Brevi note - *Short communications*

Escherichia coli infection in house sparrows: are there implications for sexual selection?

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Riassunto – *Prevalenza di* Escherichia coli *nella passera europea e potenziali implicazioni per la selezione sessuale*. In questo studio si riporta la prevalenza di infezioni cloacali da *E. coli* nella passera europea. Su un campione di 30 individui (17 maschi, 13 femmine), il 33.3% è risultato infettato da *E. coli*. Non è stata riscontrata una significativa differenza nella prevalenza di infezione di maschi (41.1%) e femmine (23%), nonostante le cospicue differenze osservate. Inoltre, le femmine con la placca incubatrice sviluppata mostravano una tendenza verso una maggiore prevalenza di infezione (37.5%, 3/8) rispetto a femmine senza placca (0%, 0/5), e i maschi infetti tendevano a mostrare un 'bavaglino' di dimensioni ridotte. Le tendenze osservate in questo studio meritano ulteriori approfondimenti, in quanto coerenti con la '*Contagion Indicator hypothesis*'.

Sexually transmitted diseases (STDs) may have important consequences for the evolution of behaviour, reproductive physiology, and some secondary sexual characteristics (Sheldon 1993). In birds, the prevalence of sexually transmitted cloacal microorganisms (STCMs), some of which may be pathogenic and therefore cause STDs, is in part determined by the level of sexual contact, and individuals that are promiscuous are more likely to become infected with a STCM (Thrall *et al*. 1997). If the disease has direct negative fitness consequences on sexual partners, this may lead to a large cost of individual behaviour that then is likely to lead to strong mating skews.

The Contagion Indicator hypothesis (CIH) (Able 1996) proposes that the degree of expression of variable male traits indicates the infestation by 'associatively transmittable' parasites. An associatively transmittable parasite can be transmitted during mating or courtship (Able 1996). Thus, individuals with more conspicuous secondary sexual traits should be less parasitised.

In this work we studied *Escherichia coli* infection of house sparrows *Passer domesticus*, in order to specifically test the predictions derived from the Contagion Indicator hypothesis. *E. coli* is a gram-negative bacterium that is normally found in the intestinal flora of vertebrates, but can still cause colibacillosis (i.e. a localised or systemic infection) (Barnes and Gross 1997), and it can be transmitted sexually through contamination of semen (see Poiani 2003 for a recent review). The house sparrow is a dimorphic semi-colonial passerine, which is socially monogamous, but can be facultatively polygynous (Whitekiller *et al*. 2000). Males develop a bib of black feathers on their throat and chest during their annual moult in autumn, which signals social dominance (Møller 1987a, b, 1988). In some populations females seem to prefer large-bib males (Møller 1988), while in others the small-bib males are preferred by females (Griffith *et al*. 1999).

Field-work was conducted between mid September and mid December 2002 at Chesterfield Farm, about 30 km East of Melbourne, Australia. Adult birds were trapped by mist-netting and metal banded for future identification. After banding, some morphometric and other measurements were taken from each bird. Body mass was measured (to the nearest 0.1g) with a Pesola spring balance. Wing length and tail length were measured with a metallic ruler to the nearest 1 mm, whereas tarsus length and culmen depth were measured with a calliper to the nearest 0.1mm. Brood patch was given a score from 0-3 depending on its development (Poiani 1993).

Male house sparrows have a black throat and upper breast feathers forming a bib, whereas females

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have a dull white breast and underparts. The bib core area (darkest area of the bib) and bib total area were measured by tracing them onto acetate with a permanent fine-point marker. The total bib contour and core bib contour were then cut out and the acetate weighed to the nearest 0.1 mg. A standard curve for acetate area (cm^2) vs. weight (g) was constructed (Area = 0.451 + 53.317 $\log_{10}$(Weight + 1), $r^2 = 0.946$, $n = 9$, $F_{1,7} = 145.99$, $P = 0.00001$). The curve was then used to determine bib area from the weight of the acetates. Total bib area showed a repeatability (Lessells and Boag 1987) of 75% and core bib area of 78%. Bib measurements were taken by the same observer. Mean ± standard deviation are given throughout, all tests are one-tailed.

A cloacal swab was also taken from each bird to test for the presence of *E. coli,* and stored in a 1ml cryotube with 0.5 ml of RLT buffer solution (4M Guanidine thiocyanate, 15mM Pipes, pH 6.7). The cryotubes were transported to the laboratory approximately 2 hours after collection and frozen at –70°C. A 5 μL aliquot of β-mercaptoethanol was added to each cloacal swab sample in the RLT buffer. They were subsequently vortexed, and a 250 μL aliquot was added to tubes containing 300 μL of 70% ethanol. A total of 15 μL of QiaexII matrix (Qiagen) was then added to each tube. The matrix was resuspended by gently pipetting, and the entire volume was added to a spin column. Columns were centrifuged for 30 seconds at 10000 rpm in a microcentrifuge, and the flow-through discarded. The columns were placed into fresh 2 ml microfuge tubes, and washed twice with 500 μL of RPE buffer (10mM Tris, pH 7.6, 1mM EDTA, pH 8.0, 80% ethanol). The tubes were centrifuged each time for 30 seconds at 10000rpm. The tubes were then centrifuged for an additional 2 minutes at 13000 rpm to remove any excess ethanol. The columns had 30 μL of double distilled water (ddH_2O) added to them, with tips changed each time to avoid contamination. Tubes were incubated for 1 minute at room temperature, followed by centrifugation for 1 minute at 10000 rpm to elute the nucleic acid from the column. The final product was stored at 4°C temporarily or at –70°C for long-term. *E. coli* oligonucleotide primers (Genbank accession number: AJ404543): 16Srev and 16Sfwd used for PCR were acquired from a commercial source (Geneworks Pty Ltd).

PCR was carried out in 25 μL reactions containing 5 μL extracted DNA sample in 1 × *Taq* DNA polymerase buffer, 2.0 mM $MgCl_2$, 200 mM of each dNTP, 1.0 mM of each primer, and 1.25 U *Taq* DNA polymerase. Contamination of reactions by PCR product was avoided by using different working areas. Reactions underwent an initial denaturation period of 3 minutes at 94°C, then were subjected to 35 cycles of one minute at 94°C, one minute at 55°C, and one minute at 72°C, and then one cycle of ten minutes at 72°C, using a Bio-Rad iCycler thermal cycler (Hercules, California). The *E. coli* strain DH5 α/5 (Ginns *et al.* 2000) was used as a positive control, and double distilled water was used as a negative control. A 5 μL aliquot of each reaction was mixed with 2 μL of loading buffer (50% glycerol, 0.125M EDTA, 0.25% Bromophenol Blue) and subjected to electrophoresis through a 2% agarose gel. Samples were considered positive when a band of the expected size was clearly visible after staining with ethidium bromide and viewing with ultraviolet transillumination. Molecular weight markers λ*Hind* digested with (*Hae*)III, and a 2-Log DNA ladder were used to estimate the size of expected band.

We sampled 30 individuals, with a total of 10 individuals testing positive to *E. coli*, resulting in a prevalence of 33.3%. None of the birds were symptomatic for colibacillosis, that is they did not display symptoms such as salpingitis (i.e. caseous mass visible in the cloaca), ocular lesions such as panophthalmitis or swollen head syndrome.

Tail length was chosen as the indicator of body size as it was the body size variable with the highest mean correlation coefficient with all other morphometric variables. The CIH predicts that larger males should be less parasitised than smaller males. Tail length was not significantly different between males infected with *E. coli* (57.0 mm ± 1.6, $n = 7$) and not infected males (58.0 mm ± 2.1, $n = 10$) ($t_{15} = 1.032$, $P = 0.159$). Body condition was also calculated, by regressing the cube root of body mass against tail length (cube root of body mass = 2.274 + 0.013 tail length, $r^2 = 0.135$, $F_{1,15} = 2.349$, $P = 0.146$). Body condition was expressed as the residual of cube root body mass (expected value – observed value). Mean residual body mass (cube root) was calculated for males infected with *E. coli* (0.012 $g^{1/3} \pm 0.023$, $n = 7$) and non-infected males (-0.032 $g^{1/3} \pm 0.019$, $n = 10$). The CIH predicts that infected males should have a poorer body condition. Infected males did not significantly differ in their residual body mass from non-infected males ($t_{15} = -1.465$, $P = 0.164$), although the trend was for infected males to have a larger body mass than expected from their tail length, whereas