# Prevalence of avian influenza and sexual selection in ducks

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Investigations of avian influenza have so far focused on the global circulation and conversion of virus strains and showed that wild waterfowl and especially ducks represent the reservoir and source of virus strains that can become highly pathogenic in domestic species. Information is largely missing regarding the routes of transmission between individuals and the species of concern for transmission. Moreover, evolutionary comparative studies only considered ecological factors and ignored other potential determinants of virus transmission. Such determinants include the mating strategies of hosts because links between sexual selection and parasites are well known. Here, we show that morphological adaptations associated with copulation frequency in both male and female hosts strongly explain differences in low-pathogenic influenza prevalence among wild duck species. Prevalence is negatively related to male phallus length and female vaginal complexity, traits that evolved due to sexual conflict over forced copulations. This pattern suggests a hitherto unrecognized transmission route of the virus via copulation and subsequent mother–offspring transfer. Due to a relationship between forced copulations and the expression of white wing covert patches, male covert patch expression and sexual dichromatism in covert patch expression are positively related to influenza prevalence. Our results suggest that the arms race between male and female reproductive tracts had epidemiological consequences. Our findings further suggest that morphological correlates of sexual selection in ducks, including conspicuous plumage ornamentation, could be robust clues to identify high-risk host species during the large-scale monitoring of avian influenza. *Key words:* forced copulation, plumage ornament, reproductive anatomy, viral transmission, waterfowl. *[Behav Ecol]*

It has been known for a long time that influenza A viruses are present in wild birds, and that waterfowl (Anatidae) are the principal hosts (Webster et al. 1992). Transfer between major taxonomic groups of hosts and the associated reassortment of genetic material may have promoted the recent emergence of virus strains that are highly pathogenic to poultry, and some of these strains have also infected humans with a high rate of fatality (Peiris et al. 2004). Waterfowl likely play important roles in this process. First, they evolve, circulate, and supply the low-pathogenic avian influenza (LPAI) strains (this means low pathogenicity to domestic poultry) that may evolve high pathogenicity in domestic birds (Boon et al. 2007). Second, although highly pathogenic avian influenza (HPAI) strains are not thought to be perpetuated in wild populations (Boyce et al. 2009), the role of wild birds in the dispersal of these strains cannot be ruled out. For example, of the introductions of HPAI to European countries, "20 of 23 introductions were most probably caused by migrating wild birds" (Artois et al. 2009). A recent review paper notes that "it seems unlikely that either wild birds or the poultry production units independently act as reservoir hosts" of HPAI (Caron et al. 2009).

Given that wild birds play important roles in the ecology and evolution of avian influenza, monitoring the disease in wild populations is a primary concern (Olsen et al. 2006). However, surveillance has severe logistic and other practical limitations (Guberti and Newman 2007), and the geographic coverage increases only slowly (Gaidet et al. 2007). It seems that "surveillance programs must be targeted and focused in order to obtain valuable information about disease ecology" (Guberti and Newman 2007). The classification of wild species as high versus low risk could improve the efficiency of surveillance efforts (Artois et al. 2009). High-risk species in the strict sense are those that can carry HPAI virus without severe symptoms. Due to the very low prevalence of HPAI in the wild (Boyce et al. 2009), this high-risk group can be identified only by targeted infection experiments conducted in the laboratory (e.g., Brown et al. 2006; Keawcharoen et al. 2008). However, these studies are seriously limited by the capacity of high biosafety level facilities. Moreover, the genetic source of HPAI strains is LPAI from wild species (Chen and Holmes 2009), and the relationship of wild birds and LPAI strains is a kind of evolutionary stasis with viruses having little effect on their hosts (Webster et al. 1992). Therefore, a second type of high-risk host group can be those species that carry LPAI at high prevalence.

Intensifying surveillance is difficult even with respect to HPAI, so the amount of comparative data on LPAI prevalence is not expected to increase rapidly (Guberti and Newman 2007). Therefore, a practical way of identifying the second group of high-risk host species is to look for relationships between existing LPAI prevalence data and various characteristics of the host species (Boyce et al. 2009). Because phenotypic characteristics of different species are not statistically independent observations and parasite susceptibility results from evolutionary processes, a comparative approach that controls for similarity in phenotype among hosts and parasites due to common phylogenetic descent is necessary to identify and understand the evolution of such risk factors. Discussions

of transmission risk have so far largely focused on ecological variables such as migration, foraging, and habitat use (e.g., Muzaffar et al. 2006; Olsen et al. 2006). It is therefore not surprising that the only evolutionary comparative study of LPAI prevalence conducted so far focused on ecological predictors (Garamszegi and Møller 2007), whereas other potential risk factors remain unexplored. Among others, these factors include "mating behavior" (Spackman 2009), or in a broader sense, sexual selection.

Sexual selection has intimate proximate (Spencer et al. 2005) and evolutionary links to parasites (Møller et al. 1999). For example, mating strategies may increase contact rates among individuals (McKinney et al. 1983; Thrall et al. 2000) and may result in parasite transfer during copulation between host individuals (Lombardo 1998; Kulkarni and Heeb 2007). However, aspects of sexual selection in hosts may also coevolve with parasite defense mechanisms (Poiani 2002; Garamszegi et al. 2003). Ducks (Anatini), the main hosts of avian influenza in the wild (Webster et al. 1992), are also known for their variable mating strategies. These birds are predominately monogamous, but the frequency of mate change differs among species (Scott and Clutton-Brock 1989). Moreover, they have diverse copulation strategies that also include forced extrapair copulations (FEPCs): a behavior rarely found in other birds (McKinney et al. 1983).

Ducks mainly copulate in water (McKinney et al. 1983), and water is regarded as an important medium of influenza transmission (Ito et al. 1995; Lang et al. 2008). The HPAI strains have been isolated from the eggshell of ducks (Li et al. 2006) and from egg contents and oviduct in Japanese quail (Promkuntod et al. 2006). The LPAI strains have not yet been confirmed from egg contents (Spickler et al. 2008), but they are known to cause lesions in the reproductive tract (Hooper and Selleck 1998), so internal inoculation of the eggs is possible. Moreover, LPAI strains are principally shed through the cloaca (Chon et al. 2008; Ellström et al. 2008) so they are almost certainly smeared on the egg surface at laying. The combination of copulation in contaminated water and shedding through the intestinal and reproductive tracts raises the possibility of viral transmission to offspring through the egg. It therefore seems logical to assume that variation in mating strategies of hosts contributes to differences in susceptibility to influenza among duck species (Kulkarni and Heeb 2007).

Here, we use comparative data from ducks to investigate the prevalence of LPAI in relation to different aspects of sexual selection in these well-known hosts of the virus. We primarily examine reproductive strategies related to copulation (Kulkarni and Heeb 2007). We use residual testis size (corrected for body mass) as an indicator of general copulation rates (positive correlation; Møller and Briskie 1995; Pitcher et al. 2005) and phallus length as an index of FEPC rates (positive correlation; Coker et al. 2002). We also examine the morphological adaptations of females related to forced copulation (Brennan et al. 2007) because such morphological changes may influence the efficiency of sexually mediated virus transmission (Briskie and Montgomerie 1997). We assess white wing ornamentation as a conspicuous attribute associated with copulation behavior (Hegyi et al. 2008) and immune defense in ducks (Hanssen et al. 2006, 2008), which could serve as a useful indicator of transmission risk among species. Finally, we analyze social mating frequency (Scott and Clutton-Brock 1989) as a "control" trait, that is, an aspect of sexual selection less expected to correlate with LPAI transmission than copulation rate or the related traits. In ducks, social mate change is a relatively rare event and therefore involves fewer individuals per unit time compared with actual copulation events (McKinney et al. 1983), so the dominant determinants of contact rates should be alternative mating tactics rather than social mating frequency.

## METHODS

### Comparative data

Most of our data on LPAI prevalence (the proportion of sampled birds found to be positive) came from a review (Olsen et al. 2006). More species, and additional data from Africa for the existing species, were added from 2 recent large-scale studies (Gaidet et al. 2007; Munster et al. 2007). Because zero prevalence may occur due to an absence of sampling, low sampling effort, or true lack of infection, we excluded zero prevalence data from the analyses, which implies that our conclusions refer to the rate of infection and not the presence of infection (Garamszegi and Møller 2007). Population-level nonzero prevalence data were log transformed and averaged to get a species-specific value. A previous study (Garamszegi and Møller 2007) used prevalence data that were corrected for geographical variation. We could not use such data in the present study due to our restricted taxon sampling, but the prevalence measure of Garamszegi and Møller (2007) highly positively correlated with the simple averages we use here ($r = 0.736$, $N = 15$, $P = 0.002$). Note that, in spite of seasonal fluctuations, point estimates of prevalence are repeatable within species (Garamszegi and Møller 2007). The total number of birds sampled in all populations was also noted. For social mating system, a binary scoring was used (Scott and Clutton-Brock 1989). A score of 1 was given to species with social pair bonds longer than a year and 2 to those pairing at least once a year. Data on testis size were residuals of testes mass on body mass (Pitcher et al. 2005). For male intromittent organ length (hereafter phallus length), we used data measured on formalin-preserved specimens (Coker et al. 2002; Brennan et al. 2007). The amount of data on vaginal length and complexity was very limited (Brennan et al. 2007). Therefore, to avoid bias due to single data points, we calculated a composite index by obtaining the first principal component of vagina length, number of spirals, and number of pouches (unrotated principal components analysis conducted in Statistica 5.5, loadings $r = 0.918$, $r = 0.964$, and $r = 0.930$, respectively) and converting this to a binary measure (1 for negative and 2 for positive scores, representing low and high elaboration, respectively).

As a measure of plumage ornamentation, we focused on the size of white patches on the wing coverts. These patches are frequently sexually dichromatic and they have been suggested to correlate with social competition and forced copulation rates (Hegyi et al. 2008). Moreover, white wing patches have been found to reflect the status of the immune system in female eiders (*Somateria mollissima*) (Hanssen et al. 2006, 2008). Covert patches were scored from paintings of flying birds in a global guide (Madge and Burn 1987) as 0 if absent and 1 or 2 if less or more than half the area of coverts was white, respectively. Therefore, these scores quantify covert patch size relative to the wing surface. Covert patch size was considered a continuous variable. The scoring method has previously been validated using both other guidebooks and museum specimens (Hegyi et al. 2008). We used male scores, female scores, and binary covert patch dichromatism (0 if absent, 1 if males were more ornamented than females). The data set can be found in the online Supplementary material.

### Statistical analyses

Species-specific data were analyzed by using mean log-transformed LPAI prevalence as dependent variable in univariate linear regressions (continuous predictors) or Student's *t*-tests (binary predictors). To investigate the magnitude of bias introduced by the huge variation of sampling effort for virus among species, we also made weighted analyses, using

the log-transformed total number of sampled individuals as weight factor (Garamszegi and Møller 2007). Finally, to take the nonindependence of species-specific data into account, we also assessed the relationships using generalized least squares methods on unweighted data, as implemented in the program Continuous (Pagel 1999). We used a trimmed version of a recently outlined phylogenetic tree of ducks that combines molecular and morphological information (Hegyi et al. 2008). The lack of sufficient information forced us to use unit branch lengths. We first investigated the role of phylogenetic inertia by estimating the phylogenetic scaling parameter lambda (λ) by means of maximum likelihood. If λ was significantly higher than zero, we calculated a correlation between the variables of interest that took into account the estimated degree of phylogenetic effect. If λ did not differ significantly from zero, we considered the data phylogenetically independent and used the species-level results as phylogenetically corrected estimates. When multiple correlated predictors were significant, we used multivariate tests to assess their relative importance. Previous analyses of a larger data set have identified migration distance and the degree of surface feeding as robust predictors of LPAI prevalence (Garamszegi and Møller 2007). Controlling for these factors using multivariate models did not change our conclusions but reduced the sample size, so we present the uncorrected results here. All statistical results were converted to effect sizes (Pearson *r*) and are presented with their 95% confidence ranges (Nakagawa and Cuthill 2007). The weighting procedure increases the degrees of freedom (df) associated with individual data points, so the error df are higher in these models.

## RESULTS

The 3 statistical approaches we applied (analysis of species-specific data, weighted regression, and unweighted, phylogenetically corrected analyses) gave remarkably similar results in all cases (see details of univariate tests in Table 1). Social mating frequency of hosts did not predict LPAI prevalence, and all effect sizes for this relationship were small (Figure 1A). Residual testis size and phallus length, on the other hand, were both negatively related to LPAI prevalence (Figure 1B,C). All effect sizes were medium or large, and 5 of the 6 were also significant. The size of white wing covert patches in males was positively related to virus prevalence with small or medium effect sizes (Figure 1D). The weighted and the phylogenetically corrected results were significant, whereas the relationship for species-specific data approached significance. All 3 methods yielded small effect sizes for the relationship of prevalence with female covert patch expression, and all effects were nonsignificant. Covert patch dichromatism, however, was positively associated with LPAI prevalence using the weighted data, and the other 2 approaches yielded positive but nonsignificant correlations with medium effect sizes (Figure 1E). Vagina elaboration was negatively related to LPAI prevalence with large effect sizes for all 3 approaches, but only the weighted relationship was significant due to small sample sizes (Figure 1F). Finally, testis size and phallus length are positively correlated (Coker et al. 2002; Hegyi et al. 2008), whereas wing patch size and phallus length are negatively correlated (Hegyi et al. 2008), so we also used multivariate models to disentangle their effects. In these models, the effect sizes of phallus length remained medium or large with 5 of the 6 tests being significant, whereas those of testis size and wing patch size considerably decreased (see Table 2 for details).

## DISCUSSION

We examined potential sexual selection correlates of LPAI prevalence in ducks, the most important hosts of avian influ-

**Table 1**

**Effect sizes and 95% confidence intervals for relationships between avian influenza prevalence and measures of sexual selection in ducks**

| Effect | Data | *r* | *N* | CI lower | CI upper | λ |
|---|---|---|---|---|---|---|
| Social mating frequency | Species | 0.087 | 28 | −0.296 | 0.446 | |
| | Weighted | 0.132 | 75 | −0.098 | 0.349 | |
| | Phylogeny | 0.141 | 28 | −0.245 | 0.488 | 0.734 |
| Residual testis size | **Species** | **−0.440** | **14** | **−0.787** | **0.118** | |
| | **Weighted** | **−0.428*** | **33** | **−0.673** | **−0.099** | |
| | **Phylogeny** | **−0.558*** | **14** | **−0.840** | **−0.040** | **0.712** |
| Phallus length | **Species** | **−0.521*** | **15** | **−0.816** | **−0.012** | |
| | **Weighted** | **−0.420*** | **34** | **−0.664** | **−0.095** | |
| | **Phylogeny** | **−0.521** | **15** | **−0.816** | **−0.012** | **0** |
| Male covert patch size | **Species** | **0.366** | **28** | **−0.008** | **0.650** | |
| | Weighted | 0.274* | 64 | 0.030 | 0.487 | |
| | **Phylogeny** | **0.434*** | **28** | **0.073** | **0.695** | **0.551** |
| Female covert patch size | Species | 0.248 | 28 | −0.138 | 0.568 | |
| | Weighted | 0.135 | 64 | −0.115 | 0.369 | |
| | Phylogeny | 0.247 | 28 | −0.139 | 0.567 | 0.812 |
| Covert patch dichromatism | Species | 0.287 | 28 | −0.096 | 0.596 | |
| | **Weighted** | **0.307**** | **75** | **0.086** | **0.499** | |
| | Phylogeny | 0.287 | 28 | −0.096 | 0.596 | 0 |
| Vagina elaboration | **Species** | **−0.601** | **7** | **−0.932** | **0.278** | |
| | **Weighted** | **−0.508*** | **21** | **−0.771** | **−0.098** | |
| | **Phylogeny** | **−0.507** | **7** | **−0.912** | **0.398** | **0.409** |

Medium or large effects (*r* > 0.3) are shown in bold. The 3 types of analyses used species-specific data (Species), data weighted by log-transformed sample size (Weighted), and phylogenetic correction (Phylogeny). CI, confidence interval. λ, phylogenetic scaling parameter. *P < 0.05; **P < 0.01.

enza in the wild (Webster et al. 1992; Olsen et al. 2006). Our results show that copulation-related morphological attributes and plumage ornamentation of hosts strongly predict susceptibility to influenza at the species level. These findings have important implications because wild duck species are key players in the evolution and propagation of low-pathogenic strains from which HPAI strains evolve (Artois et al. 2009), but the screening of wild populations is slow and demanding (Munster et al. 2007), so clues that highlight particularly likely hosts are very important at this stage (Muzaffar et al. 2006; Garamszegi and Møller 2007; Boyce et al. 2009). Moreover, our results may contribute to the explanation of the poorly understood temporal and spatial cycling of influenza prevalence in the wild (Ito et al. 1995; Olsen et al. 2006). We detail these points below.

A mechanism that may link parasite susceptibility and sexual selection in hosts is copulation rate. Here, we used residual testis size as a general measure of copulation rates (Pitcher et al. 2005). We also used the length of phallus as a specific index of FEPC rates (Coker et al. 2002). Copulation may itself transmit diseases (Kulkarni and Heeb 2007), but FEPCs in ducks may further promote transmission through the respiratory route (Sturm-Ramirez et al. 2005), by creating several additional opportunities for close contact. Males looking for FEPC opportunities often form groups that aggregate around fertile females unguarded by their mates. Even if only a single male initiates a sexual chase of a female, other males may join quickly, and the result is a group of males struggling to copulate with the female (McKinney et al. 1983). We found that social mating frequency, probably a minor determinant of viral transmission rates (Scott and Clutton-Brock 1989), did not significantly predict LPAI prevalence. However, relative testis size and phallus length both showed negative relationships with prevalence, opposite to the predictions of the
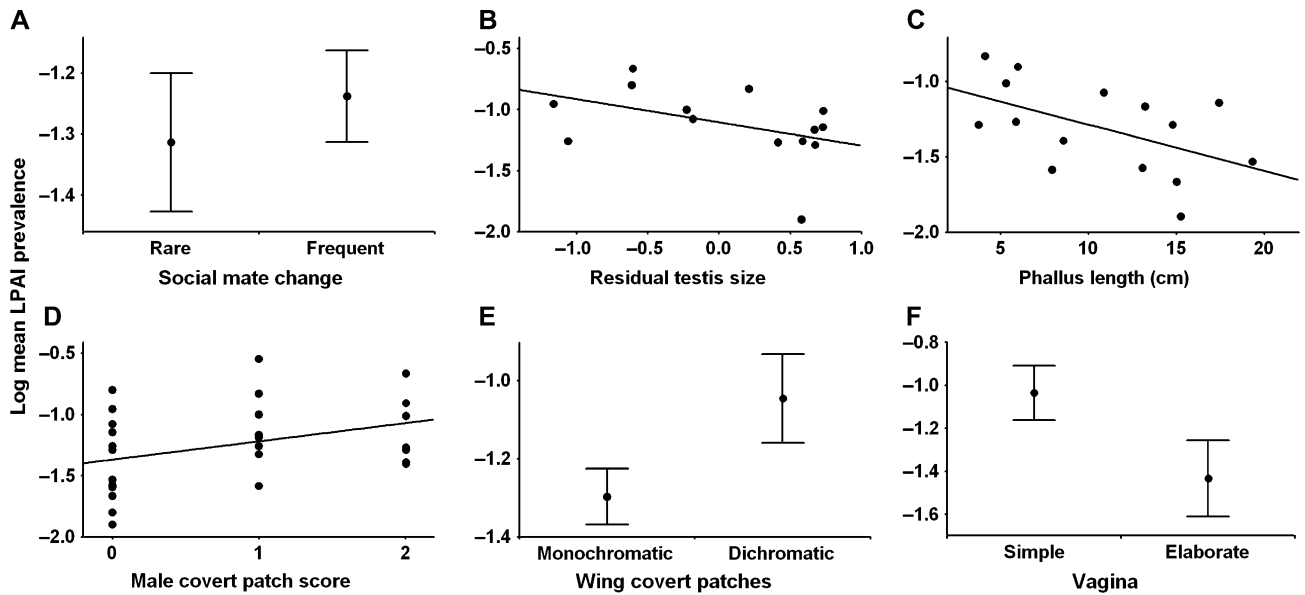
**Figure 1**
Relationships between the prevalence of LPAI and aspects of sexual selection in ducks. The variables plotted include the frequency of social mate change (A), residual testis size (B) and phallus length in males (C), white patch expression on the wing coverts of males (D), covert patch dichromatism (E), and vagina elaboration (F). Error bars denote 1 standard error.

transmission rate hypothesis. Multivariate tests with the correlated predictors of testis size and phallus length (Coker et al. 2002; Hegyi et al. 2008) entered together yielded phallus length as a dominant predictor. This suggests that the important determinant of prevalence is FEPC rate or the related morphology and not copulation rate in general.

A mechanism that may explain low parasite prevalence in species with high FEPC rates is the direct costs of forced copulations. FEPC enormously increases the stress levels of females, and it is known to lead to elevated rates of female mortality (McKinney et al. 1983). This behavior is also very costly to males that must endure continuous search for females and long chases and struggles (Sorenson 1994). If the given influenza strain is pathogenic to even a small degree (Keawcharoen et al. 2008), infected females may be those that die during FEPC attempts or whose breeding fails afterward. Infected males, on the other hand, may prove unable

to secure FEPCs because of the high costs and violent contests involved. This may lead to relatively low influenza prevalence by the time of postbreeding aggregation, the presumed principal moment of viral transfer (Hinshaw et al. 1985).

Alternatively, a high rate of infection in species with a high mating rate may select for mechanisms that reduce the impact of parasites. For example, sexual ornaments may evolve to allow females to assess the parasite load of males or the strength of their immune defense (Møller et al. 1999). In this case, we would expect a greater expression of sexually selected plumage ornaments in species with high sexual contact rates. In ducks, however, the expression of white patches on the wing coverts is negatively correlated with FEPC activity as measured by phallus length (Hegyi et al. 2008). Moreover, our results show that white wing ornamentation in males but not in females is positively related to LPAI prevalence, and species that are sexually

**Table 2**
Effect sizes and 95% confidence intervals from multiple regressions and multivariate generalized least squares models that tested 2 individually significant, correlated predictors of avian influenza prevalence in ducks together

| Model | Effect | Data | $r$ | $N$ | CI lower | CI upper | $\lambda$ |
|---|---|---|---|---|---|---|---|
| Testis size and phallus length | Testis size | Species | −0.276 | 8 | −0.821 | 0.532 | |
| | | Weighted | −0.102 | 20 | −0.521 | 0.357 | |
| | | Phylogeny | −0.440 | 8 | −0.874 | 0.384 | 0.875 |
| Testis size and phallus length | Phallus length | Species | −0.545 | 8 | −0.903 | 0.259 | |
| | | **Weighted** | **−0.488** | **20** | **−0.765** | **−0.058** | |
| | | **Phylogeny** | **−0.739** | **8** | **−0.949** | **−0.072** | **0.913** |
| Male covert patch and phallus length | Male covert patch | Species | 0.010 | 15 | −0.505 | 0.520 | |
| | | Weighted | −0.025 | 34 | −0.360 | 0.316 | |
| | | Phylogeny | 0.010 | 15 | −0.505 | 0.520 | 0 |
| Male covert patch and phallus length | Phallus length | **Species** | **−0.521** | **15** | **−0.816** | **−0.012** | |
| | | **Weighted** | **−0.420** | **34** | **−0.664** | **−0.095** | |
| | | **Phylogeny** | **−0.521** | **15** | **−0.816** | **−0.012** | **0** |

Backward stepwise selection with reintroduction ($P = 0.05$), terms in bold were retained in the final model. The 3 types of analyses used species-specific data (Species), data weighted by log-transformed sample size (Weighted), and phylogenetic correction (Phylogeny). CI, confidence interval. $\lambda$, phylogenetic scaling parameter.

dichromatic in covert patch expression have a higher LPAI prevalence than monochromatic species. Under the hypothesis of parasite-mediated sexual selection, our data suggest that male ornaments are indeed larger in species with currently high disease transmission risk, whereas duck species with high general copulation or FEPC rates are characterized by low transmission risk. Using male wing patch size and phallus length as simultaneous predictors in a multivariate model indicated that phallus length explained the relationship of wing patch size with LPAI prevalence. This may indicate that communication associated with copulation behavior has been more important for the evolution of ornamentation than infection risk (Hegyi et al. 2008). However, irrespective of the causal pathway, white wing ornamentation is currently a reliable indicator of LPAI prevalence patterns among duck species.

Mechanisms underlying low transmission risk in species with high copulation rates may be physiological or morphological. High rates of sexual contact may promote increased immune activity in the female reproductive tract (Poiani 2002). A long phallus in itself should enhance rather than reduce parasite transfer during copulation, by prolonging the period of contact (Briskie and Montgomerie 1997). However, forced copulations by males have also led to counteradaptations by females that may have profound implications for the circulation of influenza virus. The length of the vagina and its surface complexity increases significantly with the prevalence of forced copulations (Brennan et al. 2007). If the virus received during copulation does not cause infection, it may quickly clear from the cloaca with feces. However, if it enters the oviduct, it may potentially settle there for a longer time and may contaminate eggs either externally (Li et al. 2006; Ellström et al. 2008) or internally (Hooper and Selleck 1998; Promkuntod et al. 2006). In species with high prevalence of FEPC, we expect that long and highly complex vaginas (Brennan et al. 2007) will reduce the probability that viruses from the cloaca reach the site of egg formation. Indeed, despite the small amount of data available, we found that a binary composite measure of vaginal elaboration was negatively related to influenza prevalence.

Our results suggest that sexual transmission and the associated adaptations are important determinants of the prevalence of avian influenza in ducks: the principal hosts of the virus. This finding may represent important guidance for future screening efforts of species with currently unknown influenza prevalence (Garamszegi and Møller 2007; Keawcharoen et al. 2008). Individual behavioral and morphological predictors we tested here explained up to 35% of the variance in virus prevalence, so they may prove reliable indicators of interspecies differences in transmission risk (Keawcharoen et al. 2008). Virus screening should particularly focus on host species with simple genital tracts, low prevalence of forced copulations, and larger and more dichromatic white patches on the wing coverts. Among these data, white wing ornamentation represents the most widely available information (Hegyi et al. 2008). According to our results, species that are most likely to host avian influenza at high prevalence are those with large male covert patches and covert patch dichromatism. These include, for example, mergansers (*Mergus* spp.) and eiders (*Somateria* spp. and *Polysticta stelleri*), which are also migratory so they may circulate the virus strains to large distances. There is currently little information on influenza prevalence from these species, but all 4 species in our data set with both large male covert patch and covert patch dichromatism (*Anas americana*, *Anas penelope*, *Bucephala albeola*, *S. mollissima*) showed average or large prevalence.

In addition to surveillance, sexual transmission of LPAI also has implications for seasonal prevalence cycles (Olsen et al. 2006). In the case of internal inoculation of eggs from the oviduct (Promkuntod et al. 2006), less pathogenic virus strains may persist and affect the chick. However, if the virus is on the eggshell, it may infect the chick only at hatching. Adult birds may shed the virus for more than a week after inoculation (Sturm-Ramirez et al. 2005). A similar pattern of shedding in chicks with a benign infection (Laudert et al. 1993) may lead to high levels of contamination of the water with feces at the breeding habitat, and subsequent transmission to other immunologically naive chicks by the fecal–oral route (Garamszegi and Møller 2007).

Indeed, high virus titers detected in lake water at Alaskan breeding areas suggested that the large numbers of infected juvenile birds found at the staging areas had already acquired the virus at their site of hatching (Ito et al. 1995). One pathway through which viruses that survived the winter in frozen water (Ito et al. 1995) may rapidly infect many susceptible juveniles is copulation of the mother in the water, followed by contamination of the eggs (Li et al. 2006; Promkuntod et al. 2006). In other words, mother–offspring transmission through the egg may represent an important contribution to the yearly peak in LPAI prevalence observed at the postbreeding staging of waterfowl (Hinshaw et al. 1985). This route of transmission does not strictly require the actual infection of the female due to copulation, or even that the male is infected before copulation (i.e., sexual transmission sensu stricto). However, it is necessary that 1) copulation, either in the contaminated water or with an infected male, introduces the virus into the female, 2) the virus is stored in the oviduct, and 3) it contaminates the eggs and infects the chicks at or before hatching, resulting in viral transmission to other individuals.

We conclude that, in addition to ecological factors (Garamszegi and Møller 2007), certain aspects of sexual selection may play a major role in determining the worldwide patterns of transmission of influenza virus. In particular, behavioral, physiological, and morphological adaptations associated with copulation rates (McKinney et al. 1983; Poiani 2002; Brennan et al. 2007), and potential transmission from mother to offspring (Li et al. 2006; Promkuntod et al. 2006; Ellström et al. 2008), deserve further attention as risk factors. Finally, our results suggest that, in addition to the fecal–oral and the respiratory routes, copulation in water represents a potent third, intergenerational route of virus transmission (Kulkarni and Heeb 2007). This may further illuminate the spatial and temporal dynamics of virus prevalence, and may also have implications for the management of domestic populations (Gilbert et al. 2006; Olsen et al. 2006).

## SUPPLEMENTARY MATERIAL

Supplementary Material can be found at http://www.beheco.oxfordjournals.org/.

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