected. Buffy coat was measured using digital sliding callipers and a magnifying glass. All measurements were taken by the same person (S.V.) without knowledge of mass or plumage score of the sampled bird. Haematocrit and buffy coat were expressed as proportion of blood volume taken by red or white blood cells respectively. Repeatability (r) of both values were found to be high in another study by S.V. of European oystercatchers (haematocrit: r (s.e.) = 0.98 (0.007), buffy coat: r = 0.94 (0.02), n = 30 duplicate samples, both $F_{1,29} > 34.6$, P < 0.0001). We therefore collected single samples, rather than duplicates, to minimise blood loss. During capture session II (mid April) we

also measured haemoglobin concentration with a haemoglobin photometer (Haemocue, Angelholm, Sweden) based on the sodium nitrite method (Vanzetti 1966).

Haematocrit and buffy coat were square-root arcsine transformed prior to statistical analysis, but for ease of interpretation untransformed values are shown in the graphs. Seasonal variation was tested with linear regression, using Julian date as the independent variable. Individual variation within capture sessions was tested using partial correlations, using dummy variables to control for capture session.

Results

Seasonal variation

Independently of body size, which did not differ between capture sessions, mass increased prior to departure (Table 1). Over the whole capture period the rate of mass gain was 0.10 g / day, exactly equal to the value reported by Goede et al. (1990a) for the same study site based on data collected in earlier years. Haematocrit increased prior to departure (Table 1; slope (s.e.) = 0.062 (0.014) % / day, $F_{1,88} = 18.7$, P < 0.001), while buffy coat decreased (Table 1; slope (s.e.) = –0.0019 (0.0006) % / day, $F_{1,88} = 9.42$, P < 0.003). The proportion of birds moulting (body moult score > 0) increased from 7 % in mid March, to 86 % in mid April, and 100 % in late April. Although 7 % of the birds had started moulting in mid March, plumage score was still 0 for all birds, and the proportion of birds with a plum-

Table 1. Characteristics (+ s.e.) of dunlins at different capture sessions. Haematocrit and buffy coat are % of blood volume; haemoglobin is mmol / l; mass in g. Body size is the first principal component of a PCA involving five body size parameters, high values indicating larger body size (see text for details). Statistical results are from a regression of the focal trait on Julian capture date.

	mid March	(s.e.)	mid April	(s.e.)	late April	(s.e.)
Haematocrit***	45.92	(0.005)	47.21	(0.623)	48.77	(0.377)
Haemoglobin			11.06	(0.11)		
Buffy coat**	0.683	(0.027)	0.659	(0.020)	0.593	(0.014)
Plumage score***	0.0	(0.0)	0.59	(0.16)	2.04	(0.15)
Mass***	47.9	(0.64)	49.18	(0.80)	52.05	(0.51)
Body size	0.14	(0.29)	–0.07	(0.29)	–0.06	(0.27)
n	29		22		40	

Significance of seasonal trend: ** P < 0.01, *** P < 0.001
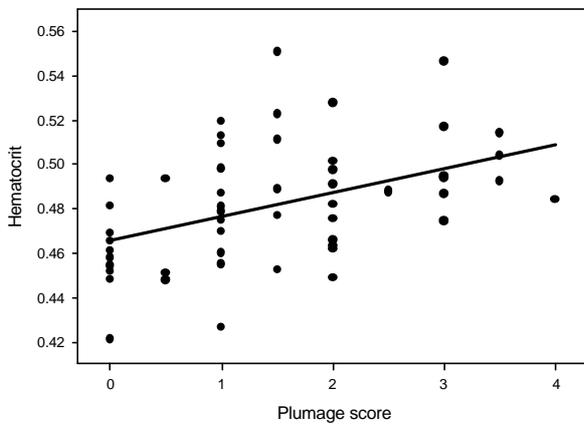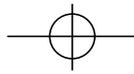
Figure 1. Breeding plumage score (0 = winter plumage, 4 = complete breeding plumage) and haematocrit values (proportion of blood volume taken by red blood cells) of dunlins.

age score of 0 had decreased to 52 % in mid April. In late April all birds had a plumage score greater than zero.

Individual variation

While a comparison between capture sessions reflects changes in the population mean over time, comparisons within capture sessions (Table 2) can reveal differences between individuals. Birds had a higher haematocrit and higher breeding plumage score later in the season, suggesting a positive correlation between these parameters. Indeed, also within capture sessions there was a positive correlation between plumage score and hae-

matocrit (Fig. 1; capture session I excluded because all birds were still in winter plumage). This relationship did not change over time, in the sense that there was no significant difference in haematocrit between capture sessions when plumage score was controlled for statistically ($F_{1,58} = 0.01$, P > 0.9). This indicates that early and late moulting birds follow on average the same trajectory, increasing haematocrit as they moult into their breeding plumage. However, the rate of moult as indicated by the moult score was not correlated with haematocrit ($F_{1,58} = 0.7$, P = 0.6). Haemoglobin concentration (Hb; measured only during capture session II) was strongly correlated with haematocrit ($r^2 = 0.74$, Hb = 4.407 + 14.216 * (untransformed) haematocrit; P < 0.0001, n = 20, one outlier for Hb was excluded). Correlations with haemoglobin concentration per volume of red blood cell were tested using partial correlations with haemoglobin, controlling for haematocrit. There was a trend for the density of haemoglobin to decrease with increasing plumage score (partial correlation: r = –0.4, n = 20, P = 0.08). Within capture sessions (residual) mass was not correlated with plumage score or haematocrit (Table 2).

Buffy coat decreased with time, while mass increased (Table 1), and also within each capture session birds with high mass had low buffy coat (Fig. 2). This suggests that birds with high mass were in better health. The level of this relationship did not differ statistically between the three capture sessions ($F_{2,87} = 0.1$, P = 0.9), indicating that there was a constant relationship between mass and buffy coat with respect to Julian date, at least over the period studied. Plumage and moult score (partial correlation, controlling for capture ses-

Table 2. Correlations between plumage score, residual mass, haematocrit and buffy coat of dunlins within capture sessions.

Capture period	Correlations of plumage score with			Correlations of residual mass with	
	Mass[1]	Haematocrit	Buffy coat	Haematocrit	Buffy coat
mid March				–0.11	–0.34[+]
mid April	–0.08	0.48*	–0.12	–0.42[+]	–0.40[+]
late April	0.15	0.35*	–0.15	–0.04	–0.45**
Combined[2]	0.13	0.38***	–0.14	–0.17	–0.37***

[+] P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.005
[1]Mass is here the residual of a regression of mass on body size.
[2]Combined r-values are from partial correlations controlled for capture session using dummy variables.
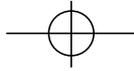
sion; r < 0.15, n = 62, P > 0.2) were not related to buffy coat. Controlling for (residual) mass did not change this result.

In total 15 % (14/91) of the captured birds were yearlings, and this proportion was independent of capture session (χ^2 = 4.38, df = 2, P = 0.1). Yearlings did not differ from older birds in body size, haematocrit, buffy coat or moult (controlling for capture session, all P > 0.1), but there was a trend for yearlings to have a lower plumage score (P = 0.08). Yearlings also had significantly lower residual mass (–1.8 (s.e. = 0.6) g, P < 0.005). Controlling for age did not change the main results and age was not significantly correlated with haematocrit when plumage score was controlled for (P = 0.1), or with buffy coat when mass was controlled for (P = 0.5).

Figure 2. Residual mass and buffy coat (proportion of blood volume taken by white blood cells) of dunlins. Residual mass was calculated from a regression of mass on body size.

Discussion

Haematocrit increased prior to migration (Table 1), a pattern that was also found in other migrating species (Banerjee & Banerjee 1977, based on haemoglobin measurements; Morton 1994, Piersma et al. 1996). Further support for the association between migration and an elevated haematocrit has been found in dunlins, curlew sandpipers *Calidris ferruginea* and little stints *C. minuta* on their Siberian breeding grounds, where haemoglobin concentration (which we found to be strongly correlated with haematocrit) was higher during spring and/or autumn migration than during the reproductive period (Tulp & Schekkerman 2001). The premigratory increase in haematocrit enhances oxygen transport capacity (Hammond et al. 2000), and is therefore likely to be an adaptation to the anticipated metabolic demands of migration. This interpretation is supported by the positive correlation found in an interspecific comparison of haematocrit and 'strength of flight' in different bird species (Carpenter 1975).

Buffy coat decreased prior to departure, suggesting an increase in health but further study (on e.g. parasite loads) is required to verify this interpretation. We are not aware of other studies of the buffy coat in relation to migration in free-living birds. Birds with high mass appear to be in better health, because they had a relatively low buffy coat (Fig. 2), but there was no evidence for a relationship between buffy coat and the development of the breeding plumage (Table 2).

The primary aim of this study was to test the hypothesis that earlier moulting birds, with a more developed breeding plumage, were in better condition, as was previously found in bar-tailed godwits (Piersma et al. 1996). The positive correlation between plumage score and haematocrit (Fig. 1) appears to confirm this result. However, the haematocrit associated with a particular plumage score was independent of the date at which a bird attained that plumage score, which implies that early and late moulting birds followed on average the same trajectory, increasing haematocrit as they moulted into their breeding plumage. There is no indication from our data that individuals that started moulting earlier are characterised by a higher haematocrit. (Such a relationship would have resulted in significant differences in level between capture sessions in the relationship between plumage score and haematocrit, but there was no evidence for such variation.) Haematocrit was not correlated with moult rate. This suggests that the increase in haematocrit was not associated with the moult process *per se*, but rather that the increase in haematocrit is a physiological adaptation to migration which coincides with the moult. It is not known whether dunlins delay their departure until their breeding plumage is complete, and their haematocrit sufficiently high, but this is a hypothesis worth testing. If dunlins delayed their departure until their breeding plumage is complete, late moulting individuals are likely to breed relatively late, resulting in lower reproductive success
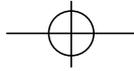
(Tulp & Schekkerman 2001). Alternatively, the variation in timing of moult may be associated with geographic variation in breeding sites, late moulting individuals breeding in areas where arrival and breeding occurs later in the season. In the latter case it is less likely that late moulting associates with lower reproductive success. These two possibilities are not mutually exclusive.

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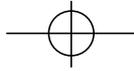
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