PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2007) **274**, 2003–2012
doi:10.1098/rspb.2007.0124
Published online 30 May 2007

Prevalence of avian influenza and host ecology

László Zsolt Garamszegi[1,*] and Anders Pape Møller[2]

[1]*Department of Biology, University of Antwerp, Campus Drie Eiken Universiteitsplein 1, 2610 Wilrijk, Belgium*
[2]*Laboratoire de Parasitologie Evolutive, Université Pierre et Marie Curie, CNRS UMR 7103, Bâtiment A, 7ème étage, 7 quai St Bernard, Case 237, 75252 Paris Cedex 05, France*

Waterfowl and shorebirds are common reservoirs of the low pathogenic subtypes of avian influenza (LPAI), which are easily transmitted to poultry and become highly pathogenic. As the risk of virus transmission depends on the prevalence of LPAI in host-reservoir systems, there is an urgent need for understanding how host ecology, life history and behaviour can affect virus prevalence in the wild. To test for the most important ecological correlates of LPAI virus prevalence at the interspecific level, we applied a comparative analysis by using quantitative data on 30 bird species. We controlled for similarity among species due to common descent, differences in study effort and for covariance among ecological variables. We found that LPAI prevalence is a species-specific attribute and is a consequence of virus susceptibility, as it was negatively associated with the relative size of the bursa of Fabricius, an estimate of juvenile immune function. Species that migrate long distances have elevated prevalence of LPAI independent of phylogeny and other confounding factors. There was also a positive interspecific relationship between the frequency of surface feeding and virus prevalence, but this was sensitive to phylogenetic relatedness of species. Feeding in marine habitats is apparently associated with lower virus prevalence, but the effect of water salinity is likely to be indirect and affected by phylogeny. Our results imply that virus transmission via surface waters and frequent intra- and interspecific contacts during long migration are the major risk factors of avian influenza in the wild. However, the link between exploitation of surface waters and LPAI prevalence appears to be weaker than previously thought. This is the first interspecific study that provides statistical evidence that host ecology, immunity and phylogeny have important consequence for virus prevalence.

Keywords: Anatidae; bird flu; feeding ecology; migration; prevalence; risk assessment

1. INTRODUCTION

The low pathogenic subtypes of avian influenza (LPAI), consisting of different combinations of haemagglutinin (H) and neuraminidase (N) glycoproteins, cause little harm in the wild (Webster *et al.* 1992; Fouchier *et al.* 2005). However, certain subtypes, particularly H5 and H7, may become highly pathogenic if they are transmitted to domesticated birds (Shortridge *et al.* 1998; Spackman *et al.* 2003; Aubin *et al.* 2005). The switch into a high pathogenic subtype (HPAI) in poultry is achieved via the insertion of multiple acid residues into the cleavage site of the polypeptide precursor of H, the subunit that facilitates virus replication (Alexander 2000). As HPAI outbreaks not only lead to enormous economical problems but also threaten human health and the risk is dependent on the prevalence of LPAI in the natural host-reservoir systems, it is very important to understand the biology of virus prevalence in the wild (Webster *et al.* 1992; Suarez 2000; Webby & Webster 2003; Capua & Alexander 2004; Tracey *et al.* 2004; Clark & Hall 2006; Olsen *et al.* 2006).

Several observations suggest that host ecology is associated with LPAI virus prevalence (Stallknecht & Shane 1988; Stallknecht 1998; Alexander 2000). For example, many infected bird species are from wetlands and aquatic environments, because such habitats are likely to involve favourable circumstances for virus transmission and survival (Webster *et al.* 1978, 1992). The virus is easily transmitted among aquatic birds via the faecal–oral route, as it replicates in the epithelial cells of the intestinal tract and is excreted from the cloaca in high concentrations into water, thereby infecting other animals that drink the contaminated water (Webster *et al.* 1978). In addition, other routes, such as oral–oral, respiratory route and uptake via the cloaca may also be in effect (e.g. Sturm-Ramirez *et al.* 2005). In any case, the virus seems to survive and remain infectious under aquatic environmental conditions, whereas it is less stable in solid material (Lu *et al.* 2003). Another ecological factor that has long been suspected as the principal risk factor of LPAI infection is migration (Hinshaw & Webster 1982; Ito *et al.* 1995; Krauss *et al.* 2004; Tracey *et al.* 2004; Olsen *et al.* 2006). Long-distance migration is a common phenomenon in Anseriform and Charadriiform birds (Cramp & Perrins 1985–1994; Poole *et al.* 1993–2002), which can distribute LPAI over large geographical regions. Migrant species often form intra- and interspecific flocks at suitable stopover and wintering sites, which enhances parasite transmission within and among species. Peak prevalence in wild ducks in the temperate zone coincides with the preparation for migration, when birds rely on intense aquatic feeding and start forming aggregations. In addition, migrants tend to occupy northern latitudes for breeding, where colder temperatures may favour virus survival in fresh water (Stallknecht *et al.* 1990*a*). Consequently, migration, aquatic lifestyle and

* Author for correspondence (laszlo.garamszegi@ua.ac.be).

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2007.0124 or via http://www.journals.royalsoc.ac.uk.

Received 29 January 2007
Accepted 9 May 2007

This journal is © 2007 The Royal Society

particularly surface feeding have been identified as the key ecological factors mediating LPAI prevalence in the wild (Olsen *et al.* 2006).

However, our understanding of the exact role of different ecological factors in determining the prevalence of avian influenza is incomplete, and risk assessment based on pure observations may be misleading (Shortridge *et al.* 1998; Spackman *et al.* 2003; Webby & Webster 2003; Aubin *et al.* 2005). A typical problem could be that there is an interaction between different factors and an apparent association between LPAI prevalence and a host trait is mediated by a third factor. For example, although migratory habits seem to have consequences for infection rate (Hinshaw & Webster 1982; Ito *et al.* 1995; Krauss *et al.* 2004; Tracey *et al.* 2004; Olsen *et al.* 2006), the high LPAI prevalence in migratory birds may be caused by several factors such as frequent aggregations, intense surface feeding prior to departure, interspecific contacts and colder temperatures at the breeding sites. Moreover, closely related species often share ecological and life-history traits, and similarity in phenotype may be caused by common phylogenetic descent rather than adaptation. For example, owing to their feeding habits on surface waters, dabbling ducks are generally thought to have higher LPAI infection than diving ducks (Clark & Hall 2006; Olsen *et al.* 2006). However, common ancestry may cause virus infection to be more prevalent in dabbling ducks independent of selection pressures arising from surface feeding, as these birds share other life-history or ecological traits that may also be relevant for disease susceptibility. Therefore, to assess the importance of different ecological factors in mediating LPAI prevalence in wild birds, statistical analyses that control for covariation of traits and for similarity due to common phylogenetic descent would be required.

Here, we present the results of such analyses, in which we investigated the fundamental hypotheses of LPAI prevalence (Tracey *et al.* 2004; Clark & Hall 2006; Olsen *et al.* 2006) in a comparative study of birds. Using descriptions from handbooks, we extracted information on the key ecological variables that are generally thought to mediate LPAI prevalence and could be estimated for the majority of species in a standard way. We tested the following simple but important predictions that are overwhelmingly present in the bird flu literature (see above), but so far have not seen proper statistical tests. First, if the ecology of aquatic feeding is an important determinant of prevalence through efficient virus transmission via the faecal–oral route for example, we predicted that species that rely on extensive surface-related foraging should have higher virus prevalence than species feeding in deeper waters or on land. Second, if habitat preference affects virus prevalence, because virus transmission is linked to water habitats, species that show preference for water habitats should suffer from higher infection rates than terrestrial species. Additionally, waters with lower temperatures and salt concentrations can also enhance the virus's ability to remain infectious and thus have consequences for interspecific patterns. Finally, if migration is a key determinant of prevalence, species that migrate longer distances or have relatively larger migrating populations should have relatively high prevalence, if these traits reflect a high risk of intra- and interspecific virus transmission. To test the above predictions, we used data for 30 bird species with non-zero prevalence (from Olsen *et al.* 2006) and calculated species-specific values of prevalence that were controlled for differences in sampling effort and locality. After validating the biological meaning of LPAI prevalence by showing that it may reflect disease susceptibility, we applied a comparative approach to determine the most important ecological factors accounting for variation among species. This approach allowed us to control for the effect of phylogenetic relationships among hosts and for covariation among predictor variables. We also assessed the importance of differences of data quality across studies by weighting each datum by the associated sample size.

2. MATERIAL AND METHODS

(a) *Dataset: LPAI prevalence*

We used information from the extensive list of world-wide surveillance of Olsen *et al.* (2006). However, this dataset should be treated with caution, as although it seems extensive, it is still incomplete (Olsen *et al.* 2006). For many bird species, prevalence data were collected at limited time points and sampling sites, whereas the data are probably biased towards reporting positive results (Olsen *et al.* 2006). Therefore, to deal with the underrepresentation of species without infection, we excluded zero prevalence data. Accordingly, with this smaller but supposedly less biased dataset, we focused only on those species that are known hosts of LPAI virus, and thus any conclusion should be carefully drawn when making generalizations.

As the prevalence of influenza virus in general may vary between different surveillance studies depending on species, time and place, we first tested for such confounding effects by analysing the raw dataset by focusing on species for which multiple records were available. Multiple records are likely to cover samples at different parts of the year and can be used to assess the importance of intraspecific variation in LPAI prevalence due to within-year fluctuations (Okazaki *et al.* 2000; Hanson *et al.* 2003; Munster *et al.* 2005). In addition, as many observations originate from different localities, information from different study sites for the same species can be used to identify geographical effects. Moreover, statistical approaches are available that allow control for differences in surveillance methods, as data points in the analyses can be weighted based on the corresponding sample size. We were especially interested in finding significant taxonomic effects, because we aimed to explain interspecific variation. For comparative purposes, such variation among species should be larger than variation within species (e.g. due to time and place effects or methodological differences between studies), which makes comparisons of species-specific values biologically meaningful.

Therefore, we analysed multiple data on positive prevalence at the level of individual surveys using a GLM approach. LPAI prevalence was log-transformed to achieve a linear distribution. We then built a model with LPAI prevalence as the dependent variable, and tested for the effect of host species (main effect), while involving geographical region (Europe, North America, Asia, Australia; data from African sites were excluded due to small sample size) as random factors. To account for differences in surveillance studies in terms of the numbers of animals monitored, the model was weighted by sample size. After identifying the

determinants of LPAI prevalence at the study level, we calculated species-specific estimates of peak prevalence that are independent of sampling effects (sample size and locality) in the form of least square (LS) means from the most appropriate model. We used these estimates in a set of phylogenetic analyses to test for the determinants of LPAI prevalence at the interspecific level (see Lucas *et al.* (2004) and Garamszegi (2006*b*) for similar approaches).

To assess the biological relevance of LPAI prevalence, we used available information ($N=8$) on the mass of the bursa of Fabricius of juvenile birds from other studies (Møller *et al.* 2005; Garamszegi *et al.* 2007). The bursa of Fabricius is the most important immune organ in juvenile birds, as it synthesizes antibody, and is responsible for differentiation of the repertoire of B-cells (Toivanen & Toivanen 1987; Glick 1983, 1994). The relative size of this immune defence organ in birds may reflect the ability to respond to an infection (Rose 1981; Glick 1983; John 1994). For example, selection for increased and decreased immune responses to an immune challenge by sheep red blood cells resulted in correlated response to selection for the size of the bursa of Fabricius (Parmentier *et al.* 1995). In addition, relative bursa size has been found to relate to factors that involve high parasite pressure (Møller & Erritzøe 1996, 1998). Immunologically naive young birds are particularly susceptible to LPAI (Alfonso *et al.* 1995; Jørgensen *et al.* 1998; Capua *et al.* 2000; Hanson *et al.* 2003), and thus the immune organ that mediates immune defence in young birds should be an important component of LPAI resistance. Therefore, we predicted that if the use of species-specific prevalence data makes sense and it is mediated by disease susceptibility, species with relatively smaller bursa of Fabricius should have higher virus prevalence than species with larger organs, owing to the weakly functioning immune defence in juveniles. To estimate the importance of bursa mass relative to body mass, we obtained body mass from Dunning (1993) and calculated residuals from the allometric regression.

(b) *Dataset: ecological variables*

As comparative datasets for our specific purposes and data at hand were basically lacking, we used information from standard handbooks (Dementiev & Gladkov 1967; Cramp & Perrins 1985–1994; Urban *et al.* 1986; MacLean 1988; Marchant & Higgins 1990–1993; Clement *et al.* 1993; MacKinnon & Phillipps 1993; Poole *et al.* 1993–2002; Higgins & Davies 1996). These handbooks provide detailed descriptions of behavioural and ecological traits including migration, habitat preference and feeding methods. Although each section was written by a different group of authors, they all follow a standard format, which facilitates cross-species comparisons, as frequently seen in the comparative literature (e.g. Yanes & Suarez 1997; Rolland *et al.* 1998; Iwaniuk & Nelson 2003; Hall & Tullberg 2004; Mougeot 2004; Ekman & Ericson 2006; Figuerola & Green 2006). If quantitative information was unavailable, based on the detailed verbal descriptions, we used scores for the particular trait and averaged them for species. These scores were treated as continuous variables, because intermediate states are biologically meaningful, and different states are thus arbitrary points along a continuum (Sokal & Rohlf 1995). In addition, in an evolutionary context, a transition between two states of these variables follows non-discrete gradual evolutionary changes (see also Harvey & Pagel 1991; Bennett & Owens 2002). However, we always verified the reliability of these scores by calculating repeatability across sources and observers (based on the intraclass correlation method; Van Vleck 1993) to provide statistical evidence that these measurements were biologically meaningful species-specific attributes. Note that any uncertainty due to imprecise estimation of the scores should produce random errors and not systematic bias, because scoring was done blindly with respect to the prevalence data. The following variables were estimated.

— *Surface feeding*. Virus transmission is predicted to be associated with the rate of contact with surface waters. Hence, we scored main feeding methods by assuming that the rate at which the bill of the animal is in physical contact with surface water reflects the involved risk of the feeding method for parasite transmission (highest risk, score 3: surface-feeding methods (e.g. dabbling, skimming); intermediate risk, score 2: aquatic foraging with less contact with the water surface (e.g. diving, up-ending, hovering); no risk, score 1: feeding on land). These scores were averaged by species, which was biologically reliable as indicated by significant repeatability ($R=0.730$, $p<0.001$). We predicted that surface feeding is positively related to LPAI prevalence.

— *Water habitat use*. Inhabiting aquatic environments is thought to involve higher prevalence, while the frequency at which such habitats are exploited accentuates the importance of surface feeding. We counted the number of aquatic habitats during the yearly cycle listed in the habitat sections of the handbooks in relation to the total number of habitats used (%). Relative water habitat use had moderate repeatability ($R=0.331$, $p=0.136$), and thus corresponding results should be treated with caution. However, as could be predicted, average water habitat use was a significant predictor of surface feeding ($r=0.505$, $N=30$, $p=0.004$), which indicates that this measure is biologically meaningful. We predicted that water habitat use is positively related to LPAI prevalence.

— *Water salinity*. Salt concentrations affect the virus's ability to remain infectious, which may have consequences for the interspecific patterns of LPAI prevalence. We scored the salinity of water habitats involving breeding and winter habitats (score 3: salt water; score 2: brackish water; score 1: fresh water), and calculated species means, which were repeatable ($R=0.841$, $p<0.001$). We predicted that water salinity is negatively related to LPAI prevalence.

— *Temperature during breeding and wintering*. Water temperature may also determine virus survival, and the exploitation of colder waters may potentially increase prevalence. To reflect the importance of water temperature, we calculated the absolute mean of the latitudinal distribution during breeding and wintering. We assumed that in breeding and wintering sites at higher latitudes, water temperature is lower than in sites at lower latitudes. Latitudinal distributions were determined by using maps to the nearest 1°, on which we projected information on the northernmost and the southernmost distribution limits for the breeding season and the winter as obtained from our sources. Means of the latitudinal distributions were the average of the relevant northernmost and the southernmost limits (repeatabilities: northernmost breeding latitude, $R=0.966$, $p<0.001$; southernmost breeding latitude, $R=0.962$, $p<0.001$; northernmost winter latitude, $R=0.476$, $p=0.029$; southernmost winter latitude: $R=0.809$, $p<0.001$). We predicted a positive relationship between latitudes and LPAI prevalence.

— *Migration distance*. Migration involves several risk factors for virus infection, such as intense feeding during preparation for migration, aggregations at stopover and wintering sites, breeding sites at higher latitudes and frequent contacts with other species. Unfortunately, we could not obtain quantitative estimates for these risk factors for a large number of species to allow interspecific comparisons. However, we could measure migration distance and assumed that it reflected the risks of virus transmission. The risk factors can be assumed to increase linearly with migration distance, because species with long migratory routes breed at high latitudes, may be expected to feed intensively prior to departure and at stopover sites due to their increased metabolic needs, and they may have elevated risk of getting in contact with other migratory species (see also Meiri & Yom-Tov 2004). Accordingly, a genetic analysis revealed that interregional transmission of influenza viruses occurred between North American and Eurasian birds (Liu *et al.* 2004), which is probably enhanced by species that fly long distances between continents. Moreover, recovery rates and isolation studies suggest incidences of intercontinental spread of LPAI by migratory birds (Hurt *et al.* 2006; Muñoz *et al.* 2006). Therefore, for migrating species, we calculated the mean breeding latitude minus the mean winter latitude, which were already shown to be repeatable, multiplied by 111 km. We predicted that migration distance is positively related to LPAI prevalence.

— *Migration propensity*. The risk factors are likely to increase with migration propensity, because species showing little tendency to migrate will have small populations that effectively encounter no risk during migration. At the species level, the generally low prevalence detected in large resident populations would potentially blur any pattern occurring in the small migrating populations. Therefore, we calculated migration propensity (%) based on digital distribution maps originating from the electronic versions of Cramp & Perrins (1985–1994) and Poole *et al.* (1993–2002) as (summer distribution range of migrating populations)/(summer distribution range of migrating and resident populations). These underlying variables were highly repeatable (summer distribution range of migrating populations, $R=0.841$, $p=0.009$; distribution range of resident populations: $R=0.748$, $p=0.033$). We predicted a positive relationship between migration propensity and LPAI prevalence.

We also used *body mass* from Dunning (1993) in our multivariate analysis to control for the potentially confounding effect of the most important life-history traits associated with body mass (e.g. survival, reproductive rate; Bennett & Owens 2002). Moreover, the relative importance of the ecological factors may be proportional to body mass due to physiological effects. For example, risk factors involved in migration distance or surface feeding are likely to depend on metabolic needs, which mediate the number of stopovers or feeding rates. Body mass is a good predictor of such metabolic needs, as it is the key determinant of field metabolic rates reflecting food requirements (Nagy 1987; Tieleman & Williams 2000). In general, the control for body mass is important to understand interspecific variation in parasite prevalence, as shown by a study of avian blood parasites (Scheuerlein & Ricklefs 2004).

The full dataset is reported in the electronic supplementary material. All variables were appropriately transformed for the phylogenetic analyses.

(c) *Phylogenetic and statistical methods*

To control for similarity in phenotype among species due to common phylogenetic relatedness (summarized in the electronic supplementary material), we applied the general method of comparative analysis for continuous variables based on generalized least squares (GLS) models using the statistical software CONTINUOUS (Pagel 1997, 1999). First, we investigated the role of phylogenetic inertia by estimating the phylogenetic scaling parameter lambda (λ) that varies between 0 (phylogenetic independence) and 1 (species' traits covary in direct proportion to their shared evolutionary history; Freckleton *et al.* 2002). We permitted λ to take its maximum-likelihood value, and tested whether there was any evidence for $\lambda>0$, which indicates that trait variation is dependent on phylogeny. After adjusting for phylogenetic effects through λ, we calculated the phylogenetically corrected correlation between variables of interest.

To determine the strength and direction of the predicted relationships, we estimated effect sizes (such as Pearson's correlation coefficient *r sensu* Cohen 1988), and the associated 95% CI for each particular relationship. We preferred reporting and focusing on effect sizes, instead of using Bonferroni correction for significance levels, because the latter approach has been criticized in the field of ecology and behavioural ecology for mathematical and logical reasons (Perneger 1998; Moran 2003; Nakagawa 2004; Garamszegi 2006*a*). Therefore, to balance Type I and II errors, we followed the recent recommendations of Nakagawa (2004) who emphasized the importance of unbiased reports of effect sizes.

One of the common assumptions underlying most statistical approaches is that each data point provides equally precise information about the deterministic part of the total process variation, i.e. the standard deviation of the error term is constant over all values of the predictor or explanatory variables (Sokal & Rohlf 1995). This assumption, however, clearly does not hold, even approximately, in association with LPAI prevalence data, as there is a huge variation between studies and species in the number of individuals sampled (range 1–15 407). In such situations, the equal treatment of every observation would be misleading, and thus weighted analyses can be used to maximize the efficiency of parameter estimation. To give each data point its proper amount of influence over the parameter estimates, we used the corresponding sample sizes as a weight in our analyses of the raw species data.

In cases where we found evidence for the strong effects of weights on the raw data and the common descent in the phylogenetic analysis ($\lambda\approx1$), we simultaneously assessed the role of these factors. We combined variance factors due to phylogenetic and weight effects as error terms in a form of a matrix using the $Q=V+cW$ equation, where V is the phylogeny matrix; W is the diagonal matrix of 1/weights; and c is constant (Martins & Hansen 1997). By varying c constant, we calculated the maximum likelihood of different combinations of the phylogeny and weight matrices. At the combination, which resulted in the highest maximum likelihood, we determined the slope of the effect in focus and compared it with slopes estimated at $Q=V$ (only phylogenetic effect) and $Q=W$ (only weight effect). This additional GLS exercise was performed in the R statistical

Table 1. Pairwise relationships between LPAI prevalence and ecological variables, when analyses were based on the raw species data and when controlled for common ancestry. For analyses relying on the raw species data, effect sizes are estimated as Spearman correlations that were calculated without and with sample size as a weight. Effect sizes corresponding to the phylogenetic models are the phylogenetic correlations as obtained from the program CONTINUOUS. Effect size conventions: $r=0.10$, small effect; $r=0.30$, intermediate effect; and $r=0.50$, large effect (Cohen 1988). Signs of effects show the untransformed direction of the relationship, i.e. they do not indicate whether the patterns are in the expected or the opposite direction. Sample sizes and lower/upper 95% CI are presented. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

	raw data						
	without weighting		with weighting		phylogenetic models		
predictor variables	r	95% CI	r	95% CI	λ	r	95% CI
migration distance ($N=30$)	0.400^{*}	0.046/0.665	0.571^{**}	0.265/0.772	0.000	0.349^{*}	−0.013/0.630
migration propensity ($N=27$)	0.269	−0.124/0.589	-0.401^{*}	−0.678/−0.025	0.000	0.361	−0.022/0.652
water habitat use ($N=30$)	−0.072	−0.421/0.296	−0.227	−0.543/0.145	0.024	0.136	−0.236/0.473
water salinity ($N=30$)	-0.368^{*}	−0.643/−0.009	-0.670^{***}	−0.830/−0.408	1.000	−0.201	−0.523/0.172
surface feeding ($N=30$)	0.396^{*}	0.042/0.662	0.494^{**}	0.163/0.725	1.000	0.116	−0.255/0.457
body mass ($N=30$)	−0.048	−0.401/0.318	0.110	−0.261/0.452	1.000	0.014	−0.348/0.372
breeding latitude ($N=30$)	0.157	−0.215/0.490	0.299	−0.069/0.595	0.017	0.208	−0.165/0.529
winter latitude ($N=30$)	0.081	−0.288/0.429	−0.148	−0.483/0.224	0.084	0.214	−0.158/0.533

computing environment, with additional unpublished functions by R. Freckleton (University of Sheffield, available upon request) for the phylogenetic procedures. In this modelling, we used estimates of λ as obtained from program CONTINUOUS for the correlated evolution of traits in focus.

3. RESULTS

(a) *Intraspecific variation in LPAI prevalence*

There was consistent variation in LPAI prevalence among host species, when geographical origin was controlled and analyses were weighted by sample size ($F_{29,124}=3.455$, $p<0.001$). The model revealed a significant effect for geographical region ($F_{3,124}=3.897$, $p=0.011$), suggesting that prevalence varies systematically among continents (back-transformed LS means $\pm$ s.e. when differences between species were held constant: Asia, $1.53\pm0.15\%$, 38 studies, 6540 birds; Australia, $1.88\pm0.27\%$, 5 studies, 1602 birds; Europe, $2.18\pm0.19\%$, 37 studies, 13 622 birds; North America, $3.94\pm0.37\%$, 77 studies, 26 285 birds).

We further dealt with the potentially confounding effect of study intensity by repeating the above analysis by focusing on studies that relied on at least 10 or 100 individuals. (We could not proceed with $N>500$ as the entering criterion, because the resulting dataset had low sample size for such multivariate analyses.) These analyses confirmed the previous observations ($N>10$ criterion: species, $F_{28,113}=3.301$, $p<0.001$; geographical region, $F_{3,113}=3.646$, $p=0.015$; $N>100$ criterion: species, $F_{14,98}=4.766$, $p<0.001$; geographical region, $F_{3,98}=3.424$, $p=0.020$). Therefore, from the model relying on the largest sample and weighted by sample size, we determined the LS means of prevalence (hereafter LS prevalence) and used them in our interspecific analyses as host-specific estimates that are independent of geographical origin.

(b) *Interspecific variation in LPAI prevalence: biological relevance*

We tested the biological relevance of LS prevalence. For each species we extracted data on maximum prevalence and, using the rest of the data, calculated LS prevalence as described above. Maximum prevalence and LS prevalence based on the remaining data are thus independent, but should correlate if they represent the same biological phenomenon. Indeed, these independent estimates were strongly positively correlated (raw data: $r=0.572$, 95% CI$=0.159/0.815$, $N=19$, $p=0.011$; weighted analyses based on the number of birds sampled at the maximum prevalence: $r=0.736$, CI$=0.423/0.892$, $N=19$, $p<0.001$; controlling for the geographical origin of the weighted maximum prevalence data: $F_{1,14}=28.617$, $p<0.001$; phylogenetically adjusted correlation at $\lambda=0.010$: $r=0.581$, CI$=0.172/0.819$, $N=19$, $p=0.005$).

Across the few species for which data were available, LS prevalence was negatively related to the relative size of the bursa of Fabricius (raw data: $r=-0.784$, 95% CI$=-0.959/-0.177$, $N=8$, $p=0.021$; weighted data: $r=-0.900$, CI$=-0.982/-0.534$, $N=8$, $p=0.002$; phylogenetically adjusted correlation at $\lambda=1$: $r=-0.808$, CI$=-0.964/-0.240$, $N=8$, $p=0.004$). Hence, LS prevalence is a species-specific attribute and it mirrors the susceptibility of hosts to LPAI.

(c) *Interspecific variation in LPAI prevalence: ecological predictors*

We tested whether our predictor variables can explain variation among species in LS prevalence. When using raw species data migration distance, water salinity and surface feeding each predicted 13–16% of the variance in LS prevalence (table 1). This explanatory power was manifested in the form of intermediate effect sizes in the expected directions. Given the limited data availability, these effect sizes could be estimated with broad CI. However, the available data for 30 species showed that species migrating long distances, frequently feeding on fresh waters or applying surface-feeding foraging methods have higher LPAI prevalence than short distance migrants or species exploiting more saline or non-surface waters (figure 1). On the other hand, these data do not exclude that migration propensity, water habitat use, body mass and latitudes could have consequences for LPAI prevalence (table 1).

There is vast heterogeneity in data quality, as information on prevalence for different species originates from different studies relying on very different sample sizes. To accommodate this problem, we also tested our

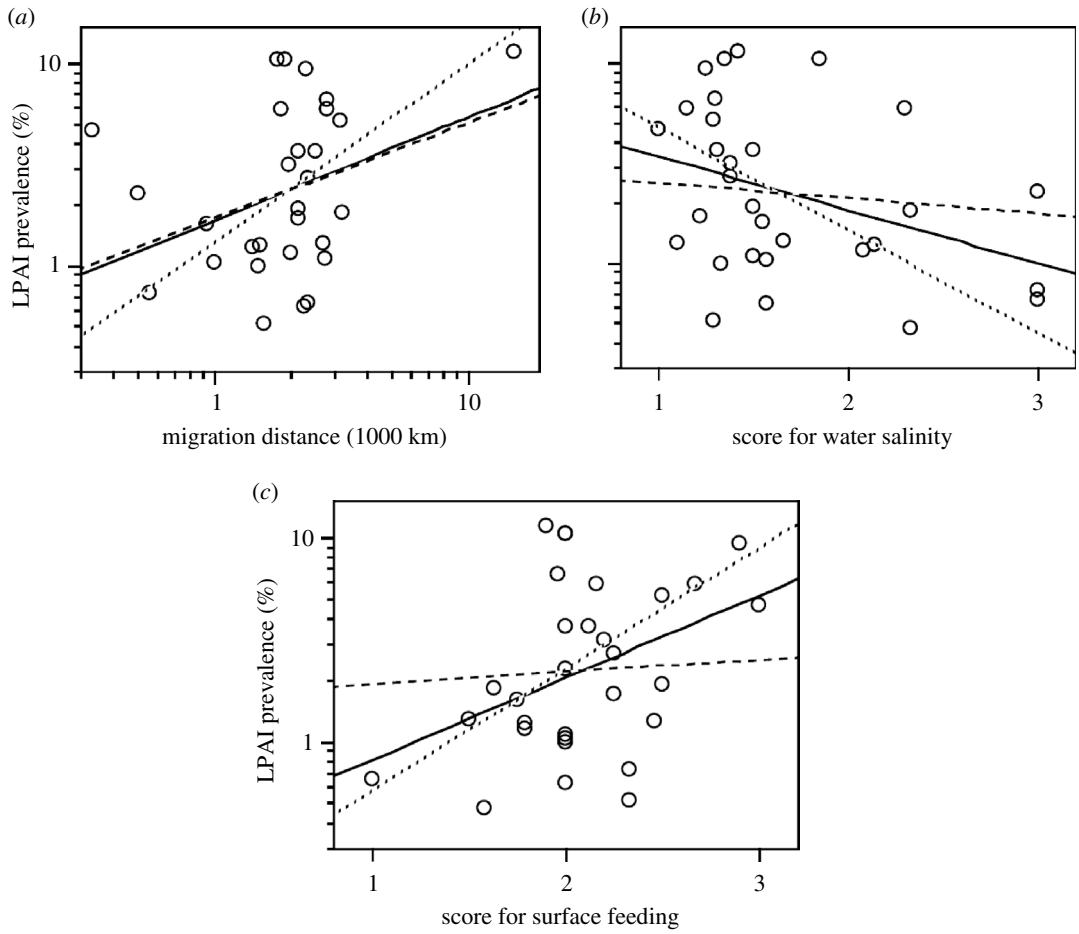

Figure 1. The relationship between the prevalence of low pathogenic avian influenza (LPAI) virus and host ecology. Open circles are species-specific data. Solid lines show the regression lines that were calculated without considering phylogenetic relationships and weights. Dotted lines represent the regression lines laid on the raw species data when using sample size as a weight. Dashed lines are the non-weighted, phylogenetically corrected regression lines as calculated from the program CONTINUOUS. (*a*) Migration distance (1000 km), (*b*) surface feeding and (*c*) water salinity. For the associated statistics, see table 1.

predictions by weighting each species in our analyses based on the number of birds that were sampled for LPAI prevalence in the given species. This approach yielded stronger effects for migration distance, water salinity and surface feeding (table 1 and figure 1). In addition, the use of weights also modified effect sizes and conclusions in association with the other variables. This change in some cases (e.g. migration propensity) was inconsistent with directions found on the non-weighted data.

Moreover, any apparent correlation between variables may be caused by similarity in phenotype, as closely related species are more likely to be similar in terms of prevalence and ecology. We found no evidence for phylogenetic inertia in LS prevalence ($\lambda=0.163$ versus $\lambda=0.000$, $p=0.394$), but other studies suggested differences in prevalence between different taxonomic groups, as ducks may have higher prevalence than gulls (Clark & Hall 2006). Moreover, there was a significant support for water salinity and surface feeding being concentrated in specific avian taxa (water salinity: $\lambda=1.000$ versus $\lambda=0.000$, $p<0.001$; surface feeding: $\lambda=1.000$ versus $\lambda=0.000$, $p<0.001$). We thus assessed the relationships between variables in a phylogenetically controlled context, which revealed that phylogeny can play an important role in the correlated evolution of certain traits (see table 1 for λs). The phylogenetic correlations showed effects similar to correlations for the species-specific data for migratory behaviour, but the effect sizes for water salinity and surface feeding weakened considerably (table 1 and figure 1).

The effects of phylogeny and the use of weights for these two variables seem to be strong and act in opposite directions (table 1). When modelling LPAI prevalence as a function of surface feeding by adjusting for the combined matrix of phylogeny and weights, we achieved higher maximum likelihood (ML$=-14.49$) than by considering the phylogeny (ML$=-19.40$) or the weight (ML$=-23.06$) matrix only. This suggests that these effects should be simultaneously important when assessing the relationship between surface feeding and LPAI prevalence. However, when these effects were combined, the slope of the focal relationship was similar to what we observed when adjusting for phylogenetic effects alone (phylogeny and weights combined: slope$\pm$s.e.$=0.155\pm0.173$, $p=0.378$; only phylogeny: slope$\pm$s.e.$=0.135\pm0.219$, $p=0.542$; only weights: slope$\pm$s.e.$=0.597\pm0.196$, $p=0.005$; see also table 1 and figure 1). A similar exercise for water salinity revealed that adjustment for the combination of phylogeny and weights offered the best fit to the data, but even in the best model, the explanatory power of this variable remained weak (phylogeny and weights combined: ML$=-14.23$, slope$\pm$s.e.$=-0.153\pm0.127$, $p=0.238$; only phylogeny: ML$=-18.99$, slope$\pm$s.e.$=-0.175\pm0.161$, $p=0.287$; only weights: ML$=-18.38$, slope$\pm$s.e.$=-0.511\pm0.107$, $p<0.001$; see also table 1 and figure 1).

Table 2. Results of a multiple regression model on LPAI prevalence based on the raw species data and weighted by sample size. We did not proceed with model simplification by stepwise deletion procedures, as recommended by Whittingham *et al.* (2006). However, we derived AIC values for each trait by excluding it from the full model and calculating the AIC of the remaining model. To avoid multicollinearity, we excluded breeding latitude that strongly correlated with winter latitude; $r=0.806$, $N=30$, $p<0.001$.

dependent variable	F	d.f.	p	AIC
full model	5.122	7,19	0.002	129.24
migration distance	5.338	1,19	0.032	133.93
migration propensity	1.810	1,19	0.194	129.70
water habitat use	2.915	1,19	0.104	131.09
water salinity	0.008	1,19	0.929	127.25
surface feeding	6.750	1,19	0.018	135.45
body mass	0.565	1,19	0.462	128.03
winter latitude	0.187	1,19	0.670	127.51

Finally, we assessed covariation between traits, as the predictive power of correlating variables can be mediated by a single biological phenomenon. For example, there was a tendency for a correlation between water salinity and surface feeding (species-specific data: $r=-0.469$, CI$=-0.709/-0.131$, $N=30$, $p=0.009$, phylogenetic correlation at $\lambda=1$: $r=-0.321$, CI$=-0.611/0.044$, $N=30$, $p=0.071$), indicating that typical surface feeders prefer exploiting fresh waters. When we controlled for the correlation between predictor variables in a multiple regression model using the raw species data and weights, we found that migration distance and surface feeding independently explained some of the interspecific variances in LS prevalence (table 2). On the other hand, this model revealed no effect on water salinity, which was thus likely to be covered by surface feeding. A phylogenetic model involving migration distance, water salinity and surface feeding confirmed the independent effect of migration distance, but revealed weak effects for aquatic feeding ecology as found previously in a phylogenetic context (partial correlations at $\lambda=1$: migration distance, $r=0.441$, CI$=0.096/0.691$, $N=30$, $p=0.019$; water salinity, $r=0.041$, CI$=-0.324/0.395$, $N=30$, $p=0.836$; surface feeding, $r=0.257$, CI$=-0.114/0.565$, $N=30$, $p=0.187$).

4. DISCUSSION

The main aim of this study was to statistically identify the most important predictors of differences in LPAI prevalence among wild species. Before addressing such interspecific questions, the remarkable variation in LPAI prevalence within species (e.g. Okazaki *et al.* 2000; Hanson *et al.* 2003; Munster *et al.* 2005) warrants discussion. Intraspecific variation may occur for biological reasons, e.g. within-year fluctuations or population differences, or be artificially caused by inconsistent surveillance approaches across studies (Olsen *et al.* 2006). Here, we showed that virus prevalence, even for the same species, varies across continents. Prevalence was generally found to be the lowest in Asia and the highest in North America, for which there may be three explanations. First, this bias may be caused by differences in study efforts or data reporting practice applied by surveys from different continents. Second, continental differences in life history or behaviour of hosts (e.g. Martin & Clobert 1996; Böhning-Gaese *et al.* 2000; Garamszegi & Lucas 2005) can mediate similar differences in susceptibility to LPAI. Third, Liu *et al.* (2005) showed that avian influenza virus in Asia can become highly virulent in wild birds. Therefore, different parasite strains may be present in different continents that cause host mortality rates to vary in a continent-specific way, which in turn would cause prevalence to be detected in *surviving* animals at lower rates in regions with virulent strains (Rappole & Hubálek 2006).

In any case, our analyses revealed significant species effects when sampling locality and sampling effort were controlled. This indicated that different studies applying different samplings provide similar interspecific patterns, because they estimate the same species-specific attributes with certain errors. In addition, if multiple records cover within-year variation in prevalence, within-species variation caused by timing effects seems negligible in a between-species context. We showed that even if prevalence varies during the annual cycle, this should occur within certain species-specific limits, as lower rates still reflect maximum rates. Therefore, our statistical evidence indicates that the prevalence of LPAI virus in wild birds is a species-specific attribute. This is important, because species as a whole are subject to higher or lower LPAI prevalence due to their species-specific biology. In addition, we also showed a negative correlation between LS prevalence and the relative size of the bursa of Fabricius measured in young birds. This pattern suggests that species with less efficient immune systems in juveniles are more likely to harbour LPAI virus than species with more efficient immune defence, and that higher virus prevalence is a result of the higher susceptibility of the host. Therefore, understanding the link between interspecific differences in virus prevalence and the distribution, ecology and life history of susceptible host species can have ultimate implications for virus epidemiology.

Migration has been proposed to incur considerable cost in terms of increased parasitism in general (Møller & Erritzøe 1998; Zeller & Murgue 2001; Alerstam *et al.* 2003; Hubálek 2004; Perez-Tris & Bensch 2005), and elevated LPAI prevalence in particular (Slemons *et al.* 2003; Krauss *et al.* 2004; Tracey *et al.* 2004; Brown *et al.* 2006; Gilbert *et al.* 2006; Rappole & Hubálek 2006). Here, we found consistent patterns for migration distance, as it was positively related to LS prevalence of LPAI virus. These conclusions were robust to different approaches, because when we balanced data quality or controlled for common descent, we arrived at very similar results (figure 1*a*). In addition, when we controlled for covariance among predictor variables, and thus held constant the effect of risk factors in association with feeding ecology and latitude and the effect of body size reflecting metabolic needs, the independent effect of migration distance was still robust (table 2). This implies that the relationship between migration distance and virus prevalence seems to be caused by frequent stopovers and/or interactions with other species on long migratory routes. On the other hand, migration propensity showed weaker effects that varied inconsistently among different approaches. Perhaps, this is because surveillance studies focused on migratory populations even if these were small, and larger non-migratory populations remained underrepresented in the available data. Alternatively, resident populations may also be

infected if they are in contact with other migrating species harbouring the virus (Slemons *et al.* 2003). In any case, the probability of migration *per se* does not seem to be a strong predictor of LPAI prevalence.

Aquatic birds are hypothesized to be primordial targets of LPAI owing to the water-related survival and transmission of the virus (Webster *et al.* 1978, 1992). We tested this hypothesis by examining the interspecific relationship between LPAI prevalence and preference for aquatic environments, water salinity and foraging method. Field studies have shown that the LPAI virus remains viable in ponds even after freezing over winter and can act as a source of reinfection in waterfowl (Ito *et al.* 1995; Kida 1997). In particular, the virus can persist longer and remain infectious in cooler temperatures and at lower pH and salt concentrations (Stallknecht *et al.* 1990*a*,*b*). Hence, the increased survival and infectivity of the virus may cause birds inhabiting freshwater wetlands to have higher prevalence. We were unable to find strong support for the hypothesis that occupation of aquatic environments alone led to higher risk of infection, although this may be due to our difficulty in precisely estimating water habitat use as shown by its low repeatability. We detected intermediate-to-strong effects for water salinity when analyses were based on raw species data. This effect seems to indicate that species from marine waters suffer less from lower LPAI prevalence than species from fresh waters (figure 1*b*), implying that virus survival can play a role in mediating interspecific patterns of LPAI. However, such a conclusion should be made with caution. First, there may be a 'third variable effect', as a correlation with another factor may be responsible for the observed patterns. For example, there was an apparent interaction between surface feeding and water salinity, as species exploiting surface waters are more likely to inhabit fresh waters. When we controlled for this relationship, the predictive power of water salinity dramatically decreased (table 2). Second, the use of saline habitats is not randomly distributed on the phylogenetic tree. For example, ducks and geese generally have lower scores (mean $\pm$ s.e. $= 1.43 \pm 0.07$) than shorebirds and gulls (mean $\pm$ s.e. $= 2.12 \pm 0.20$). If LPAI prevalence also scales with phylogeny similarly, this may mediate a relationship between water salinity and prevalence as a phylogenetic artefact. Our phylogenetic modelling revealed some roles for such phylogenetic inertia (figure 1*b* and table 1). Accordingly, we conclude that salty and aquatic environments do not necessarily result in high LPAI prevalence. These factors may comprise physiological constraints for parasite survival, but they may have little effect on transmission rates.

Virus transmission may be more directly enhanced by frequent contacts with water surfaces, as the faecal–oral route is the most efficient way of virus spread (Webster *et al.* 1978, 1992). Corroborating this hypothesis, surface feeding was an important predictor of virus prevalence (table 1 and figure 1*c*), even when other variables reflecting the quality of the aquatic environment were held constant (table 2). Hence, in species that exploit aquatic habitats at a similar degree and under the same salt and temperature conditions, those that frequently adopt surface-feeding foraging methods will have higher virus prevalence. However, this relationship weakened when we controlled for similarity among taxa due to their phylogenetic relationships. The pattern appears to be partially mediated by the fact that surface-feeding dabbling ducks usually have high prevalence. Therefore, we cannot exclude the possibility that other attributes of this avian taxon (e.g. flocking or sexual behaviours) also favour virus transmission and thus high prevalence independently of surface feeding. However, although the relationship between surface feeding and LS prevalence is less pronounced in a phylogenetically controlled context, the associated effect size is still larger than 0.25 when water salinity is controlled, and it appeared to be even higher when we simultaneously controlled for differences in a study effort as reflected by the number of animals sampled. Based on our results relying on a limited sample size, it would be misleading to reject the hypothesis that predicts a link between the ecology of aquatic feeding and LPAI prevalence, although this relationship may not be as strong as appears from simple observations.

Identifying critical vulnerabilities is important in risk management. Those bird species that rely on surface waters when foraging or fly long distances during migration and thus have contact with other species should be of major concern in the management of avian influenza. However, heterogeneity in data quality and phylogenetic associations among species should be considered when we intend to understand the relationship between host ecology and virus prevalence. The identified ecological factors suggest that virus transmission may play a key role in shaping interspecific patterns of LPAI prevalence in the wild.

We are highly indebted to Rob Freckleton for his help in the GLS modelling. Two anonymous referees, M. Eens, G. Hegyi and T. Mousseau provided constructive criticism. During this study, L.Z.G. was supported by a grant from the FWO-Flanders (Belgium).

REFERENCES

Alerstam, T., Hedenström, A. & Åkesson, S. 2003 Long-distance migration: evolution and determinants. *Oikos* **103**, 247–260. (doi:10.1034/j.1600-0706.2003.12559.x)

Alexander, D. J. 2000 A review of avian influenza in different bird species. *Vet. Microbiol.* **74**, 3–13. (doi:10.1016/S0378-1135(00)00160-7)

Alfonso, C. P., Cowen, B. S. & Vancampen, H. 1995 Influenza-A viruses isolated from waterfowl in 2 wildlife management areas of Pennsylvania. *J. Wildl. Dis.* **31**, 179–185.

Aubin, J. T. *et al.* 2005 Evolution of H5N1 avian influenza viruses in Asia. *Emerg. Infect. Dis.* **11**, 1515–1521.

Bennett, P. M. & Owens, I. P. F. 2002 *Evolutionary ecology of birds*. Oxford, UK: Oxford University Press.

Böhning-Gaese, K., Halbe, B., Lemoine, N. & Oberrath, R. 2000 Factors influencing the dutch size, number of broods and annual fecundity of North American and European land birds. *Evol. Ecol. Res.* **2**, 823–839.

Brown, J. D., Stallknecht, D. E., Beck, J. R., Suarez, D. L. & Swayne, D. E. 2006 Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. *Emerg. Infect. Dis.* **12**, 1663–1670.

Capua, I. & Alexander, D. J. 2004 Avian influenza: recent developments. *Avian Pathol.* **33**, 393–404. (doi:10.1080/03079450410001724085)

Capua, I., Mutinelli, F., Bozza, M. A., Terregino, C. & Cattoli, G. 2000 Highly pathogenic avian influenza (H7N1) in ostriches (*Struthio camelus*). *Avian Pathol.* **29**, 643–646. (doi:10.1080/03079450020016913)

Clark, L. & Hall, J. 2006 Avian influenza in wild birds: status as reservoirs, and risks to humans and agriculture. *Ornithol. Monogr.* **60**, 3–29. (doi:10.1642/0078-6594(2006)60[3:AIIWBS]2.0.CO;2)

Clement, P., Harris, A. & Davis, J. 1993 *Finches and sparrows*. London, UK: Helm.

Cohen, J. 1988 *Statistical power analysis for the behavioural sciences*. Hillsdale, MI: Lawrence Erlbaum Associates.

Cramp, S. & Perrins, C. M. (eds) 1985–1994 *The birds of the Western Palearctic*. Oxford, UK: Oxford University Press.

Dementiev, G. P. & Gladkov, N. A. (eds) 1967 *Birds of the Soviet Union*. Jerusalem, Israel: Israel Program for Scientific Translations.

Dunning, J. J. B. 1993 *Handbook of avian body masses*. Ann Arbor, MI: CRC Press.

Ekman, J. & Ericson, P. G. P. 2006 Out of Gondwanaland; the evolutionary history of cooperative breeding and social behaviour among crows, magpies, jays and allies. *Proc. R. Soc. B* **273**, 1117–1125. (doi:10.1098/rspb.2005.3431)

Figuerola, J. & Green, A. J. 2006 A comparative study of egg mass and clutch size in the Anseriformes. *J. Ornithol.* **147**, 57–68. (doi:10.1007/s10336-005-0017-5)

Fouchier, R. A. M., Munster, V., Wallensten, A., Bestebroer, T. M., Herfst, S., Smith, D., Rimmelzwaan, G. F., Olsen, B. & Osterhaus, A. D. M. E. 2005 Characterization of a novel influenza a virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J. Virol.* **79**, 2814–2822. (doi:10.1128/JVI.79.5.2814-2822.2005)

Freckleton, R. P., Harvey, P. H. & Pagel, M. 2002 Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**, 712–726. (doi:10.1086/343873)

Garamszegi, L. Z. 2006*a* Comparing effect sizes across variables: generalization without the need for Bonferroni correction. *Behav. Ecol.* **17**, 682–687. (doi:10.1093/beheco/ark005)

Garamszegi, L. Z. 2006*b* The evolution of virulence and host specialization in malaria parasites of primates. *Ecol. Lett.* **9**, 933–940. (doi:10.1111/j.1461-0248.2006.00936.x)

Garamszegi, L. Z. & Lucas, J. R. 2005 Continental variation in relative hippocampal volume in birds: the phylogenetic extent of the effect and the potential role of winter temperatures. *Biol. Lett.* **1**, 330–333. (doi:10.1098/rsbl.2005.0328)

Garamszegi, L. Z., Erritzøe, J. & Møller, A. P. 2007 Feeding innovations and parasitism in birds. *Biol. J. Linn. Soc.* **90**, 441–455. (doi:10.1111/j.1095-8312.2007.00733.x)

Gilbert, M., Xiao, X. M., Domenech, J., Lubroth, J., Martin, V. & Slingenbergh, J. 2006 Anatidae migration in the western palearctic and spread of highly pathogenic avian influenza H5N1 virus. *Emerg. Infect. Dis.* **12**, 1650–1656.

Glick, B. 1983 Bursa of Fabricius. In *Avian biology* (eds D. S. Farner & J. R. King), pp. 443–500. New York, NY: Academic Press.

Glick, B. 1994 The bursa of Fabricius: the evolution of a discovery. *Poult. Sci.* **73**, 979–983.

Hall, K. S. S. & Tullberg, B. S. 2004 Phylogenetic analyses of the diversity of moult strategies in Sylviidae in relation to migration. *Evol. Ecol.* **18**, 85–105. (doi:10.1023/B:EVEC.0000017848.20735.8b)

Hanson, B. A., Stallknecht, D. E., Swayne, D. E., Lewis, L. A. & Senne, D. A. 2003 Avian influenza viruses in Minnesota ducks during 1998–2000. *Avian Dis.* **47**, 867–871.

Harvey, P. H. & Pagel, M. D. 1991 *The comparative method in evolutionary biology*. Oxford, UK: Oxford University Press.

Higgins, P. J. & Davies, S. J. J. F. (eds) 1996 *Handbook of Australian, New Zealand and Antarctic birds*. Melbourne, Australia: Oxford University Press.

Hinshaw, V. S. & Webster, R. G. 1982 The natural history of influenza A viruses. In *Basic and applied influenza research* (ed. A. S. Beare), pp. 79–104. Boca Raton, FL: CRC Press.

Hubálek, Z. 2004 An annotated checklist of pathogenic microorganisms associated with migratory birds. *J. Wildl. Dis.* **40**, 639–659.

Hurt, A. C., Hansbro, P. M., Selleck, P., Olsen, B., Minton, C., Hampson, A. W. & Barr, I. G. 2006 Isolation of avian influenza viruses from two different transhemispheric migratory shorebird species in Australia. *Arch. Virol.* **151**, 2301–2309. (doi:10.1007/s00705-006-0784-1)

Ito, T., Okazaki, K., Kawaoka, Y., Takada, A., Webster, R. G. & Kida, H. 1995 Perpetuation of influenza-A viruses in Alaskan waterfowl reservoirs. *Arch. Virol.* **140**, 1163–1172. (doi:10.1007/BF01322743)

Iwaniuk, A. N. & Nelson, J. E. 2003 Developmental differences are correlated with relative brain size in birds: a comparative analysis. *Can. J. Zool.-Revue Canadienne De Zoologie* **81**, 1913–1928. (doi:10.1139/z03-190)

John, J. 1994 The avian spleen: a neglected organ. *Q. Rev. Biol.* **69**, 327–351. (doi:10.1086/418649)

Jørgensen, P. H., Nielsen, O. L., Hansen, C., Manvell, R. J., Banks, J. & Alexander, D. J. 1998 Isolation of influenza A virus, subtype H5N2, and avian paramyxovirus type 1 from a flock of ostriches in Europe. *Avian Pathol.* **27**, 15–20.

Kida, H. 1997 Ecology of influenza viruses in animals and the mechanisms of emergence of new pandemic strains. *Nippon Rinsho* **10**, 2521–2526.

Krauss, S., Walker, D., Pryor, S. P., Niles, L., Li, C. H., Hinshaw, V. S. & Webster, R. G. 2004 Influenza A viruses of migrating wild aquatic birds in North America. *Vector-Borne Zoonot. Dis.* **4**, 177–189. (doi:10.1089/vbz.2004.4.177)

Liu, J. H., Okazaki, K., Bai, G. R., Shi, W. M., Mweene, A. & Kida, H. 2004 Interregional transmission of the internal protein genes of H2 influenza virus in migratory ducks from north America to Eurasia. *Virus Genes* **29**, 81–86. (doi:10.1023/B:VIRU.0000032791.26573.f1)

Liu, J. *et al.* 2005 Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science* **309**, 1206–1206. (doi:10.1126/science.1115273)

Lu, H., Castro, A. E., Pennick, K., Liu, J., Yang, Q., Dunn, P., Weinstock, D. & Henzler, D. 2003 Survival of avian influenza virus H7N2 in SPF chickens and their environments. *Avian Dis.* **47**, 1015–1021. (doi:10.1637/0005-2086(2003)047[0361:ALSOAN]2.0.CO;2)

Lucas, J. R., Brodin, A., de Kort, S. R. & Clayton, N. S. 2004 Does hippocampal size correlate with the degree of caching specialization? *Proc. R. Soc. B* **271**, 2423–2429. (doi:10.1098/rspb.2004.2912)

MacKinnon, J. & Phillipps, K. 1993 *A field guide to the birds of Borneo, Sumatra, Java, and Bali*. Oxford, UK: Oxford University Press.

MacLean, G. L. 1988 *Roberts' birds of South Africa*. London, UK: New Holland.

Marchant, S. & Higgins, P. J. (eds) 1990–1993 *Handbook of Australian, New Zealand and Antarctic birds*. Melbourne, Australia: Oxford University Press.

Martin, T. E. & Clobert, J. 1996 Nest predation and avian life-history evolution in Europe versus North America: a possible role of humans? *Am. Nat.* **147**, 1028–1046. (doi:10.1086/285891)

Martins, E. P. & Hansen, T. F. 1997 Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**, 646–667. (doi:10.1086/286013)

Meiri, S. & Yom-Tov, Y. 2004 Ontogeny of large birds: migrants do it faster. *Condor* **106**, 540–548. (doi:10.1650/7506)

Moran, M. D. 2003 Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **102**, 403–405. (doi:10.1034/j.1600-0706.2003.12010.x)

Mougeot, F. S. 2004 Breeding density, cuckoldry risk and copulation behaviour during the fertile period in raptors: a comparative analysis. *Anim. Behav.* **67**, 1067–1076. (doi:10.1016/j.anbehav.2003.10.011)

Møller, A. P. & Erritzøe, J. 1996 Parasite virulence and host immune defense: host immune response is related to nest reuse in birds. *Evolution* **50**, 2066–2072. (doi:10.2307/2410763)

Møller, A. P. & Erritzøe, J. 1998 Host immune defence and migration in birds. *Evol. Ecol.* **12**, 945–953. (doi:10.1023/A:1006516222343)

Møller, A. P., Erritzøe, J. & Garamszegi, L. Z. 2005 Covariation between brain size and immunity in birds: implications for brain size evolution. *J. Evol. Biol.* **18**, 223–237. (doi:10.1111/j.1420-9101.2004.00805.x)

Muñoz, M. J., Sanchez-Vizcaino, J. M. & Peris, S. 2006 Short communication. Can highly pathogenic avian influenza (HPAI) reach the Iberian Peninsula from Asia by means of migratory birds? *Spanish J. Agr. Res.* **4**, 140–145.

Munster, V. J., Wallensten, A., Baas, C., Rimmelzwaan, G. F., Schutten, M., Olsen, B., Osterhaus, A. & Fouchier, R. A. M. 2005 Mallards and highly pathogenic avian influenza ancestral viruses, northern Europe. *Emerg. Infect. Dis.* **11**, 1545–1551.

Nagy, K. A. 1987 Field metabolic rate and food requirement scaling in mammals and birds. *Ecol. Monogr.* **57**, 111–128. (doi:10.2307/1942620)

Nakagawa, S. 2004 A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav. Ecol.* **15**, 1044–1045. (doi:10.1093/beheco/arh107)

Okazaki, K. *et al.* 2000 Precursor genes of future pandemic influenza viruses are perpetuated in ducks nesting in Siberia. *Arch. Virol.* **145**, 885–893. (doi:10.1007/s007050050681)

Olsen, B., Munster, V. J., Wallensten, A., Waldenström, J., Osterhaus, A. D. M. E. & Fouchier, R. A. M. 2006 Global patterns of influenza A virus in wild birds. *Science* **312**, 384–388. (doi:10.1126/science.1122438)

Pagel, M. 1997 Inferring evolutionary processes from phylogenies. *Zool. Script.* **26**, 331–348. (doi:10.1111/j.1463-6409.1997.tb00423.x)

Pagel, M. 1999 Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884. (doi:10.1038/44766)

Parmentier, H. K., Kreukniet, M. B., Goeree, B., Davison, T. F., Jeurissen, S. H. M., Harmsen, E. G. M. & Nieuwland, M. G. B. 1995 Differences in distribution of lymphocyte antigens in chicken lines divergently selected for antibody responses to sheep red blood cells. *Vet. Immunol. Immunopathol.* **48**, 155–168. (doi:10.1016/0165-2427(94)05411-K)

Perez-Tris, J. & Bensch, S. 2005 Dispersal increases local transmission of avian malarial parasites. *Ecol. Lett.* **8**, 838–845. (doi:10.1111/j.1461-0248.2005.00788.x)

Perneger, T. V. 1998 What's wrong with Bonferroni adjustments. *Br. Med. J.* **316**, 1236–1238.

Poole, A., Stettenheim, P. & Gill, F. (eds) 1993–2002 *The birds of North America*. Philadelphia, PA: The American Ornitologists' Union and The Academy of Natural Sciences of Philadelphia.

Rappole, J. H. & Hubálek, Z. 2006 Birds and influenza H5N1 virus movement to and within North America. *Emerg. Infect. Dis.* **12**, 1486–1492.

Rolland, C., Danchin, E. & de Fraipont, M. 1998 The evolution of coloniality in birds in relation to food, habitat, predation, and life-history traits: a comparative analysis. *Am. Nat.* **151**, 514–529. (doi:10.1086/286137)

Rose, M. E. 1981 Lymphatic system. In *Form and function in birds*, vol. 2 (eds A. S. King & J. McLelland), pp. 341–384. London, UK: Academic Press.

Scheuerlein, A. & Ricklefs, R. E. 2004 Prevalence of blood parasites in European passeriform birds. *Proc. R. Soc. B* **271**, 1363–1370. (doi:10.1098/rspb.2004.2726)

Shortridge, K. F. *et al.* 1998 Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. *Virology* **252**, 331–342. (doi:10.1006/viro.1998.9488)

Slemons, R. D., Hansen, W. R., Converse, K. A. & Senne, D. A. 2003 Type A influenza virus surveillance in free-flying, nonmigratory ducks residing on the eastern shore of Maryland. *Avian Dis.* **47**, 1107–1110.

Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*. New York, NY: W. H. Freeman & Co.

Spackman, E., Senne, D. A., Davison, S. & Suarez, D. L. 2003 Sequence analysis of recent H7 avian influenza viruses associated with three different outbreaks in commercial poultry in the United States. *J. Virol.* **77**, 13 399–13 402. (doi:10.1128/JVI.77.24.13399-13402.2003)

Stallknecht, D. E. 1998 Ecology and epidemiology of avian influenza viruses in wild populations. In *Fourth Int. Symp. Avian Influenza* (eds D. E. Swayne & R. D. Slemons), pp. 61–69. Athens, GA: Animal Health Association.

Stallknecht, D. E. & Shane, S. M. 1988 Host range of avian influenza-virus in free-living birds. *Vet. Res. Commun.* **12**, 125–141. (doi:10.1007/BF00362792)

Stallknecht, D. E., Kearney, M. T., Shane, S. M. & Zwank, P. J. 1990*a* Effects of PH, temperature, and salinity on persistence of avian influenza-viruses in water. *Avian Dis.* **34**, 412–418. (doi:10.2307/1591429)

Stallknecht, D. E., Shane, S. M., Kearney, M. T. & Zwank, P. J. 1990*b* Persistence of avian influenza-viruses in water. *Avian Dis.* **34**, 406–411. (doi:10.2307/1591428)

Sturm-Ramirez, K. M. *et al.* 2005 Are ducks contributing to the endemicity of highly pathogenic H5N1 influenza virus in Asia? *J. Virol.* **79**, 11 269–11 279. (doi:10.1128/JVI.79.17.11269-11279.2005)

Suarez, D. L. 2000 Evolution of avian influenza viruses. *Vet. Microbiol.* **74**, 15–27. (doi:10.1016/S0378-1135(00)00161-9)

Tieleman, B. I. & Williams, J. B. 2000 The adjustment of avian metabolic rates and water fluxes to desert environments. *Physiol. Biochem. Zool.* **73**, 461–479. (doi:10.1086/317740)

Toivanen, P. & Toivanen, A. 1987 *Avian immunology: basis and practice*. Boca Raton, FL: CRC Press.

Tracey, J. P., Woods, R., Roshier, D., West, P. & Saunders, G. R. 2004 The role of wild birds in the transmission of avian influenza for Australia: an ecological perspective. *Emu* **104**, 109–124. (doi:10.1071/MU04017)

Urban, E. K., Fry, C. H. & Keith, S. (eds) 1986 *The birds of Africa*. London, UK: Academic Press.

Van Vleck, L. D. 1993 *Selection index and introduction to mixed model methods*. Boca Raton, FL: CRC Press.

Webby, R. J. & Webster, R. G. 2003 Are we ready for pandemic influenza? *Science* **302**, 1519–1522. (doi:10.1126/science.1090350)

Webster, R. G., Yakhno, M., Hinshaw, V. S., Bean, W. J. & Murti, K. G. 1978 Intestinal influenza: replication and characterization of influenza viruses in ducks. *Virology* **84**, 268–278. (doi:10.1016/0042-6822(78)90247-7)

Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M. & Kawaoka, Y. 1992 Evolution and ecology of influenza-A viruses. *Microbiol. Rev.* **56**, 152–179.

Whittingham, M. J., Stephens, P. A., Bradbury, R. B. & Freckleton, R. P. 2006 Why do we still use stepwise modelling in ecology and behaviour? *J. Anim. Ecol.* **75**, 1182–1189. (doi:10.1111/j.1365-2656.2006.01141.x)

Yanes, M. & Suarez, F. 1997 Nest predation and reproductive traits in small passerines: a comparative approach. *Acta Oecol.* **18**, 413–426. (doi:10.1016/S1146-609X(97)80032-X)

Zeller, H. G. & Murgue, B. 2001 The role of migrating birds in the West Nile virus epidemiology. *Medecine Et Maladies Infectieuses* **31**, 168S–174S. (doi:10.1016/S0399-077X(01)80055-X)