Do Cloacal Pathogenic Microbes Behave as Sexually Transmitted Parasites in Birds?

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Abstract: In birds, microparasites found in both the reproductive and the digestive tracts may be transmitted through copulations via cloacal contact (male-to-female and vice versa) and/or through the seminal fluid (mainly male-to-female). Most importantly, such cloacal microparasites are affected by and may in turn affect sexual selection processes and the evolution of mating systems. Here I provide preliminary comparative evidence that at least some cloacal microparasites tend to be distributed in hosts according to the host's mating system and as broadly expected from predictions of sexual selection theory. The patterns, however, are more suggestive than conclusive. There is a non-significant trend for polygamy to be associated with higher richness of cloacal microparasite taxa; with body size, however, also having a positive association with both polygamy and parasite richness. Although increased sexual plumage dichromatism tends to be associated with decreased cloacal microparasite richness, indicating that secondary sexual traits may be used by sexual partners to discriminate between infected and uninfected individuals, qualitative trends also suggest that non-mating periods of the year tend to be associated with slightly higher levels of prevalence and richness of cloacal microparasites. Given this variability of results, it is suggested that future studies should focus on specialist sexually transmitted microbes, to be compared with more generalist one.

Keywords: Sexually transmitted microparasites, sexual selection, cloacal microbes.

INTRODUCTION

In the past two decades or so there has been a significant surge in the interest to study sexually transmitted diseases (STDs) and their causative agents [1]. Although such attention was undoubtedly stirred by the HIV-AIDS pandemic in humans, evolutionary biologists have also started to investigate other systems, both vertebrate and invertebrate, where transmission of pathogens among hosts may occur via sexual intercourse. Evolutionary parasitologists and epidemiologists have made significant inroads into the theoretical and empirical understanding of the behavioural, ecological, life-history, immuno-endocrinological, genetic and other mechanisms that drive this specific kind of host-pathogen system. However, our specific knowledge of avian cases remains scanty. I will start this article with a review of the major evolutionary theoretical issues regarding STDs, and then review previous ornithological empirical work carried out on sexually transmissible microbes. I will also describe the results of a comparative analysis which tests some of the major predictions of the hypothesis that at least some cloacal micropathogens behave as sexually transmitted parasites in birds.

STDs IN BIRDS: EVOLUTIONARY DYNAMICS

Early theoretical modeling of STD transmission (e.g. [2]) identified the basic issues that are relevant to understand whether a sexually-transmitted (ST) pathogen could invade a host population and be maintained over time: a) probability of initial establishment, b) probability of persistence in the host population over the long term, and c) probability of spread to other host populations. Such probabilities are critically dependent on the basic reproductive rate of the infection (Ro), defined as the average number of secondary cases of infection that are produced by a primary case in a susceptible host population. Ro can be calculated as:

$$Ro = \beta N/(\alpha + b + \upsilon)$$

where α = virulence, or level of the negative effect of the pathogen on host lifetime reproductive success, b = per capita host death rate in the absence of infection, and β = transmission rate, that is the rate of acquisition of the infection by individuals who are susceptible following their contact with an infected individual; υ = the recovery rate of infected hosts and N is the total host population. Ro is therefore calculated as the rate of production of secondary infections by infected individuals per unit time (βN), also known as the *force of infection*, over the average duration of the state of infectiousness ($\alpha+b+\upsilon$) before the host either recovers or dies [3].

A parasite population can be sustained within a population of hosts only if $Ro \geq 1$. That is, when

$$\beta N/(\alpha + b + \upsilon) \geq 1$$

From this it follows that the critical host density below which the parasite cannot be sustained in the host population (N_T) is:

$$N_T = (\alpha + b + \upsilon)/\beta$$

That is, small populations of hosts can maintain pathogens only if the latter are easily transmitted between hosts

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1874-4532/10

and/or they are not very harmful to the host (see also [1] and [4]).

If the parasite is sexually transmitted then the criterion for persistence becomes:

$$\beta(m + \sigma^2/m)/(\alpha + b + \upsilon) \geq 1$$

where m = mean number of sexual partners per individual and σ^2 = the variance in the number of sexual partners per individual [4].

Therefore, a series of characteristics are displayed by ST-parasites, with some of those characteristics being shared with other kinds of parasites, whereas others are more specific. ST-pathogens can be maintained in small host populations if they are not highly pathogenic and if they are easily transmitted. Such may be the case in hosts that have a polygamous mating system, as polygamy is associated with higher values of both m and σ^2. In addition, because such parasites are usually transmitted during very restricted and, depending on the host species, relatively infrequent circumstances (i.e. when their hosts mate), they are expected to evolve strategies to escape the immune system of the host over a relatively long period of time; possessing intracellular stages of development may help the parasite achieve this [5]. Such parasites are also expected to have lower negative effects, and perhaps higher positive effects, on those specific traits that facilitate sexual intercourse in the host [5]. If the parasite has to spend a long time dormant in the host and be dependent on good host health for successful sexual transmission, then its effects on the host's health may be expected to be relatively mild especially in systems where hosts exert strong pre-mating sexual partner choice and discrimination. Countering this, high rates of transmission are expected to be correlated with the evolution of virulence in the parasite, which leads to the prediction that mating systems characterised by promiscuity, low degree of discrimination between alternative sexual partners and higher rates of sexual intercourse per partner are expected to favour the evolution of highly pathogenic ST-parasites [5]. High virulence is also expected to evolve in the case of multiple infections, where parasites may engage in interspecific competition within the host (e.g. [6]) or, in a more synergistic scenario, when the probability of infection by a second kind of parasite is enhanced by current infection [7].

Because ST-pathogens are transmitted between individuals following processes of mate choice and opportunities to access mates willing to copulate, the density of infectious individuals in the population is not as important for the transmission of STDs as for other kinds of diseases (e.g. those caused by airborne viruses) [4,7]. Instead, STD transmission is more dependent on the frequency of infectives [8] and also copulation rate both with the same and also with various sexual partners [9,10] as suggested above.

Parasites and hosts are usually engaged in co-evolutionary processes where both exert selective pressures on each other. I have already mentioned that in the case of STDs, parasite transmission and the evolution of virulence are influenced by the host mating system; and the evolution of host mating strategies, in turn, is influenced by sexually transmitted parasites [11, 12]. Sexual selection, for instance, can decrease the evolution of virulence among ST-parasites [13], simply because if secondary sexual traits are costly (*sensu* Zahavi [14]) then virulent parasites would be associated with development of less attractive secondary sexual traits in the host, and therefore lower probability of mating. As mating rates decrease, probability of transmission of the ST-parasite will also decrease. Thrall *et al*. [15] have predicted that when prevalence of ST-pathogens is relatively low or intermediate in the host population, then discrimination of sexual partners within a polygamous mating system and/or monogamy should be selected. In the absence of STDs, promiscuity can be selected. Interestingly, if the prevalence of ST-pathogens is very high promiscuity can also be selected ([15], see also [16]) as at high prevalence values for the parasite the probability of being infected does not decrease significantly by mating with fewer partners.

Whereas hosts are under selective pressure to discriminate between sexual partners (assortative mating) in order to decrease ST-pathogen transmission (e.g. [16,17]), those pathogens, in turn, are under selective pressure to enhance sexual attractiveness and/or sexual activity of their hosts which may increase their chances of transmission [18]. It can be easily seen how this situation can jump start a runaway sexual selection process whereby parasites enhance the attractiveness of male birds whereas females become more discriminative, which may result in the prevention of infection [19]; parasites will then be selected to further increase attractiveness of males in order to overcome female choosiness. A similar mechanism is involved in the *Chase Away* sexual selection model of Holland and Rice [20] in which female avoidance of costs of mating (STDs in our case) may sustain a runaway evolutionary process for attractive males exploiting sensory preferences of females and for females to be reluctant to mate with showy males. A slight variation on this theme was proposed by Graves and Duvall [21] who suggested that female preference for more attractive males may increase the probability of transmission of STDs thus lowering female fitness. This would result in selection on females to preferentially choose males with less attractive secondary sexual traits, such a preference would be associated with higher fitness and, with time, lowered STD transmission [e.g. 156]. However, as prevalence of ST-pathogens decreases in the host population sexual selection may increase for males displaying more conspicuous secondary sexual traits, and so the cycle will start again.

Interestingly, such coevolutionary cycles based on negative feedback may be broken if the parasite and the host evolve a symbiotic mutualistic relationship, where the sexually transmissible microbe may increase its spread in the host population by a) decreasing its pathogenicity, b) increasing the attractiveness of honest secondary sexual traits and also c) increasing both male and female host's mating and reproductive success. However, such a mutualistic system can be evolutionary stable only if there is strong competition between cloacal microorganisms, and a new pathogenic mutant or invader has little chance of succeeding within a host that already carries a predominantly mutualistic cloacal microflora.

STDs can not only affect male-female mating interactions, but they may even affect sperm competition among males. For instance, an immunoreactive female reproductive tract that is competent in the defence against micropathogens carried by the semen, may also produce an immune attack

against spermatozoa thus exerting a selective pressure on ejaculates from different males, such selective pressure may affect the outcome of sperm competition (see [22, 23] and refs. therein).

STDs IN BIRDS: A REVIEW OF EMPIRICAL STUDIES

Both the avian cloaca and various sections of the reproductive and digestive systems, harbour a large variety of microorganisms that could be eventually transferred from one individual to another during copulation. Most male birds do not possess penile-like organs that are intromitted into the female cloaca during copulation, hence female-to-male transfer of ST-pathogens is only likely to occur through infection of the external surface of the female cloaca and the fluids that cover it. Male-to-female transfer of ST-pathogens, however, can also occur through contamination of the semen. Therefore in birds there is a likely bias in the transmission rate of ST-parasites from male to female. This has been recently demonstrated by Kulkarni and Heeb [24] in *Taeniopygia guttata*. Birds, both males and females, had their cloaca experimentally infected with *Bacillus licheniformis*. This allowed the measurement of the level of symmetry between male-to-female and female-to-male transmission. What Kulkarni and Heeb found was that the rate of transmission was higher from male to female than *vice versa*.

The avian semen may contain a diverse microflora that originates from various sections of the reproductive but to some extent also the digestive tract, as the cloaca is a common route of discharge of both faeces and seminal products.

Table **1** summarises the findings of some of the works that have studied seminal microflora in birds. Microorganisms in Table **1** are broadly classified as viruses, bacteria or fungi, the latter encompassing a diversity of taxa that include yeast, mould and others [34]. Although studies of seminal microflora are mainly confined to a few species, with emphasis on domestic birds, it is clear that many kinds of micropathogens could be transmitted through the semen during copulation. That some of those pathogens may be specialists on the reproductive system, but many others may originate from the digestive system was shown by Hupton *et al.* [31] who reported the prevalence of various microbes in both the cloaca and the seminal fluid in *Agelaius phoeniceus*. Although the trend was for prevalence in the cloaca to be positively correlated with prevalence in the semen, the association was not statistically significant presumably reflecting the binary origin of the cloacal microflora.

In taxa that possess a phallus (e.g. Anseriformes) STDs could be also transmitted from female to male. In fact, various microorganisms such as *Candida albicans* and *Mycoplasma* that inhabit the cloaca can infect the phallus of domestic geese (e.g. [35-37]).

After copulation has occurred, the success or failure of sexually transmitted micropathogens to establish themselves in the host and perhaps cause disease, is likely to be a complex process. Factors of potential importance include current parasitic loads in the sexual partners and how within-host pathogen community structure influences competitive exclusion or coexistence or even facilitation of the various species or strains. Of clear importance is the ability of individual host defence systems (e.g. immunity) to clear the body of invading pathogens which will be determined by host genotype as well as condition (i.e. general health). Such considerations may explain why, for instance, the level of congruence in bacterial presence or absence between mated individuals is not necessarily perfect (e.g. 36% in Hupton *et al.*'s [31] study of *Agelaius phoeniceus*), although additional effects such as extra-pair copulations may also decrease the level of intra-pair similarity.

Understanding the microflora composition of the avian semen is critical in order to unravel the mechanisms of sexual transmission of pathogens, however a broader knowledge of the cloacal microflora is also of great potential relevance. The cloacal microflora may have diverse origins within the body of the animal: faeces and reproductive system as mentioned above, but also skin and feathers from the regions surrounding the cloacal opening (e.g. [38]) and it may be affected by a variety of factors that range from genetic makeup, immune competence, body size, age and sex of the host to host population density, host habitat, food and more. The cloacal microflora is acquired very early on by young birds from their environment (e.g. [39]) and whether cloacal microorganisms persist or not in the host is critically dependent on both the host's immune system (e.g. [40]) and ecological interactions among the various microbes (e.g. [41]). Moreover, sexual transmission is not only restricted to microorganisms. Some arthropod ectoparasites such as *Menapon* and *Goniodes* feather lice may also be transmitted during copulation [42].

I have mentioned in the previous section that the ecology of the cloacal microflora, as expressed through inter-specific interactions between diverse kinds of microorganisms, can play a major role in shaping the species composition of within-host microbe communities: what kind of pathogen that was acquired through copulation will be able to survive and establish itself in the new environment and therefore what kind of pathogen will be in turn transmitted in future copulations. Being infected by more than one pathogen (i.e. superinfection) may result in decreased host fitness if pathogens have synergistic negative effects on the host. For instance, *Chlamydophila* infected turkeys may suffer greater deterioration of their health when superinfected with *Escherichia coli* [43]. Similarly, Newcastle Disease virus titres increased in chickens following superinfection with *E. coli* [44]. However STD-microbes may be also involved in competitive interactions among themselves. At one end of the spectrum of possibilities, one sexually transmissible pathogen may outcompete others (e.g. *Enterococcus faecalis* may outcompete other species of *Enterococcus* in pied flycatcher, *Ficedula hypoleuca*, [45]). At the other end of the spectrum harmless microbes may defend the organism against infections by pathogenic microbes, thus becoming, overall, beneficial to their host. *Lactobacillus* is an example of such "probiotic" bacteria which has been studied in many birds [30, 46, 47]. *Bacillus subtilis* is another probiotic bacterium able to outcompete *E. coli*, *Salmonella enterica* and *Clostridium perfringens* in chickens [48, 49].

In this work I test the hypothesis that cloacal pathogenic microorganisms are distributed across host species in a manner that is expected from them being sexually transmitted parasites. This hypothesis predicts that the more polygamous

Table 1. Some Micropathogens that have been Isolated from Avian Semen

VIRUSES		
Taxon	**Host**	**Reference**
Chicken anaemia virus	*Gallus domesticus*	[25]
Avian influenza virus	*Meleagris gallopavo*	[26]
BACTERIA		
Mycoplasma spp.	*Meleagris gallopavo*	[27]
M. gallisepticum	*Anser anser*	[28]
M. meleagridis	*Meleagris gallopavo*	[29]
Salmonella spp.	*Tachycineta bicolor*	[30]
Shigella spp.	*Tachycineta bicolor*	[30]
Vibrio spp.	*Tachycineta bicolor*	[30]
Parahaemolyticus spp.	*Tachycineta bicolor*	[30]
Yersinia spp.	*Tachycineta bicolor*	[30]
Bacillus circulans	*Agelaius phoeniceus*	[31]
B. laterosporus	*Agelaius phoeniceus*	[31]
B. licheniformis	*Agelaius phoeniceus*	[31]
Enterobacter agglomerans	*Agelaius phoeniceus*	[31, 32]
E. cloacae	*Agelaius phoeniceus*	[31]
Enterococcus gallinarum	*Agelaius phoeniceus*	[31]
Escherichia coli	*Agelaius phoeniceus*	[31]
Gardnerella vaginalis	*Agelaius phoeniceus*	[31]
Listeria spp.	*Agelaius phoeniceus*	[31]
L. denitrificans	*Agelaius phoeniceus*	[31]
L. grayi	*Agelaius phoeniceus*	[31]
Micrococcus spp.	*Agelaius phoeniceus*	[31]
M. roseus	*Agelaius phoeniceus*	[31]
Staphylococcus cohnii	*Agelaius phoeniceus*	[31]
S. epidermis	*Agelaius phoeniceus*	[31]
S. warneii	*Agelaius phoeniceus*	[31]
S. xylosus	*Agelaius phoeniceus*	[31]
Acinetobacter calcoaceticus	*Agelaius phoeniceus*	[32]
Pseudomonas spp.	*Agelaius phoeniceus*	[32]
P. putida	*Agelaius phoeniceus*	[32]
P. paucimobilis	*Agelaius phoeniceus*	[32]
P. maltophila	*Agelaius phoeniceus*	[32]
Ewingella americana	*Agelaius phoeniceus*	[32]

Table 1. contd…

BACTERIA		
Taxon	**Host**	**Reference**
Aeromonas hydrophila	*Agelaius phoeniceus*	[32]
Weeksella virosa	*Agelaius phoeniceus*	[32]
FUNGI		
Mucor janssenii	*Anser anser*	[33]

and also the more sexually dichromatic species are likely to harbour more cloacal microparasites if parasitism is a cost of multiple matings, but those host species should harbour less cloacal microparasites if mate choice mechanisms, based on secondary sexual traits, are in place that are used to select for less parasitised sexual partners. Moreover, the cloaca is expected to host more microparasites during the breeding than during the non-breeding periods of the year. The test will be done by means of carrying out a comparative analysis across 56 bird taxa pertaining to 10 orders.

MATERIALS AND METHODS

Data used in the comparative analyses were obtained from published works after a thorough bibliographical search was carried out through the Web of Science, Google Scholar, Biological Abstracts, Scopus, ProQuest and list of references taken from review articles. I was able to obtain information for a sufficient number of host species for seven variables associated directly or indirectly with reproductive behaviour (climate, time of sampling, sex of host, age of host, mating system, extra-pair copulations (EPCs) and sexual dichromatism), and two dependent variables that measure cloacal parasitism: taxa richness and mean value of prevalence, the latter being the sum of prevalence values for the parasitic taxa found in a host species divided by the number of those parasitic taxa. Host body mass (in grams) was also recorded and the value entered was the log-transformed mean value of body mass of male and female. Only values of the parasitological variables recorded from host sample sizes larger or equal than 10 were included in the analyses.

Climate data were inferred according to the study region mentioned in the original source data using the Köppen-Geiger climate classification system [50]. The following climates were represented in our sample (Köppen-Geiger code in parenthesis): semi-arid (Bsh, Bsk), humid subtropical (Cfa), oceanic (Cfb), mediterranean (Csa), humid continental (Dfa, Dfb, Dwb), subarctic (Dfc), polar (Ef). Each climate is characterised by a categorisation of the level of humidity (high, medium and low) and temperature (high, medium and low). Time of sampling of parasites could be during the breeding or during non-breeding periods of the population studied. Some studies, however reported results of year-round sampling. Sex of the host was also recorded, whether it was male or female, although most entries from the literature corresponded to combined values of parasitism for both sexes. Age of the host (adult vs. young) was also recorded. Mating system ranged from monogamy (M), facultative polygyny (FPy), polygyny (Py) and polyandry (Pa) to polygamy (Pm) and promiscuity (Pr). The "EPCs" category actually included a variety of variables ranging from actual EPC to extra-pair paternity (EPP) and forced-EPCs (FEPC). These variables are different and cannot be directly combined in the same analysis. However, in order to use all of the information available and yet account for the difference between variables, I ordered the data into broad categories: No EPC (or EPP, FEPC) (0%), Low (>0%-3%), Medium (>3%-10%), High (>10%-40%), Very High (>40%). Sexual dichromatism was measured in terms of marked plumage colouration differences between males and females and recorded as either present or absent.

With regard to cloacal parasites, they are reported in the literature at various levels of taxonomic identification: genus, species, but also broader descriptions such as "anaerobic bacteria". I measured richness as the number of different taxa recorded. For each study published on a specific host species, I also calculated the mean prevalence (percentage of hosts infected) across parasite taxa and averaged values across studies in order to obtain a mean value of prevalence of infection for each host species. In this way I smoothed the effect of the high variability in prevalence values usually found in parasitological studies, and hence decreased the effect of outliers. Studies report values of prevalence for viruses, bacteria and fungi, which in this case were combined in the analyses to provide an overall value of microparasitic richness and mean prevalence in the cloaca.

I tested the hypothesis by carrying out comparative analyses using independent contrasts [51]. "Time of sampling", however was a variable analysed through a Wilcoxon two-sample test, whereas sex of the host was not included in the analyses due to most authors lumping together the information for males and females. Most studies also focus on adults, thus limiting our ability to test for age effects across species. Calculation of phylogenetically independent contrasts was carried out using the PDAP program [52] that runs in the *Mesquite* program of Maddison and Maddison [53]. All analyses used branch lengths set following Grafen's method [54].

The 56 taxa included in the analyses pertain to the orders: Anseriformes, Galliformes, Sphenisciformes, Ciconiiformes, Gruiformes, Charadriiformes, Strigiformes, Falconiformes, Columbiformes and Passeriformes. In the comparative analysis, I used a compound phylogeny of those taxa (see

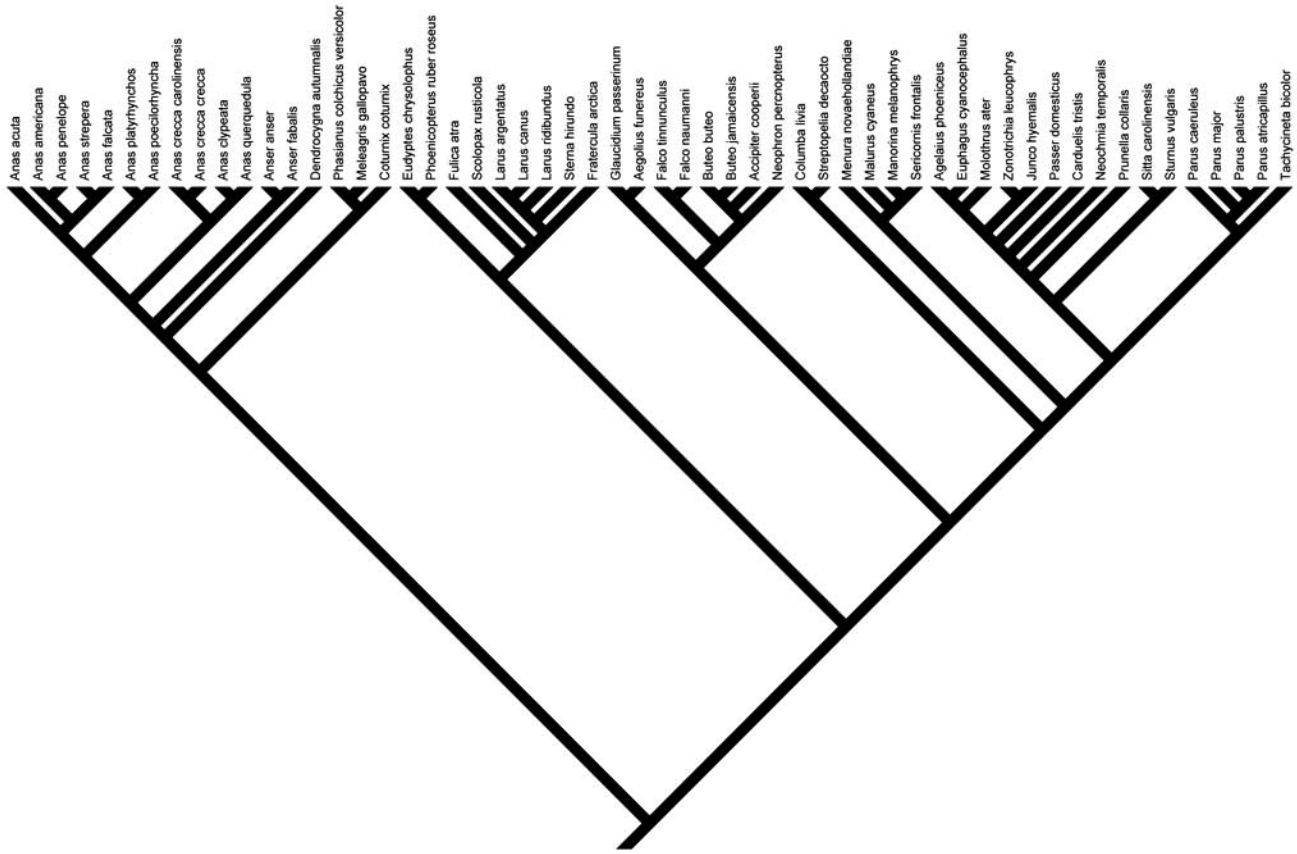

Fig. (1). Compound phylogeny of the 56 bird taxa used in the comparative analyses.

Fig. **1**) that was reconstructed on the basis of the following published information. The phylogenetic relationship among orders was based on Livezey and Zusi [55]. This order-level phylogeny allowed the easy positioning of taxa represented by only one or only two species within the phylogeny. Orders represented by more than two species required a more detailed within-order phylogeny. The phylogeny of the Anseriformes was reconstructed on the basis of Johnson and Sorenson [56] and Donne-Goussé *et al.* [57]. The Galliformes phylogeny followed Kimball and Braun [58], whereas the Charadriiformes' was based on Paton *et al.* [59] and Thomas *et al.* [60]. The Falconiformes followed Lerner and Mindell [61] and, finally, the specific phylogeny of the Passeriformes was reconstructed using information from various authors [62-72].

Continuous variables such as body mass and prevalence were log- and square-root (plus one)-transformed respectively before they were entered in the analyses. Categorical variables were coded in the following manner. Mating system: M =1, FPy = 2, Py,Pa = 3, Pm,Pr = 4; "EPCs": No EPCs = 1, Low = 2, Medium = 3, High = 4 and Very High = 5; Plumage Sex Dichromatism: Monochromatic = 1, Dichromatic = 2; Climate: each climate type was categorised high (3), medium (2), or low (1) in terms of temperature and humidity. The scores for temperature and humidity were then added to provide an overall code for each climate. These combined codes increased from Polar/Sub-arctic (2) to Humid Continental (3), Mediterranean/Semi-arid (4) to Oceanic (5) and Humid Subtropical (6) climates.

Finally Pearson's product-moment correlations were carried out between variables expressed as phylogenetically independent contrasts.

RESULTS

The full original dataset used in the comparative analyses is shown in Table **2**. For each variable only one value was entered for each bird species - after transformation or encoding as the case may be - by obtaining the mean value for the various intra-specific data. Table **3** summarises the cloacal microorganism taxa found in the various studies. The results of relevant correlations between phylogenetically independent contrasts are shown in Table **4**. The first result that I would like to highlight is the significant trend for evolutionary changes towards increased richness of cloacal microparasites to be associated with evolutionary changes towards living in more tropical climates. Prevalence contrasts, on the other hand, are not significantly associated with climate contrasts and, if anything, the trend is for decreased prevalence of cloacal microparasites as hosts tend to live in more tropical climates. Therefore, although in tropical climates there is a larger number of cloacal microparasite taxa, the level of infection is not necessarily higher than in non-tropical climates.

I also detected a highly significant positive correlation between log-body mass contrasts and mating system contrasts, indicating that evolutionary trends towards larger body sizes are associated with evolutionary trends toward a higher degree of polygamy. Moreover, as the body size tends

Table 2.

Host Species	Climate	TS	SH	AH	Mating System	EPCs	SD	BM	HSS	R	MP	Reference
Passer domesticus	Dfa-Cfa	B	M+F	A	12.3%Py, 87.7%M	8%EPY	D	28.5	16	8	70	[73-76]
	Cfb	B	M	A					17	1	41.1	[77], *E. coli* only*
	Cfb	B	F	A					13	1	23	[77], *E. coli* only*
	Csa	YR	M+F	A					65	10	6.3	[78]
Phoenicopterus ruber roseus	Csa	B	M+F	Y	M	?	M	2500	54	12	39.4	[79, 80, 81]
Euphagus cyanocephalus	Csa	YR	M+F	A	29%Py, 71%M	?	D	68	17	7	8	[78, 82, 83]
Molothrus ater	Csa	YR	M+F	A	M-Pr	Infr. EPC	M	45	56	12	6.5	[76, 78, 84, 85]
Sturnus vulgaris	Csa	YR	M+F	A	Fpy	8.6%EPY	M	85	34	9	11.3	[76, 78, 86]
	Csa	YR	M+F	A					162	1	1.3	[87] AIV only, mean value of various works*
Agelaius phoeniceus	Csa	YR	M+F	A	44%Py, 56%M	24.6%EPF	D	56	43	9	8.9	[76, 78, 88]
	Cfa	B	M+F	A					48	24	4	[31]
Zonotrichia leucophrys	Csa	YR	M+F	A	M	36%EPF	M	25.8	29	11	14.5	[76, 78, 89]
Mamorina melanophrys	Cfb	B	M	A	M	4.1%EPP	M	29.4	22	2	9	[90-92]
	Cfb	YR	M+F	A					92	5	3.6	[92]
Malurus cyaneus	Cfb	B	M	A	M-Pa	76%EGP, 15%EPP	D	9.9	20	2	3.3	[90, 92, 93]
			F						12	2	5.5	[92]
	Cfb	YR	M+F	A					73	4	5	[94]
Sericornis frontalis	Cfb	B	M	A	Pa	12%EGP	D	12.9	11	2	18.2	[92, 94, 95]
			F						14	1	4.7	[92]
	Cfb	YR	M+F	A					23	3	8.6	[94]
Neochmia temporalis	Cfb	YR	M+F	A	M	?	M	10.5	21	3	6.1	[94]
Menura novaehollandiae	Cfb	B	M	A	Pr	?	D	1022	13	1	63	[96, 97] Only chlamydial antigen in faeces*
			F	A					15	1	66.6	Only chlamydial antigen in faeces*
Dendrocygna autumnalis	Bsh	B	M+F	A	M	?	M	831	110	27	7.6	[98-100]
Anser anser	Cfb	B	M	A	M-Fpy	L?	M	3360	134	1	2.2	[28, 76, 80], *Mycoplasma gallisepticum* only*
	Cfb	YR	M+F	A					325	1	1.3	[87], AIV only, mean value of various works*
Anser fabalis	Dfb	NB	M+F	Y	?	?	M	3020	11	0	0	[101, 102], Yeasts only*
	Cfb	YR	M+F	A					74	1	0	[87], AIV only, mean value of various works*
Anas clypeata	Bsk	NB	M+F	A	M(mainly)-Fpy	Rare EPC	D	613	35	1	66	[76, 103, 103], *Campylobacter* only*
Anas acuta	Bsk	NB	M+F	A	M	Fre. FEPC	D	908	30	1	50	[76, 101, 103, 105], *Campylobacter* only*
	Cfa/Dfb	YR	M+F	A					198	1	7.9	[87], AIV only, mean value of various works*
Anas americana	Bsk	NB	M+F	A	M	Infr. FEPC	D	997.5	38	1	42	[76, 103-105], *Campylobacter* only*
	Dfb	YR	M+F	A					40	1	10.3	[87], AIV only, mean value of various works*
Anas platyrhynchos	Bsk	NB	M+F	A	M	3%EPP	D	1082	243	1	34	[76, 80, 103], *Campylobacter* only*
	Cfa	NB	M+F	A					259	0	0	[106], *Yersinia pseudotuberculosis* only*
	Cfa/Dfb	YR	M+F	A					410	1	10.1	[87], AIV only, mean value of various
Anas crecca carolinensis	Bsk	NB	M+F	A	M	Infr. FEPC	D	315.5	56	1	16	[103, 107-109], *Campylobacter* only*
Anas crecca crecca	Cfa	NB	M+F	A	M	Fre. FEPC	D	341	60	0	0	[76, 101, 105, 106, 110], *Yersinia pseudotuberculosis* only*
	Dfb	NB	M+F	A					40	1	25	[102], Yeasts only*
	Dfb	NB	M+F	Y					18	1	5.5	[102], Yeasts only*
	Cfb	YR	M+F	A					109	1	8.4	[87], AIV only, mean value of various works*
Anas poecilorhyncha	Cfa	NB	M+F	A	M	?	M	1230	24	1	4.2	[101, 106], *Yersinia pseudotuberculosis* only*
	Cfa	YR	M+F	A					189	1	3.4	[87], AIV only, mean value of various works*
Anas falcata	Cfa	NB	M+F	A	?	?	D	910	15	0	0	[101, 106], *Yersinia pseudotuberculosis* only*
	Cfb	YR	M+F	A					35	1	0	[87], AIV only, mean value of various works*
Anas penelope	Cfa	NB	M+F	A	M	?	D	800	22	1	4.5	[101, 106, 111], *Yersinia pseudotuberculosis* only*
	Cfb	YR	M+F	A					335	1	2.5	[87], AIV only, mean value of various works*
Anas querquedula	Dfb	NB	M+F	A	M	?	D	330	14	1	28.6	[101, 102, 112], Yeast only*
	Dfb	NB	M+F	Y					16	1	31.2	[102], Yeasts only*
Anas strepera	Bsk	NB	M+F	A	M	4.2%EPY	M	860	26	1	15	[103, 113], *Campylobacter* only*
	Cfa/Cfb/ Dwb	YR	M+F	A					98	1	2.2	[87], AIV only, mean value of various works*
Tachycineta bicolor	Dfb	B	M+F	A	M	51%EPC	M	19	11	8	92	[76, 80, 114]
				N					22	8	67.1	[114]
Buteo buteo	Csa	YR	M+F	A	M	0%EPC	M	800	32	3	1.3	[110, 115, 116]
Buteo jamaicensis	Csa	NB	M+F	A	M	?	M	1075	10	6	24.7	[76, 117, 118]
Falco tinnunculus	Csa	YR	M+F	A	M	2%EPC	D	170	63	4	1.6	[76, 80, 116]
Falco naumanni	Csa	YR	M+F	A	M	6.7%EPC	M	124	19	1	2.6	[80, 116, 119, 120]
Glaucidium passerinum	Csa	YR	M+F	A	M	?	M	64.7	10	1	1.2	[116, 121]
Neophron percnopterus	Bsk	B	M+F	A	M(mainly)	4.6%EPC	M	1889	11	14	25.6	[76, 122-124]
	Bsk	B	M+F	N					13	28	17	[124], Mean values for three sites*
Accipiter cooperii	Csa	NB	M+F	A	M	0%EPC	M	418	10	6	30	[117, 125-128]
Aegolius funereus	Dfc	NB	M+F	A	7.8%Pm, 92.2%M	~0.5%EPP	M	154	12	1	5.5	[76, 129-131]

Table 2. contd…

Fratercula arctica	Dfc	NB	M+F	A	M	Infr. EPC	M	490.5	76	1	17.1	[76, 129, 132]
Larus argentatus	Dfc	NB	M+F	A	76.9%Py, 23.1%M	0.5%EPC	M	1025	24	2	2.7	[76, 80, 129]
	Dfb	YR	M+F	A					153	1	2.1	[87], AIV only, mean value of various works*
Larus canus	Dfc	NB	M+F	A	M	3.6%EPF	M	432	37	1	6.3	[76, 129, 133]
	Dfb	YR	M+F	A					275	1	0	[87], AIV only, mean value of various works*
Larus ridibundus	Dfc	NB	M+F	A	M	?	M	260	53	2	5	[76, 110, 129]
	Cfa	NB	M+F	A					20	0	0	[106], *Yersinia pseudotuberculosis* only*
	Cfb	YR	M+F	A					256	1	17.7	[87], AIV only, mean value of various works*
Sterna hirundo	Dfc	NB	M+F	A	M	2.6%EPC	M	120	36	2	2.7	[76, 129, 134, 135]
	Dfb	YR	M+F	A					240	1	1.9	[87], AIV only, mean value of various works*
Prunella collaris	Dfb	YR	M+F	A+J	Pa	0%EPO	M	37.5	28	11	6.5	[133, 136-138]
Parus caeruleus	Dfb	NB	M+F	A	6%Py, 94M	10.9%EPO	D	10.6	43	1	69.8	[73, 133, 136, 139], *Chlamydia* only*
Parus major	Dfb	NB	M+F	A	M	7.3%EPO	D	17.3	318	1	53.1	[133, 136, 137, 139], *Chlamydia* only*
Parus palustris	Dfb	NB	M+F	A	M	?	M	12	32	1	37.5	[137, 139, 140, 141], *Chlamydia* only*
Parus atricapillus	Dfb	NB	M+F	A	M	8.9%EPO	M	10.4	290	5	10.1	[76, 133, 142, 143]
Junco hyemalis	Dfb	NB	M+F	A	M	25%EPP	D	19	40	3	6.6	[76, 142, 144, 145]
	Dfb	YR	M+F	A					15	1	6.6	[87], AIV only, mean value of various works*
Cardualis tristis	Dfb	NB	M+F	A	M	14.3%EPO	D	13.6	25	3	4	[76, 133, 142]
Sitta carolinensis	Dfb	NB	M+F	A	M	?	D	20	19	4	12.3	[76, 142]
Phasianus colchicus versicolor	Cfa	NB	M+F	A	Py	6%EPP	D	1600	13	0	0	[106, 146-148], *Yersinia pseudotuberculosis* only*
Meleagris gallopavo	Bsk	NB	M+F	A	Py	?	M	7300	190	1	25	[76, 148, 149], *Mycoplasma* sp. only*
Columba livia	Bsk	NB	M+F	A	M	1%EPP	D	358.7	200	1	31	[76, 80, 150], *Campylobacter* only*
	Bsk	B	M+F	A					200	1	21.5	[150], *Campylobacter* only*
	Bsk	YR	M+F	A					400	4	7	[150]
	Cfa	YR	M+F	A					47	1	0	[87], AIV only, mean value of various works*
Streptopelia decaocto	Dfb	NB	M+F	A	M	?	M	187	50	1	8	[102, 148, 151], Yeasts only*
	Dfb	NB	M+F	Y					54	1	6.2	[102], Yeasts only*
	Dfb	YR	M+F	A					19	1	5.2	[87], AIV only, mean value of various works*
Scolopax rusticola	Dfb	NB	M+F	A	Py	?	M	309	43	1	9.3	[102, 152], Yeasts only*
	Dfb	NB	M+F	Y					62	1	1.6	[102], Yeasts only*
Coturnix coturnix	Dfb	NB	M+F	A	Pm	26%EPP	M	112.5	29	0	0	[76, 102, 153], Yeasts only*
	Dfb	NB	M+F	Y					28	1	3.4	[102], Yeasts only*
	Cfa	YR	M+F	Y					145	1	0	[87], AIV only, mean value of various works*
Fulica atra	Dfb	NB	M+F	Y	M	?	M	735.3	42	1	59.5	[102, 154], Yeasts only*
	Cfb/Dfb	YR	M+F	Y					445	1	1.8	[87], AIV only, mean value of various works*
Eudyptes chrysolophus	Ef	B	M+F	Y	M	?	M	5500	100	1	3	[76, 148, 155], *Campylobacter jejuni* subsp. *jejuni* only*

* = Value of richness not used in the analyses as the study only targeted one specific pathogen. TS = Time of sampling: B (breeding), YR (year round), NB (non-breeding). SH = Sex of host. AH = Age of host: A (adult), Y (young), J (juvenile), N (nestling). SD = Sexual plumage dichromatism: M (monochromatic), D (dichromatic). BM = Body mass in grams. HSS = Host sample size, Infr. = infrequent, Fre. = frequent. R = Parasite species richness. MP = mean value of prevalence (%).

to increase, microparasite richness also tends to increase, although marginally not significantly so (P = 0.08). This suggests that the non-significant trend (P = 0.19) for an increased level of cloacal microparasite richness with polygamy shown in Table **4**, may be at least partially explained by body size effects. Given the low level of statistical significance of the results, however, this effect does not seem to be very important.

Evolutionary trends towards increased sexual dichromatism are marginally associated with evolutionary trends towards decreased microparasite richness (P = 0.08). This result would be expected if sexually dichromatic species have either a better immune system that defends them against infection by various microparasitic taxa and/or have evolved mate choice behaviour that prevents them from being infected in the first place.

Although evolutionary trends towards increased cloacal microparasite richness are associated with evolutionary trends towards increased cloacal microparasite prevalence, the association is marginally non-significant (P = 0.10).

If cloacal microparasites are mainly transmitted via the sexual route, then we would expect that both prevalence and richness would be higher during mating periods of the year. For the species that were sampled during both mating and non-mating periods (note that in some pairings either the "mating" or the "non-mating" period, but obviously not both, may have been represented by year-round data, see Table **2**) the difference in both prevalence and richness is not significant (Wicoxon signed rank test: P = 0.15 for prevalence and P = 0.26 for richness). Moreover, if anything, the qualitative trend for both variables is for the non-mating periods of the year to be associated with higher cloacal microparasite prevalence and richness values than mating periods (see Table **2**).

DISCUSSION

The main objective of this work was to answer the question of whether cloacal microbes are distributed across host species in a manner expected from the action of mechanisms of sexual transmission. The results of the comparative analysis indicate that the answer is "to some extent".

Table 3. List of the Potentially Pathogenic Microorganisms Found in the Avian Cloaca that were Included in the Analyses (see Table 2 for References).

VIRUSES
Orthomyxovirus (the bird cloaca can harbour a variety of other viruses as well)
FUNGI
Yeasts
Candida albicans, C. famata, C. tropicalis, C. pelliculosa, C. inconspicua, C. ciferri
Cryptococcus neoformans, C. laurentii
Rhodotorula rubra
BACTERIA
Gardnerella vaginalis
Listeria denitrificans, L. grayi, L. innocua, L. monocytogenes
Micrococcus lylae, M. roseus
Chlamydophila psittaci
Enterococcus faecalis, E. faecium, E. durans, E. gallinarum
Streptococcus faecium, S. faecalis
Staphylococcus warneri, S. xylosus, S. uberis, S. Simulans, S. lactis, S. aureus, S. hominis, S. epidermalis, S. cohnii
Hafnia alvei
Escherichia coli, E. vulneris, E. hermannii
Salmonella typhimurium
Bacillus circulans, B. laterosporas, B. licheniformis
Yersinia enterocolitica, Y. intermedia, Y. pseudotuberculosis
Mycoplasma gallisepticum
Enterobacter cloacae, E. sakazakii, E. agglomerans, E. aerogenes, E. hafniae
Proteus vulgaris, P. mirabilis
Providencia rettgeri, P. alcalifaciens, P. stuartii
Plesiomonas shigelloides
Pseudomonas pseudomallei, P. aeruginosa, P. putrefaciens, P. maltophila
Citrobacter freundii
Campylobacter fetus subsp. *jeujuni*
Klebsiella pneumoniae, K. ozaenae, K. oxytoca
Edwardsiella hoshinae
Alcaligenes faecalis
Pantoea agglomerans
Serratia fonticola, S. liquefaciens, S. marcescens, S. odorifera
Acinetobacter calcoaceticus anitracus
Aeromonas hydrophila
Morganella morganii
Vibrio cholerae

Some authors also mention broader categories of taxa such as fungi, yeast, anaerobic bacteria, aerobic bacteria, non-lactose fermenters, dark lactose fermenters, red lactose fermenters, coliforms. In addition, authors who do identify microorganism taxa in more detail may sometimes limit their identification to the Genus level. In this work this was the case for: *Candida* sp., *Kloeckeria* sp. among the yeasts and *Clostridium* sp., *Listeria* sp., *Micrococcus* sp., *Chlamydia* sp., *Streptococcus* sp., *Staphylococcus* sp., *Corynebacterium* sp., *Salmonella* sp., *Bacillus* sp., *Yersinia* sp., *Mycoplasma* sp., *Enterobacter* sp., *Proteus* sp., *Providencia* sp., *Pseudomonas* sp., *Citrobacter* sp., *Campylobacter* sp., *Klebsiella* sp., *Pasteurella* sp., *Pantoea* sp., *Serratia* sp. among the bacteria. Cases of infection reported in this manner were also included in the analyses.

Polygamy was expected to be associated with higher levels of richness of parasitic taxa and prevalence than mating systems characterized by more monogamous behaviour. The trends in the results are all in the expected direction, but they are not statistically significant (Table **4**). The same qualitative, but non-significant results were obtained for the positive correlation between levels of EPCs and both richness and prevalence of cloacal microparasites (Table **4**).

Sexual dichromatism is expected to be associated with both polygamy and EPCs, which is what I found if we just

Table 4. Results of Correlations Between Phylogenetically Independent Contrasts of a Series of Parasitological, Morphological and Life-History Variables in Birds

Variables	*N*	*r*	*P*
Climate *vs* Mating System	53	0.002	0.48
Climate *vs* EPCs	35	0.151	0.18
Climate *vs* Sexual Dichromatism	55	0.104	0.22
Climate *vs* Log-Body Mass	55	0.004	0.48
Climate *vs* Microparasite Richness	31	0.450	**0.005****
Climate *vs* Microparasite Prevalence[1]	55	-0.100	0.23
Mating System *vs* EPCs	35	-0.024	0.44
Mating System *vs* Sexual Dichromatism	53	0.037	0.39
Mating System *vs* Microparasite Richness	31	0.161	0.19
Mating System *vs* Microparasite Prevalence	53	0.053	0.34
EPCs *vs* Microparasite Richness	23	0.164	0.22
EPCs *vs* Microparasite Prevalence	35	0.065	0.35
Sexual Dichromatism *vs* EPCs	35	0.154	0.18
Sexual Dichromatism *vs* Microparasite Richness	31	-0.246	0.08
Sexual Dichromatism *vs* Microparasite Prevalence	55	0.028	0.41
Log-Body Mass *vs* Mating System	53	0.648	**<0.0001*****
Log-Body Mass *vs* EPCs	35	0.141	0.20
Log-Body Mass *vs* Sexual Dichromatism	55	0.081	0.27
Log-Body Mass *vs* Microparasite Richness	31	0.250	0.08
Log-Body Mass *vs* Microparasite Prevalence	55	-0.044	0.37
Microparasite Richness *vs* Microparasite Prevalence	31	0.228	0.10

[1]In fact, square root (plus one)-corrected prevalence.

look at the trends shown in Table **4**, but those trends are not statistically significant. However, they are consistent with the non-significant trend for microparasite prevalence to be positively associated with sexual dichromatism. Together these results suggest that as species evolve polygamy, EPCs and sexual dichromatism, that is, as the evolution of the host species is driven more and more by sexual selection, then evolutionary changes may also occur that could result in higher prevalence values of cloacal microparasites. This is a result that is consistent with both the facilitiation of transmission of cloacal micropathogens under polygamy and also the evolution of host's counter-adaptations that tend to limit the risk of infection during sexual intercourse. Polygamy and EPCs may increase the risk, but honest sexually selected signals may decrease it. Whether those signals may also be manipulated by the parasite in order to facilitate its transmission is certainly a possibility especially in the case of the less virulent species and/or strains. At this stage, however, these possibilities remain speculative, requiring more detailed tests than the one I carried out here.

The results for richness of cloacal microparasitic taxa complement the above results in that although sexual dichromatism is marginally non-significantly (P = 0.08) and negatively correlated with microparasite richness, a convex quadratic relationship is even better able to fit the data (r^2 = 0.12, P = 0.02). This means that higher levels of cloacal microparasite richness can be associated with either very low or very high levels of sexual dichromatism, but not so much with intermediate levels of sexual dichromatism. What can explain such a pattern? Perhaps the ability to recognise infected sexual partners is low in monochromatic species, whereas in highly dichromatic species, although such ability may be higher, the effects of EPCs, polygamy or both may nonetheless increase transmission rate and host invasion by many different parasitic taxa.

A matched-pair analysis between mating and non-mating periods of the year for both prevalence and richness of cloacal microparasites indicated that there is a non-significant trend for those parasitological values to be higher during non-mating periods. This clearly suggests that broad studies of the cloacal microflora - that includes both taxa that may be more specialised on the reproductive system and those that may be more specialised on the digestive system - although useful at the initial stages of a research program,

may be too coarse to detect the fine adaptations expected between hosts and sexually transmitted parasites. Such adaptations are more likely to be detected in host-parasite systems involving specialist sexually transmitted micropathogens such as *Chlamydia*, *Mycoplasma* and others. Future empirical studies should focus on those more specialised taxa.

CONCLUSION

Although comparative tests of the kind performed here have their limitations with regard to the quality of the data being utilised, they remain the chief approach to explore broad evolutionary trends in the evolution of specific traits and to test adaptive hypotheses in a phylogenetic perspective. In addition, comparative analyses have an exploratory function that allows the fine tuning of subsequent observational or experimental research. Although in the present study only a handful of correlations were statistically significant, some interesting trends have been uncovered. Most importantly, cloacal microparasites are affected by and may in turn affect the dynamics of sexual selection as it unfolds during the evolution of mating systems, secondary sexual traits and mate choice. On the other hand, the extent to which sexually transmissible cloacal microparasites are capable of directly modifying host secondary sexual traits and behaviour in order to enhance their probability of transmission is an area that remains poorly explored, and it should be studied experimentally using specialist ST-micropathogens.

ACKNOWLEDGMENTS

I am very grateful to Pete Thrall for his shrewd comments on an earlier version of the manuscript and to The Open Ornithology Journal for publishing this special issue.

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Received: May 10, 2009 Revised: June 15, 2009 Accepted: June 16, 2009