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 $g^{1/3}$ ± 0.023, $n = 7$) and non-infected males (-0.032 $g^{1/3}$ ± 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 non-infected males tended to have body mass values lower than expected from their tail length.

The results show that males had a larger body size (tail length: 57.5 mm ± 1.9, $n = 17$) than females (55.2 mm ± 2.1, $n = 13$), the difference being significant (two-way ANOVA: $F_{1, 26} = 7.444$, $P = 0.011$). However, $\log_{10}$-transformed tail length did not differ between *E. coli* infected (56.4 mm ± 2.3, $n = 10$) and non-infected individuals (56.6 mm ± 2.3, $n = 20$) ($F_{1, 26} = 0.570$, $P = 0.457$). The interaction between sex and infection was also not significant ($F_{1, 26} = 0.165$, $P = 0.688$).

The CIH predicts that infected males should have smaller bibs. House sparrows that tested positive to *E. coli* had slightly smaller total bib sizes (2.2 cm^2 ± 0.2, $n = 7$) than birds testing negative for the microorganism (2.5 cm^2 ± 0.3, $n = 10$). This was also true for the core bib area, with non-infected individuals having a mean of 1.1 cm^2 ± 0.4, $n = 10$, whereas infected birds had a mean of 1.0 cm^2 ± 0.3, $n = 7$. The trend approaches significance for total bib size ($t_{15} = 1.651$, $P = 0.059$) but not for core bib size ($t_{15} = 0.201$, $P = 0.421$).

Mean residual bib sizes were calculated for total bib size for males infected with *E. coli* (-0.11 ± 0.25, $n = 7$) and uninfected males (0.08 ± 0.33, $n = 10$). Residuals were also calculated for core bib size for infected males (0.01 ± 0.35, $n = 7$) and uninfected males (-0.009 ± 0.437, $n = 10$). Differences between residual bib sizes of infected and non-infected male house sparrows were marginally not significant for total bib size ($t_{15} = 1.344$, $P = 0.099$) and not significant for core bib size ($t_{15} = -0.103$, $P = 0.460$). Total bib size was not significantly correlated with tail length ($r_{15} = 0.320$, $P = 0.211$).

The CIH also predicts that males should be more parasitised than females in this species. There were 41.18% (7/17) of males infected with *E. coli* compared to only 23.08% (3/13) of females, however this difference was not significant (Fisher's exact test: $P = 0.440$). Breeding females engaged in copulations should be more parasitised than non-breeding females. Females with a brood patch score of 0 (indicating non-breeding) had 0% (0/5) prevalence of *E. coli*, whereas females with a developed brood patch (score larger than 0, indicating breeding condition) had a prevalence of 37.5% (3/8). This difference was not significant however (Fisher's exact test: $P = 0.231$). None of the birds showed evidence of primary moulting.

The trend detected in non-infected males to have larger mean bib sizes than infected males is consistent with the Contagion Indicator hypothesis, if it is assumed that females prefer large-bib males in this population. The result, however, was statistically not significant. An alternative Cost of Copulation hypothesis, which predicts that attractive partners (i.e. those displaying more conspicuous secondary sexual traits) that engage in multiple copulations may be more likely to be infected with STCMs and therefore they may be paying a cost of copulation (e.g. Thrall *et al*. 1997, Poiani and Wilks 2000) is not consistent with the observed trends.

Although it is not known which size of bib females in this population prefer, it is possible that it is the males with larger bib sizes. This hypothesis is supported by the trend detected for bib size to slightly increase with body size. It has been suggested that bib size is an indication of physical condition, and that this condition tends to improve with age (Veiga 1993). Larger males are usually older, and older males are generally preferred for copulations (Møller 1988, Veiga 1993).

The relatively poorer body condition of the non-infected males with larger bibs may be explained by their expenditure of energy in reproductive activities (Vehrencamp *et al*. 1989), as the birds were sampled during the breeding season. Courtship with females and mate guarding (Møller 1990), high levels of aggressive male-male interactions (Evans *et al*. 2000), brood care and nest defence (Montgomerie and Weatherhead 1988) may all be costly activities for large-bib males. This energetic cost may be reflected by large-bib males in a loss of fat reserves during the breeding season, whereas small-bib males that may be less successful at gaining mates may put their energy into feeding thus improving their body mass.

The difference in bib sizes may be influenced by the circulating levels of testosterone (Evans *et al*. 2000). Testosterone, an androgen mainly secreted by the male gonads, can be immunosuppressive (see Folstad and Karter's 1992 for a review), which is in contradiction with our results, as large-bib males tended to be less infected than small-bib males. Evans *et al*. 2000, however, suggested that testosterone may be immunoenhancing in *P. domesticus* males, whereas the steroid corticosterone, which has also melanogenic effects (Poiani *et al*. 2000), would be immunosuppressive.

Male *P. domesticus* tended to be more infected with *E. coli* than females in this study. This may be the result of either greater male inability to fight off an infection once it becomes in contact with a pathogen or greater exposure to *E. coli* than females, or both.

Females with developed brood patches tended to be more infected (37.5%) than females with undeveloped brood patches (0%). The brood patch is under prolactin control (El Halawani and Rozenboim 1993) and this has been reported to have immunoenhancing properties in both birds and mammals (Rodriguez *et al*. 1996), which is in contradiction with the trend found in the results. However, females with developed brood patches are likely to be engaged in copulations, which argues for a direct sexual route of transmission of *E. coli*.

In conclusion, the preliminary trends reported here are consistent with a potential relevance of sexually transmitted cloacal microparasites in sexual selection. However, firmer conclusions could be reached in the future after increasing the sample sizes and therefore the statistical power of the tests.

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Artificial lights and mortality of Cory's shearwater *Calonectris diomedea* on a Mediterranean island

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Riassunto – *Luci artificiali e mortalità della berta maggiore* Calonectris diomedea *su un'isola mediterranea*. Si riporta il rinvenimento di giovani berte maggiori, appena involate dal nido, che erano state attratte da faretti recentemente attivati all'interno del porticciolo dell'isola toscana di Pianosa. Il fenomeno, che in assenza di interventi avrebbe potuto portare alla perdita almeno del 12% del totale di giovani involati nel 2004, è ben noto per la sua gravità al di fuori del Mediterraneo. Si sono sperimentate con apparente successo forme di illuminazione alternativa.

The phototactic behaviour of many nocturnal Procellariiformes, especially fledglings on their first take off from the natal colonies, can have lethal consequences as they can be attracted by bright artificial lights. Disoriented fledglings that crash into buildings, rocks or ships often die on impact or are forced to land at unsuitable sites, where they become victims of predation, road traffic or starvation (Reed *et al*. 1985, Telfer *et al*. 1987, Le Corre *et al*. 2002). This problem is known to occur especially on foggy nights and has been recorded for several species and in different geographical situations (Warham 1996).

This source of mortality is known to affect the Cory's shearwater *Calonectris diomedea* in the Azores and Canaries (Monteiro *et al*. 1996). It has not been reported for this or other petrel species in the Mediterranean (cf. Thibault *et al*. 1997, Cadiou *et al*. 2004), although lights placed close to colonies are known to disturb incoming adults of Cory's shearwater in the Maltese islands (up to the point of site desertion, Borg and Cachia-Zammit 1998) and Balearic shearwater *Puffinus mauretanicus* in the Balearics (causing behavioural anomalies, Ruiz and Martí 2004). Dazzled juveniles of Cory's have been occasionally found on Malta (J. Borg, pers. comm.).

Here we report on a wreck of Cory's shearwater fledglings on Pianosa Island (42.35N-10.06E), 60 km off the Italian mainland coast and 40 off Corsica, France. Pianosa covers an area of 10.2 km^2 and was the site of a large state prison for more than a century, until 1998. It is now included in the Tuscan Archipelago National Park and is still closed to unauthorized visitors. The human population in the years 2000-2004 was less than 15, including personnel of different guard corps and 5-10 prisoners. Apart from the prison, there is only an abandoned village on the eastern coast, with a small port and an automatic lighthouse. Former prison buildings are scattered through the inner part of the island. The area has been totally dark at night over the last few years, except for dim street lights in the village, the nearby lighthouse and a single beam light 1 km inland, where the prisoners are presently lodged.

Pianosa has nearly lost its breeding shearwater population as a result of millennia of human presence and the consequent arrival of alien predators (cf Blondel and Vigne 1993 for details on this problem in the Mediterranean). Long-abandoned nesting sites can still be recognized in caves all around the island, but only 10-15 occupied nests of Cory's Shearwater are presently known from the southern coast (2.5 km from the village), and these may possibly represent a recent return. On the other hand, a larger colony of this species - known since 1877 (Baccetti 1989) - survives on a stack ('La Scola'), 200 m offshore and

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500 m from the village. La Scola was the most likely origin of some fledglings that were rescued while hanging around the village (5 birds) or found dead under walls (at least 2 birds), on 5-6 nights from 6-18 October 2004; feral cats, in the same period, took at least two more of the dazzled birds. Some disoriented shearwaters were also observed at night, continuously circling over the illuminated port, while it was raining; their fate could not be ascertained. No real fog was present throughout the wrecking period, but moonless nights (new moon on 14 October), overcast sky and rainy conditions prevailed. The dates coincided exactly with the usual fledging period of the Cory's shearwater on nearby Corsican islets (Thibault *et al*. 1997). All nine birds examined were fledglings. The surviving ones were successfully released from the seashore a few hours after they had been collected.

Two 400 W halogen beams, set to light the port permanently from adjacent rooftops, were the main cause of shearwater attraction. These were not directly visible from La Scola colony and had only been in use in that year, following a decision to keep a patrol vessel parked in the harbour for emergencies. Urgent requests to keep the lights switched off unless really needed, hood the lamps or replace them with less powerful ones, all received a negative response from the prison authority. As a compromise, the existing bulbs were eventually replaced on 19 October with equally powered high-pressure Sodium lamps, emitting a softer and hopefully less disturbing yellow light. No birds came ashore on the following nights till at least 1 November, but it is unclear to what extent other circumstances could have played a role in halting the phenomenon. The end of the fledging season (Thibault *et al*. 1997), a slight improvement in weather conditions, as well as a change in the phase of the moon, suggest that the losses would have diminished anyhow. Nocturnal brightness due to clear sky and waxing/full moon phase has been shown to decrease the activity of nocturnal petrels ashore (Mougeot and Bretagnolle 2000), as well as their attraction to artificial lights (Reed *et al*. 1985).

Burrow monitoring at La Scola colony in the 2005 breeding season suggested a total population size of 80-100 pairs. Breeding success of 0.85 chicks/pair was assessed one week before the earliest chicks fledged (P. Sposimo, unpubl. data). Assuming that all the stranded birds were found and that none of them would have been able to reach the sea without human intervention, birds dazzled by artificial lights on their maiden flights would represent at least 12% of the juveniles fledged in 2004. The local topography suggests that both assumptions are probably realistic, or at least that missed birds were mainly those which managed to orient themselves correctly among buildings and reach the sea on their own before dawn. Although the potential loss for the population nearly equalled all sources of breeding failure recorded at the egg/chick stage in the same year, it was lower than in other case studies (e.g. 20% for *Pterodroma baraui* on Réunion island, Le Corre *et al*. 2002).

Much heavier losses, moreover, occurred on La Scola until recently. The productivity of the colony had been restored in 2001 by means of a successful campaign to eradicate the black rat *Rattus rattus* (Perfetti *et al*. 2001). No fledged birds were recorded in 1999 and 2000, or on an earlier occasional survey in 1989 (i.e. possibly in most years since the rats reached this stack, around 1980). After the rats were poisoned, the breeding success suddenly reached values very close to the theoretical maximum in all the following seasons (0.77-0.90 fledged chicks/pair, 2001-2004). No shearwaters were wrecked on Pianosa village in autumns 2001-2003, when breeding was successful, qualified observers were present, but the harbour lights were not being used (the lighthouse and street lights, of course, were in use). In addition, no relevant wrecks could have occured in the previous two decades, because hardly any chick fledged at La Scola. Much more powerful and widespread lights must have been present in 1980-1989 when the prison was at its full capacity (human population size: 1500) and especially in 1992-1997 when a strictly surveyed confinement section was present (Piga and Foresi 2001). Memory about possible seabird wrecks in earlier times, i.e. before the rats colonized the stack, is lost. But, then, powerful lamps were probably not as easily available as at present and the electric energy was produced on the island in the strictly needed amount (a submarine connection has allowed a large energy supply only since 1990).

The future destiny of the island of Pianosa is still a matter of political discussion. Whatever decisions are taken, it is very unlikely that the human population will remain for a long time at its present low level. New inhabitants will most probably imply that the existing buildings will be restored and obsolete street lights replaced. A new hazard might, thus, face