Chapter 6. Morphological associations with haemoparasitism: cause or consequence?

Abstract

Avian haemoparasites are found worldwide at high prevalence and yet sub-clinical consequences for their hosts are rarely reported. Here I investigate parasite prevalence, abundance and associations with host and environmental variables in a population of Yellowhammer, *Emberiza citrinella* over-winter (November-May). Two lineages of *Haemoproteus* spp. and a novel *Leucocytozoon* lineage were identified from sequence data, with an overall prevalence of 50%. *Haemoproteus* infection varied temporally, with a peak prevalence during mid-December during one year but not the next; *Leucocytozoon* infection showed no clear peak. I discuss possible reasons behind the peak of *Haemoproteus* infection, including stress response associated with a reduction in food availability. Infection rates did not differ between age classes or between the sexes but were higher during the colder winter of 2008/09 than during the winter of 2007/08. Nearly double the numbers of birds were caught during 2007/08 despite similar sampling effort. I found temporally variable associations between infection with *Haemoproteus* and wing and tail lengths: wing lengths were shorter in infected birds than in uninfected birds during 2007/08 but not during 2008/09; tail lengths were shorter in infected birds during all months except January (data from this month were only available during 2008/09). Although temporal variability clouds these relationships, I use these data to test two hypotheses: 1) Parasite infection influenced tail length, and conversely, 2) Smaller birds were more likely to be infected, or more likely to suffer from patent infection if infected. I found no support for hypothesis 2, as none of tarsus length, head-beak length, or the interactions of either with month or year were related to parasite status, suggesting that parasites influence feather growth during moult, but that this relationship can be influenced by mortality induced by severe weather conditions. Finally, I detected parasite infection in 60% of seven-day-old nestlings, indicating that the nestling stage may be when most birds become infected with haemoparasites. To my knowledge, this is the youngest age at which parasite infection has been detected in wild birds.

Introduction

Avian haemoparasites are widely distributed and found at high prevalence within many species (Schueurlein and Ricklefs 2004; 2005). As parasites compete with their hosts for resources they are generally detrimental: the lethal consequences of avian malaria where it has been introduced to naïve host species are well-documented (e.g. Warner 1968). However, research on the impacts of sub-clinical avian malaria infections has been largely restricted to impacts on sexually-selected traits, after Hamilton and Zuk (1982) proposed the 'good genes' hypothesis. They proposed that birds with brighter plumage were more resistant to parasites, and thus that plumage colouration and other sexually selected traits could be used reliably as a mate choice cue to allow females to select for males with genes conferring parasite resistance (Hamilton and Zuk 1982). Much evidence has been found in support of this hypothesis: for example, yellow plumage colouration in both the Cirl Bunting *Emberiza cirlus* and the Yellowhammer *Emberiza citrinella* are reliable indicators of health status (Sundberg 1995; Figuerola et al. 1999), and haemoparasitic infection of White-Crowned Sparrows *Zonotrichia leucophrys* and Canaries *Serinus canaria* is associated with reduced song performance (Spencer et al. 2005; Gilman et al. 2007). However, evidence is not entirely conclusive (e.g. Read and Harvey 1989) and the results of some studies are contradictory: for example, Red-Backed Shrikes *Lanius collurio* infected by haematozoa have larger tail colour patterns (a sexually-selected trait) than uninfected males (Votypka et al. 2003).

More recent work has focussed on the possible implications of haemoparasitisic associations with sexually selected traits in terms of reproduction and life-history. Haemoparasites have been associated with a delay in the onset of breeding (Allander and Bennett 1995), reduced clutch size (Korpimaki et al. 1993), egg volume and hatching success (Dufva 1996), lower provisioning rate (Tomas et al. 2005), reduced chick condition and size (Dufva 1996) and reduced fledging success (Sundberg 1995; Dyrcz et al. 2005); again, some studies are contradictory: for example Davidar and Morton (1993) found a higher breeding success in Purple Martins *Progne subis* infected with *Haemoproteus* spp. Experimental work involving brood manipulation indicates a trade-off between parental effort and immune defence against parasites, with parents of experimentally enlarged broods across a range of species generally showing a higher prevalence and higher intensity of parasitic infection (Richner et al. 1995; Allander 1997; Siikamäki et al. 1997; Nordling et al. 1998; Wiehn et al. 1999; Knowles et al. *In press*). Parasite infection is not necessarily causal in this relationship: increased stress

due to an increased reproductive output may render an individual more likely to become infected, or less able to suppress a chronic infection. For example, Dawson and Bortolotti (2001) found that male American Kestrels *Falco sparverius* whose mates had high reproductive outputs were more likely to become parasitized or remain parasitized, and were subject to higher intensity infections than those males whose mates had lower reproductive outputs.

Haemoparasite infections vary temporally (Bensch and Åkesson 2003; Cosgrove et al. 2008) and the relapse of chronic infections generally coincides with the onset of the breeding season when infections are found at high prevalence (Sundberg 1995; Allander and Sundberg 1997; Cosgrove et al. 2008). These relapses are associated with an increased day length and are thought to be caused by hormones and stress associated with the onset of breeding (Valkiunas et al. 2004). Prevalence tends to decrease throughout the breeding season (Hasselquist et al. 2007; Cosgrove et al. 2008) but is followed by another peak of infection post-breeding (Cosgrove et al. 2008), thought to be due to infection of newly fledged naïve individuals (Beaudoin et al. 1971). However, little work has been carried out on the impacts that blood parasites may have on their hosts outside the breeding season (Allander and Sundberg 1997).

Associations between haemoparasites and the morphology of their hosts have been occasionally reported. Infected birds often have a reduced body condition (Figuerola et al. 1999; Schrader et al. 2003) and a lower mass than uninfected individuals (Schrader et al. 2003; Dyrcz et al. 2005), supported by an experimental infection of Blackcaps *Sylvia atricapilla* (Valkiunas et al. 2006; but see also Bennett et al. 1988). These effects on mass do not generally appear to impact upon survival (Weatherhead 1990; Schrader et al. 2003; but see also Davidar and Morton 1993). Some studies have also shown parasitized birds to have shorter wing and tail lengths compared to uninfected birds (Rätti et al. 1993; Hatchwell et al. 2001; but see also Votypka et al. 2003) with impacts upon arrival date in a migratory species (Rätti et al. 1993). Although parasitism is not associated with differential timing of moult (Allander and Sundberg 1997) it is possible that parasites may compete for host resources post-breeding, thus restricting moult (Rätti et al. 1993; Hatchwell et al. 2001); conversely it may be that smaller birds are somehow more susceptible to parasitism (Weatherhead 1990; Rätti et al. 1993). It is important to establish this causality: if the former is true and parasites impact upon feather growth then they could cause important population-level consequences for their

hosts which may previously have been underestimated. However, if the latter is true and smaller birds are more susceptible to parasitism, effects of parasites on host populations may conversely have been over-estimated: smaller individuals tend to have lower dominance rankings (Lindström et al. 2005), reduced survival (Braasch et al. 2009) and consequently a reduced reproductive output. If parasitized birds tend to be smaller then this may confound previous studies investigating life-history implications of parasitism.

Here I investigate parasite prevalence, identity and association of infection with host and environmental variables. I investigate morphological associations with parasite infection and test two contrasting hypotheses regarding the causality of the predicted associations with morphological variables: firstly, that parasites affect feather growth (feather length measured through both wing length and tail length) and secondly, conversely, that smaller birds are more likely to be infected by parasites (body size measured through both tarsus length and head-beak length).

Methods

Study population and blood sampling

Work was carried out within an individually marked population of Yellowhammers near Tadcaster, North Yorkshire (lat. 53˚ 53'N, long. 1˚ 15'W). Birds were caught in static mist nets and whoosh nets at an established supplementary feeding site baited with wheat and weed seeds, within an experimental agroforestry block surrounded by arable farmland. 203 birds were caught on 30 sampling occasions between November 2007 and April 2009. Sixteen birds were caught and sampled on two occasions within this period and three birds were caught and sampled on three separate occasions (these occasions were more than two months apart, as required by the terms of the HO licence).

Blood was taken through venipuncture of the brachial vein and stored with EDTA prior to freezing. Blood samples were also collected from 10 seven-day-old nestlings from four broods (three broods of three nestlings and one brood of one nestling) from other sites in Gloucestershire, Wiltshire and Hampshire used as part of a separate project during June and July 2008.

Morphometrics

Birds were aged and sexed according to plumage variation (Svensson 1992; Dunn and Wright In press). Morphometrics were taken as detailed by Dunn and Wright (In press): wing length and tail length were used as measures of feather growth, and head-beak length and tarsus length as measures of size. Measurements of wing length were taken using a standard metal wing rule and rounded up to the nearest mm; other measurements were taken using digital callipers (± 0.1 mm).

DNA Extraction and detection of blood parasites

DNA was extracted from 30µl of whole blood using a standard phenol-chloroform extraction followed by ethanol precipitation (Sambrook et al. 1989). Successful DNA extraction was confirmed by using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE) and diluted to a working concentration of 25 – 100 ng/µl.

Blood parasite presence or absence was determined through PCR using established protocols. The presence of *Plasmodium* and *Haemoproteus* was established using primers HaemNF and HaemNR2 nested within HaemF and HaemR2 (Waldenström et al. 2004), and *Leucocytozoon* spp. were detected using primers HaemFL and HaemR2L nested within primers HaemNFI and HaemNR3 (Hellgren et al. 2004). All protocols were carried out in a working volume of 25μl containing 50 – 200 ng template DNA, 1.25mM dNTPs, 3mM $MgCl_2$, 0.4μM of each primer, 1 x GoTaq Flexi Buffer (Promega, Madison, WI) and 1 U GoTaq Flexi (Promega, Madison, WI); a positive control of DNA from a bird with known infection and a negative control containing deionised water in place of DNA were included with each PCR reaction to ensure successful amplification and lack of contamination respectively.

The PCR protocol for first round reactions consisted of a denaturation step of 94°C for 3 minutes followed by 20 cycles of 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 45 seconds, with a terminal extension step of 72°C for 10 minutes; the protocol for second round reactions contained 35 cycles but otherwise consisted of an identical thermal profile. PCR protocols were carried out on a GeneAmp PCR System 9700 (Applied Biosystems).

As non-target DNA can be amplified with nested PCR methods (Szöllösi et al. 2008) a subsample of positive samples were sequenced using an ABI sequencer at the Core Genomic Facility, Sheffield University, to confirm the identity of parasites.

Statistical analyses

All analyses were carried out in R version 4.2.1 (www.R-project.org). Chi-squared tests were used to determine whether expected numbers of infected and non-infected individuals differed from observed numbers between the sexes, age classes, or between years. General linear models with binomial error structures and infection status (with each parasite separately) as the response variable were used for each of known age, day and month as the chi-squared test assumptions of expected values greater than 5 were not met for these variables. Data were then grouped into three time groups according to season (Nov – Dec 2007; Jan – May 2008; Jan – May 2009) in order to determine whether visible differences according to time were statistically significant (Crawley 2007).

To determine whether feather length was associated with infection by either *Haemoproteus* spp. or *Leucocytozoon* spp, four general linear models with gaussian error distributions were constructed with either wing length or tail length as the response variable. Age, sex, month, year and infection status (two separate models were constructed for each parasite) were designated as response variables along with two-way interactions between age and sex, and between infections status and each of age, sex, month and year to determine whether any associations differed between age classes, sexes, months or years. Data from only one sampling occasion was included where birds had been samples more than once, in order to avoid pseudoreplication. These models were repeated for *Haemoproteus* spp. only, with head-beak length and tarsus length as response variables to determine whether any associations with parasitism were with size or with feather length. Model comparisons using AIC values were used to determine whether terms significantly improved the fit of the model; those that didn't were removed in a stepwise fashion until only those terms that improved the fit of the model at $p<0.1$ remained; only terms that influenced the response variable at $p<0.05$ were considered to influence the response variable. Following model simplification, each term was reinserted into the minimum adequate model (MAM) in turn and compared with the MAM using AIC comparisons to ensure lack of association with the response variable. Statistics presented throughout are mean ± 1 SE.

For recaptured birds, associations between parasite status and feather length were tested using generalised linear mixed effect models with either wing length or tail length as the response variable, and infection by *Haemoproteus* spp. as the predictor variable, designating bird ID as a random factor. The infection by *Haemoproteus* term was then removed from the maximal model and the two models with and without the term compared to deduce the significance of the term.

Results

Parasite prevalence and identity

225 blood samples from 203 birds were screened for the presence of *Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp. Using the protocol of Waldenstrom *et al* (2004), for detection of *Plasmodium* spp. and *Haemoproteus* spp., 105 of 225 samples (47%) tested positive for parasites. A subset of samples were tested using the protocol of Hellgren *et al* (2004), for detection of *Leucocytozoon* spp., and 52 of 195 samples (27%) were found to contain parasites. Of the 52 birds testing positive for *Leucocytozoon* spp., only 7 were uninfected with *Haemoproteus* spp. giving an overall parasite prevalence of 50%.

Thirty-eight sequences were obtained from 34 birds. Of these, one sequence was identified as a novel *Leucocytozoon* lineage and the remainder were identified as *Haemoproteus* lineages DUNNO01 and EMRUT01 (Genbank accession numbers DQ991080 and EF380192). The *Leucocytozoon* lineage was amplified using Hellgren et al (2004) and all the *Haemoproteus* lineages were amplified using Waldenström et al (2004).

Associations with host and environmental variables

Haemoproteus infection showed no association with age (χ^2=0.02, df=1, p=0.90), known age (GLM, $Dev_{1,148}$=0.01, p=0.94) or sex (χ^2=1.02, df=1, p=0.31). *Leucocytozoon* infection showed no association with age (χ^2=0.85, df=1, p=0.36), known age (GLM, $Dev_{1,125}$=0.01, p=0.93) or sex (χ^2=0.01, df=1, p=0.90).

After removing data from days where fewer than five birds were caught, prevalence of *Haemoproteus* and *Leucocytozoon* both varied with day (*Haemoproteus*: GLM, $Dev_{12,134}$=25.16, p=0.01; *Leucocytozoon*: GLM, $Dev_{12,115}$=30.27, p<0.01). Infection rates of both parasites also varied with month (*Haemoproteus*: GLM, $Dev_{5,197}$=14.47, p=0.01; *Leucocytozoon*: GLM, $Dev_{5,168}$=11.90, p=0.04), and between years (*Haemoproteus*: χ^2=11.78, df=1, p<0.01; *Leucocytozoon*: χ^2=10.42, df=1, p<0.01), with prevalence of *Haemoproteus* increasing from 40% in the winter of 2007/08 to 68% in the winter of 2008/09, and *Leucocytozoon* prevalence rising from 20% in the winter of 2007/08 to 50% in the winter of 2008/09.

Haemoproteus showed a peak of infection in mid-December 2007, with over 50% of birds infected: prevalence then declined to 10% in April 2008. No clear pattern was shown during 2008/09 (Figure 1a), although only three sampling days yielded more than five birds. No clear peak was shown for *Leucocytozoon* infection during 2007/08, although infections with this parasite were detected throughout December and into February, but were not found after mid-February during 2007/08 (Figure 2b); during 2008/09 the parasite was found into April (Figure 2b). To confirm the statistical significance of these patterns, data were grouped into three time periods: November - December 2007; January - May 2009; and January 2009 – April 2009. Days on which fewer than five birds had been captured were reincluded in this analysis. *Haemoproteus* infection differed in prevalence between the three time periods (GLM, $F_{2,196}$=9.07, p<0.01), with the second (GLM, z=2.44, p=0.02) and third (GLM, z=-2.10, p=0.04) time periods differing from the first, and from each other (GLM, z=4.074, p<0.01) confirming a winter peak of infection between November and December 2007, followed by a period of lower prevalence between January and May 2008, and a higher prevalence between January and May 2009. This trend was slightly different for *Leucocytozoon* infection (GLM, $F_{2,168}$=4.61,

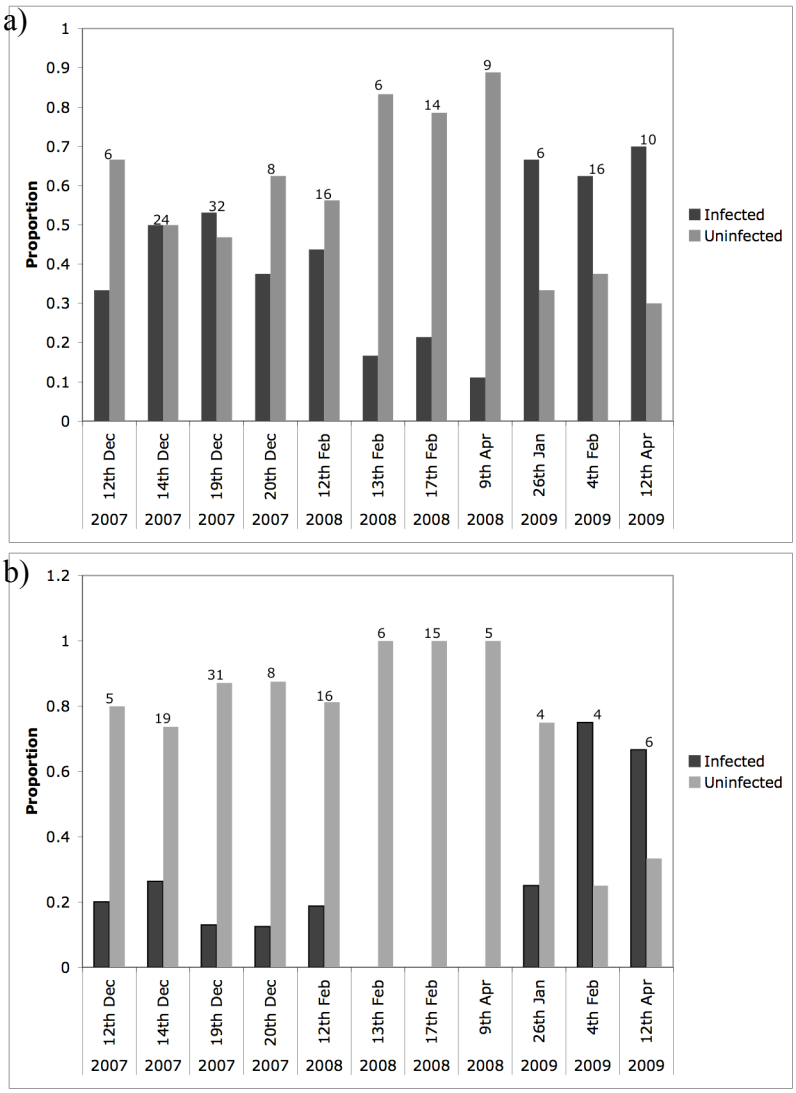

Figure 1. Prevalence of a) *Haemoproteus* (GLM, $Dev_{12,134}$=25.16, p=0.01) and b) *Leucocytozoon* (GLM, $Dev_{12,115}$=30.27, p<0.01) varied with day. Prevalence of both parasites was higher during 2008/09 than during 2007/08. Bars show proportions of infected and uninfected birds; numbers above bars show sample size

p<0.01) with the second time period showing no difference in prevalence from the first (GLM, t=0.53, p=0.59); however, prevalence was higher during the third time period than both the first (GLM, t=-2.58, p<0.01) and second (GLM, t=2.89, p<0.01) time periods.

Morphological associations with parasitism

Wing length was associated with both an interaction between age and sex, and an interaction between infection with *Haemoproteus* infection status and year (Table 1; Figure 2a). Males had longer wings than females and adult birds had longer wings than juveniles (Figure 2b). In the winter of 2007/08, birds infected with *Haemoproteus* had shorter wings than uninfected birds; however, in the winter of 2008/09, this was not the case and the wing lengths of infected birds were slightly longer than those of uninfected birds (Figure 2a). Tail length was influenced by interactions between age and year, and between month and infection by *Haemoproteus* (Table 3; Figure 3). During all months apart from January, infected birds had shorter tails than uninfected; however in January the opposite was true (Figure 3a). Tails of adult birds were longer in 2008 than in 2007; however tails of first year birds were shorter (Figure 2b). Neither *Leucocytozoon* infection nor any of its interactions were associated with wing length (Table 2) or tail length (Table 4).

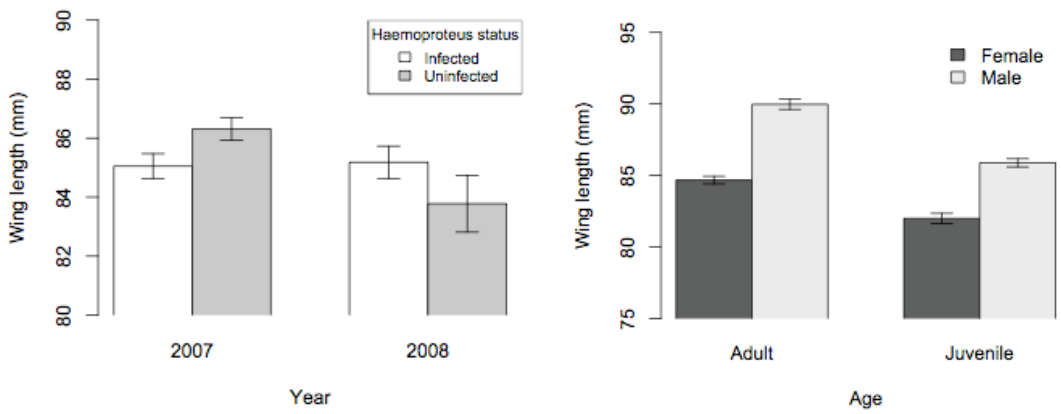

Figure 2. Wing length was influenced by interactions between a) *Haemoproteus* infection status and year, and b) Age and Sex (Table 1). Bars show mean ± 1 SE.

Table 1. Results from a GLM to determine whether infection by *Haemoproteus*, or any interactions therewith, are associated with wing length. For significant terms, parameter estimates with SE are presented; for non-significant main effects, statistics are following reinsertion of the term into the minimum adequate model (MAM) and subsequent model comparison. Two-way interactions of sex x infection status (F_1=0.97, p=0.33) and month x infection status (F_5=1.42, p=0.22) neither significantly influenced the fit of the model nor significantly influenced the response variable and thus were removed from the MAM.

Variable	**df**	**F**	**p**	**Estimate**	**SE**
Sex (Male)	1, 200	10.236	<0.001	5.231	0.511
Age (Juvenile)	1, 200	-5.214	<0.001	-2.580	0.495
Haemoproteus infection (uninfected)	1, 200	2.057	0.041	0.807	0.393
Year (2008)	1, 200	0.904	0.367	0.434	0.480
Sex x Age	1, 200	-2.184	0.030	-1.455	0.666
Haemoproteus infection x Year	1, 200	-2.143	0.033	-1.638	0.764
Variable	**df**	**F**	**p**		
Month	5, 189	0.545	0.742		

Table 2. Results from a GLM to determine whether infection by *Leucocytozoon*, or any interactions therewith, are associated with wing length. For significant terms, parameter estimates with SE are presented; for non-significant main effects, statistics are following reinsertion of the term into the minimum adequate model (MAM) and subsequent model comparison. *This term approached significance in influencing the fit of the model (F_1=3.09, p=0.08); thus, this term remains in the MAM but is not considered to significantly influence the response variable. Two-way interactions of sex x infection status (F_1=0.91, p=0.34), age x infection status (F_1=1.32, p=0.25), year x infection status (F_1=0.24, p=0.63) and month x infection status (F_4=1.18, p=0.32) neither significantly influenced the fit of the model nor significantly influenced the response variable and thus were removed from the MAM.

Variable	**df**	**F**	**p**	**Estimate**	**SE**
Sex (Male)	1, 171	123.174	<0.001	5.119	0.528
Age (Juvenile)	1, 170	99.639	<0.001	-2.768	0.547
Sex x Age	1, 169	3.089	0.081*	-1.254	0.714
Variable	**df**	**F**	**p**		
Month	5, 164	0.996	0.422		
Leucocytozoon infection	1, 168	0.333	0.565		
Year	1, 168	0.083	0.773		

Table 3. Results from a GLM to determine whether infection by *Haemoproteus*, or any interactions therewith, are associated with tail length. For significant terms, parameter estimates with SE are presented (contrasts for Month are against the mean, contrasts for factors with two levels are for the level stated and compared to the other level); for non-significant main effects, statistics are following reinsertion of the term into the minimum adequate model (MAM) and subsequent model comparison. Two-way interactions of sex x infection status (F_1=0.97, p=0.33) and month x infection status (F_5=1.42, p=0.22) neither significantly influenced the fit of the model nor significantly influenced the response variable and thus were removed from the MAM.

Variable	**df**	**F**	**p**	**Estimate**	**SE**
Month (Dec)	4, 146	1.752	0.142	0.171	0.419
Month (Jan)				-1.078	0.688
Month (Feb)				0.088	0.397
Month (Mar)				0.366	0.492
Month (Apr)				0.483	0.463
Haemoproteus infection (uninfected)	1, 145	3.497	0.064	-0.150	0.961
Sex (Male)	1, 144	65.287	<0.001	4.582	0.653
Age (Juvenile)	1, 143	56.908	<0.001	-2.505	0.605
Month (Dec) x *Haemoproteus* infection	4, 149	2.450	0.049	0.055	0.412
Month (Jan) x *Haemoproteus* infection				1.381	0.678
Month (Feb) x *Haemoproteus* infection				-1.032	0.395
Month (Mar) x *Haemoproteus* infection				-0.376	0.487
Month (Apr) x *Haemoproteus* infection				-0.028	0.462
Sex x Age	1, 138	4.178	0.043	-1.762	0.862
Variable	**df**	**F**	**p**		
Year	1, 137	0.808	0.370		

Table 4. Results from a GLM to determine whether infection by *Leucocytozoon*, or any interactions therewith, are associated with tail length. For significant terms, parameter estimates with SE are presented; for non-significant main effects, statistics are following reinsertion of the term into the minimum adequate model (MAM) and subsequent model comparison. Two-way interactions of sex x infection status (F_1=0.91, p=0.34), year x infection status (F_1=0.05, p=0.82), age x infection status (F_1=0.01, p=0.93) and month x infection status (F_4=0.80, p=0.53) neither significantly influenced the fit of the model nor significantly influenced the response variable and thus were removed from the MAM.

Variable	**df**	**F**	**p**	**Estimate**	**SE**
Sex (Male)	1, 122	46.112	<0.001	4.079	0.459
Age (Juvenile	1, 121	79.036	<0.001	-3.659	0.452
Variable	**df**	**F**	**p**		
Year	1, 120	0.307	0.581		
Month	4, 117	0.437	0.782		
Leucocytozoon infection	1, 120	0.118	0.731		

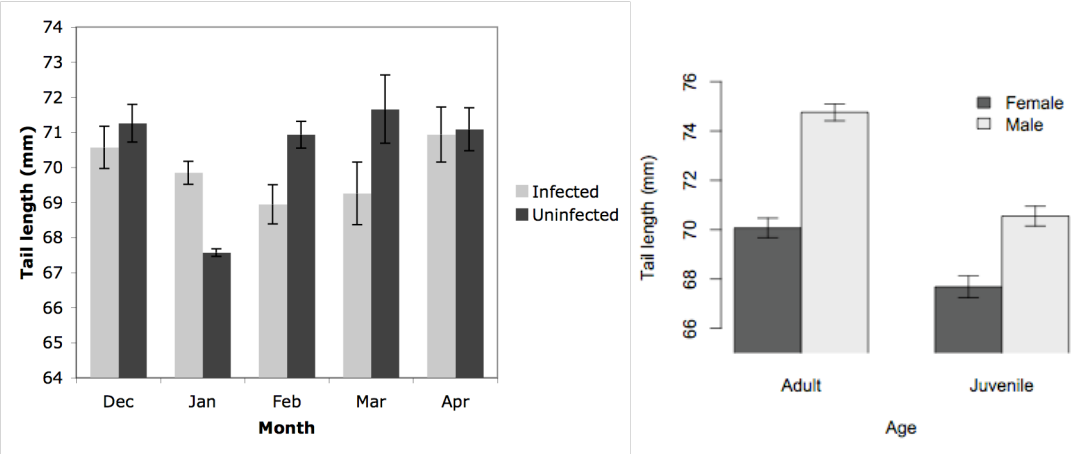

Figure 3. Tail length was influenced by interactions between a) Month and *Haemoproteus* infection status, and b) Age and sex (Table 3). Bars show mean values ± 1 SE.

To determine whether the associations between year, *Haemoproteus* infection status and wing length were a consequence of body size, further analyses were carried out to determine whether these associations were consistent with an association with body size generally, or specifically with feather length. No associations with either head-beak length ($F_{1,109}$=0.31, p=0.58; Appendix 1) or tarsus length ($F_{1,150}$=0.68, p=0.41; Appendix 2) were found (Figure 4).

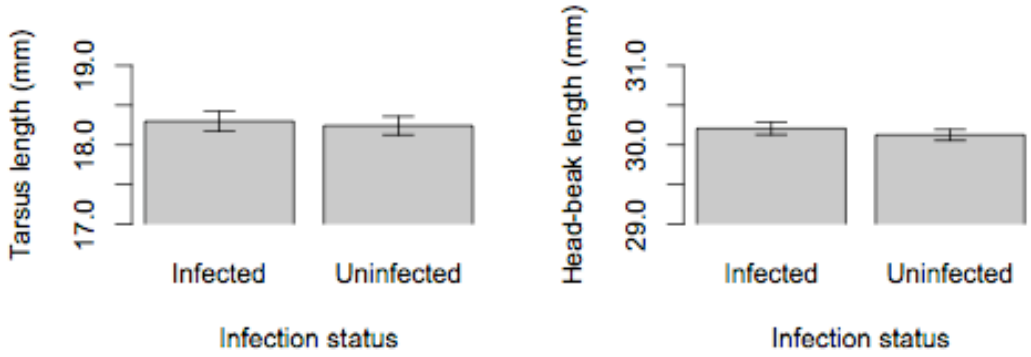

Figure 4. No difference in a) tarsus length or b) head-beak length was found between birds infected or uninfected by *Haemoproteus* spp.

Within recaptured birds, infection by *Haemoproteus* spp. had no effect upon either wing length (χ^2_1=0.11, p=0.74) or tail length (χ^2_1=0.16, p=0.69).

Infection in Yellowhammer nestlings

Of 10 chicks from four broods (three broods of three chicks and one brood of one chick) from which blood samples were obtained, 7 samples tested positive for parasites using the protocol of Waldenström et al (2004). Two chicks from each broods of three tested positive, as did the chick from the brood of one.

Discussion

Parasite prevalence, identity and association with environmental variables

Multiple sequences were obtained from three distinct haemoparasite lineages. Of the two *Haemoproteus* lineages, one had previously been isolated from a Dunnock *Prunella modularis* in the UK, and seems likely to be a generalist parasite (Cosgrove et al. Unpublished data), and the other had previously been isolated from a Chestnut Bunting *Emberiza rutila* in South Korea, and may be an *Emberiza* specialist (Ishtiaq et al. 2007). The *Leucocytozoon* sequence was from a novel lineage and more work is required to determine whether this parasite is a specialist or a more generalist parasite.

The haemoparasite prevalence of 50% found here is low when compared to a previous study of Yellowhammers sampled during the breeding season, which found 70% of birds to be infected by *Haemoproteus coatneyi*, but high when compared to other species sampled during the winter months where the prevalence of patent infections (infections where parasites remain circulating in the blood) approaches 0% (Schrader et al. 2003; Cosgrove et al. 2008). Infection of Blue Tits *Parus caeruleus* by *Plasmodium* spp. shows an increase in prevalence from February through to the start of the breeding season (Cosgrove et al. 2008), thought to be a relapse of current infection caused by stress due to the onset of breeding (Applegate 1971; Beaudoin et al. 1971; Allander and Sundberg 1997). This is in contrast to our results, which show the lowest detected prevalence on the latest sampling date during 2007 (9th April); however, it must be considered that Yellowhammers commence breeding later than Blue Tits and a previous study of Yellowhammers indicated that parasite intensity was low at the beginning of April and peaked at the beginning of May, outside our sampling period and coincidental with the onset of the breeding season (Allander and Sundberg 1997).

Our data appear to show a novel peak of *Haemoproteus* prevalence between mid-December 2007 and mid-February 2008; *Leucocytozoon* prevalence showed no clear peak but was not detected beyond mid-February in 2007. This peak is not apparent from other studies of haemoparasitism in temperate species (Schrader et al. 2003; Cosgrove et al. 2008; but see also Klei and DeGiusti 1975). Transmission of malaria parasites in temperate regions is thought to be negligible throughout the winter due to a cessation of vector activity (Cosgrove et al. 2008) but parasites remain dormant in host tissues (Valkiunas 2005) and are activated by stress hormones, usually at the onset of breeding (Applegate 1971; Allander and Sundberg 1997). Two hypotheses are

proposed for this winter peak in prevalence of circulating parasites. Firstly, host immunity can be lowered during the winter (Hasselquist et al. 1999; Møller et al. 2003; Hasselquist 2007), which may allow a relapse of existing infections as reduced immune function is associated with increased parasite prevalence (Ots and Hõrak 1998). However a multi-species study that included yellowhammers provided unconvincing evidence for a reduction in winter immunity for this species, although the sample size was small (Møller et al. 2003). The second possibility is that a reduction in over-winter food availability may trigger a relapse of infection through increased circulating corticosterone levels. A reduction in over-winter food availability has been linked to population declines in many farmland bird species, including Yellowhammers (Peach et al. 1999; Robinson and Sutherland 1999; Bradbury et al. 2000) and corticosterone levels have been shown to increase at times of low food availability (Clinchy et al. 2004). Increased corticosterone levels have been experimentally linked to both an increased parasite prevalence and an increased intensity of infection (Applegate 1970) so it appears plausible that a reduced food supply may induce relapses of haemoparasite infection, resulting in the observed peak in prevalence during winter. The potential implications of this require further exploration.

Parasite prevalence was higher during 2008 than during 2007, coincidental with lower bird numbers despite similar sampling effort. This may be due to an early cold spell during the autumn of 2008 (National Climate Information Centre 2008), which is likely to have caused high mortality and may have caused increased stress causing a relapse of infection as described previously.

Our data provide no evidence for sex or age differences in parasite infection. During the breeding season, difference in parasite prevalence between the sexes are frequently seen, with a higher prevalence and intensity of infection in females (Norris et al. 1994; Hasselquist et al. 2007), although other studies indicate that the relative prevalence between the sexes varies temporally, with a peak in males when territories are being established, and a peak in females when chicks have hatched (Applegate 1971; Allander and Sundberg 1997). In flocking situations and competitive interactions, males are usually more dominant (Domenech and Senar 1999; Seibert and Crowell-Davis 2001) and monopolise preferred roosting habitat (Mezquida et al. 2005). In American Kestrels, where size dimorphism is reversed, the smaller males suffer energetic consequences as a result of exclusion from preferred habitat by females (Ardia 2002).

However, yellowhammers form mixed age and sex flocks over-winter and size differences between the ages and sexes are restricted to feather length and not body size (Dunn and Wright *In press*), which may explain the lack of association with parasitism over-winter found here.

Morphological associations with parasitism

I demonstrate associations between *Haemoproteus* infection and wing and tail length, which differ between years and months. Previous studies have also shown associations between feather length and haemoparasites (Rätti et al. 1993; Hatchwell et al. 2001; Votypka et al. 2003): of these, Hatchwell et al. (2001) analyse data from only one year, and Votypka et al. (2003) do not appear to consider the potential influence of inter-year variation upon their analyses. Whilst Rätti et al. (1993) found an interactive effect of year and age on arrival date in a model containing parasite status and suggest that the interaction between all three terms may be significant (although they do not test for this), they did not consider the potential effect of year upon their associations between parasite status and wing and tail length (Rätti et al. 1993).

Whilst the association between *Haemoproteus* infection and feather length does not appear straightforward, no association was found between parasitism and measures of size, indicating that smaller birds are no more likely to be parasitized, and suggesting an effect of parasitism on feather growth during moult, as proposed by Rätti et al. (1993). However, this effect may well be complicated by environmental factors that differ between years, and consequently may be less exaggerated, or even entirely absent, in some years. In this case it is possible that the relatively mild autumn of 2007 (National Climate Information Centre 2008) led to a relatively high yellowhammer survival rate, and that birds susceptible to the effects of parasitism during moult survived over-winter, at least during early winter when the majority of birds sampled were caught. The autumn of 2008 was quite severe (National Climate Information Centre 2008) and it could be that birds that were susceptible to the effects of parasitism suffered high mortality prior to the sampling period, explaining both the observed year-dependent association between parasite infection and wing length, and the decreased sample size during the second winter of the study.

Infection in Yellowhammer nestlings

Parasite infection was found in 60% of yellowhammer nestlings screened. Sample size was only ten chicks and thus little can be read into the prevalence of infection; however, that infections were found at all in seven-day-old nestlings is worthy of note. Cosgrove et al (2006) found no evidence of infection by either *Plasmodium* or *Haemoproteus* in 195 fourteen-day-old nestling blue tits, although they did find evidence of one infection with *Leucocytozoon*. Indeed, prior to Cosgrove et al. (2006), only one study had found evidence of haemoparasitic infection in passerine nestlings, this being in ten-day-old Red-Winged Blackbird *Agelaius phoeniceus* nestlings (Weatherhead and Bennett 1991). This had led to the question of whether the absence of infection was due to birds being infected only following fledging, or whether birds were infected in the nest but that the prepatent period of the parasites was too long to allow the infection to reach patency prior to fledging (Cosgrove et al. 2006). Whilst it must be noted that many avian haemosporidia are species-specific (Valkiunas 2005) and that there is a wide variety in the prepatent period of haemosporidians, it is possible that the difference in detection of parasites between the studies of Cosgrove et al. (2006), Weatherhead and Bennett (1991), and this study may be due to the ecology of the avian species, rather than their parasites. The Blue Tits screened within the study of Cosgrove et al. (2006) are a box and hole-nesting species which may lead to a reduced accessibility of chicks to the vectors. Conversely, both Yellowhammers and Red-Winged Blackbirds are open-nesting species, which may increase the accessibility of nestlings to the vectors of haemosporidians.

I show a novel winter peak of infection in Yellowhammers, suggesting that the effects of blood parasites in avian life-history may not just be restricted to the breeding season. I find time-specific associations of *Haemoproteus* spp. with feather length, but not size, which suggest associations with environmental stressors. Finally, I detect blood parasite infection in 7-day-old nestlings, suggesting that haemoparasites may play a part in the ecology of very young birds, potentially playing a role in post-fledging survival or condition. This work highlights the importance of blood parasites in the ecology of their hosts, and emphasises the need for a greater understanding of the associations between haemoparasites and their hosts, especially in species under environmental pressures, where the effects of parasitism may be emphasised.

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Appendix 1. Results from a GLM to determine whether infection by *Haemoproteus*, or any interactions therewith, is associated with head-beak length. For significant terms, parameter estimates with SE are presented; for non-significant main effects, statistics are following reinsertion of the term into the minimum adequate model (MAM) and subsequent model comparison. Two-way interactions of sex x infection status (F_1=0.77, p=0.38), age x infection status (F_1=0.61, p=0.43), year x infection status (F_1=1.23, p=0.27), month x infection status (F_4=0.39, p=0.82), and sex x age (F_1=0.61, p=0.43) neither significantly influenced the fit of the model nor significantly influenced the response variable and thus were removed from the MAM.

Variable	**df**	**F**	**P**	**Estimate**	**SE**
Age (Juvenile)	1, 151	6.211	0.014	-0.256	0.103
Variable	**df**	**F**	**p**		
Month	4, 147	0.383	0.821		
Year	1, 150	0.026	0.870		
Haemoproteus infection status	1, 150	0.682	0.410		
Sex	1, 150	0.821	0.366		

Appendix 2. Results from a GLM to determine whether infection by *Haemoproteus*, or any interactions therewith, is associated with head-beak length. For significant terms, parameter estimates with SE are presented (contrasts for Month are against the mean, contrasts for factors with two levels are for the level stated and compared to the other level); for non-significant main effects, statistics are following reinsertion of the term into the minimum adequate model (MAM) and subsequent model comparison. Two-way interactions of sex x infection status (F_1=0.28, p=0.60), age x infection status (F_1=0.01, p=0.91), year x infection status (F_1<0.01, p=0.97) and month x infection status (F_4=0.44, p=0.78) neither significantly influenced the fit of the model nor significantly influenced the response variable and thus were removed from the MAM.

Variable	**df**	**F**	**p**	**Estimate**	**SE**
Sex (Male)	1, 116	1.939	0.167	0.691	0.281
Age (Juvenile)	1, 115	0.063	0.802	0.430	0.247
Month (December)	1, 111	2.735	0.032	-0.652	0.235
Month (January)				-0.038	0.196
Month (February)				-0.081	0.138
Month (March)				0.670	0.183
Month (April)				0.101	0.162
Age x Sex	1, 110	5.028	0.027	-0.795	0.355
Variable	**df**	**F**	**p**		
Year	1, 109	1.905	0.170		
Haemoproteus infection	1, 109	0.305	0.582		