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Faecal *Escherichia coli* and *Chlamydophila psittaci* in the Superb Lyrebird *Menura novaehollandiae*: host sex and age effects

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Abstract. The Superb Lyrebird is a sexually dimorphic passerine that although is not considered endangered, it has been declining in population size since the 1940s due primarily to urban development. Recent reports suggest that lyrebirds may be threatened by chlamydial infection. We studied levels of faecal infection by two microparasites in lyrebirds: *Chlamydophila psittaci* and *Escherichia coli* in the Sherbrooke Forest, south-eastern Australia. Fresh faecal samples were obtained from 33 lyrebirds (15 adult females, 13 adult males and 5 juveniles) — estimated of 27.5% of the population, all of them tested negative to *Ch. psittaci*. *E. coli* prevalence was compared between adult males and females and no difference was found. This result is expected, for instance, if *E. coli* is sexually transmitted and lyrebirds are promiscuous. Trends for juveniles to be more parasitized than adults were detected, but they were statistically not significant. Behavioural analyses of video footage indicate that *E. coli* infected birds did not allocate more or less time to any of the activities considered than did non infected birds. This might suggest that *E. coli* infection in lyrebirds is relatively benign, and behavioural effects may thus be subtle. No significant differences were found in specific measurements of foraging behaviour but non infected birds tended to scratch more frequently than infected birds.

Key words: Superb Lyrebird, *Menura novaehollandiae*, pathogens, *Escherichia coli*, bacteria, *Chlamydophila psittaci*, Chlamydia

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INTRODUCTION

The relationship between animals and their parasites can be very complex. All animals have parasites, but the level and ways parasites impact on their hosts vary (Loye & Zuk 1991, Clayton & Moore 1997). The study of host-parasite relationships has gone through important developments in the last decade (Zuk 1992, Loye & Carrol 1995, John 1997). Research has focussed on the effect parasites have on physiology, survival, reproduction and behaviour of their hosts and the defences hosts develop to protect themselves against parasite infection (see Clayton & Moore 1997 for a review). Some general trends indicate that hosts and parasites undertake co-evolutionary arms-races which may lead to extinction of the parasite, the host, or both, or to the coexistence of the two (Hamilton & Zuk 1982, Fenner & Kerr 1994, Waldenstrom et al. 2002). Co-evolutionary arms races may result in adaptations of the taxa engaged in the interaction, thus the parasite may evolve new mechanisms that enhance its ability to exploit its host (Dwyer et al. 1990, Fenner & Kerr 1994) and the host may evolve new or better defence mechanisms against the parasite (Van Riper et al. 1986, Moore 1995).

Through their negative effects on reproduction and survival of individuals, parasites may have negative effects on population sizes and viability of a range of animals, including birds (Spalding & Forrester 1993, Dobson & Hudson 1995, Banko et al. 2001, Biggins & Kosoy 2001, Cassinello et al. 2001, Baker et al. 2002; but see Shutler et al. 1996).

Male birds seem to be more heavily parasitised than females in some species (Borgia &

Collis 1989, Rozsa et al. 1996), perhaps as a result of interactions between steroid hormones and the immune system (Zuk & Maclean 1996, Hillgarth & Wingfield 1997). However, other studies have not detected sex biased parasitism in birds (Potti & Merino 1995, Lee et al. 2004, Proctor & Jones 2004), whereas other works have found a female bias in parasitism (Wheeler & Threlfall 1986).

One category of parasites that has received relatively little attention so far is that represented by the sexually transmitted cloacal microorganisms. Harmful bacteria, viruses and yeast are known to reside in the cloaca of avian species. The cloaca is the route for faecal as well as semen discharge, giving cloacal microorganisms the potential to be sexually transmitted in birds (Poiani & Wilks 2000a, 2000b, Poiani & Gwozdz 2002, Poiani 2002). Cloacal contact during copulation in birds tends to bias transmission of venereal microorganisms from male to female, although the final level of infection detected in both sexes will be also a function of the number of partners individuals of each sex have and the ability of males and females to clear their body from invading microparasites (e.g. through activation of their immune system).

As well as sex, age classes can also show differences in the level of parasite infection. Juvenile birds may be more infected than adult birds, due, for instance, to their immune system not being fully developed (Lung et al. 1996, Zheng et al. 1997, Zheng & Yoshimura 1999). Borgia & Collis (1989), studying male Satin Bowerbirds *Ptilonorhyncus violaceus* found older males to have fewer parasites than younger males. In addition to sexual transmission, cloacal microorganisms also have the potential to be transmitted through faecal contamination in the soil, especially at high population densities of the host (Poiani & Wilks 2000b). This effect will be compounded in species that are ground foragers. Moreover, young birds may also have had parasites passed on to them from infected mothers through egg contamination. This is most commonly documented in poultry in regards to *Salmonella enteritidis* infection (Suzuki 1994).

Parasites may affect physiological and/or morphological traits of their hosts, but they may also affect the host's behaviour (e.g. Latta 2003). For instance, parasitized birds at risk of decreasing their body condition are expected to increase the time spent in foraging at the expense of other activities such as sexual displays (e.g. Belthoff & Duffy 1998).

Behavioural responses to parasitism have also been studied in the context of sexual selection (Kirkpatrick & Ryan 1991). An infected male may be less successful at attracting females than a parasite-free male, and studies have shown that often females prefer males that are less parasitised (Clayton 1990). If parasitised birds are less efficient at obtaining mates, then they are expected to divert their time and energy to activities more beneficial than sexual behaviours. Activities aimed at overcoming the infection and maintain body condition, such as foraging, should be given preference by parasitized individuals. Finally, if parasitised birds have decreased immune competence due to microbial infection, then they would be more susceptible to secondary infections by ectoparasites, and would hence preen more than uninfected birds. Preening is known to be an anti-ectoparasite defence mechanism in birds (Clayton 1991, Rozsa 1993, Christe et al. 1996).

We aim at studying the degree of *Chlamydophila psittaci* and *Escherichia coli* faecal infection in the Superb Lyrebirds, and to determine the age, sex and behavioural correlates of infection.

MATERIALS AND METHODS

Species and study site

The Superb Lyrebird is a passerine belonging to the family Menuridae that can be found from southern Queensland to Victoria (Australia) in subtropical and temperate rainforests, and in dry sclerophyll forests (Robinson & Curtis 1996, Higgins et al. 2001). Lyrebirds are considered a polygynous species, exhibiting a dispersed lek behaviour, although there is some speculation in that they may be promiscuous (Kenyon 1972, Robinson & Frith 1981, Robinson 1991). The females care for the young unaided, the male having no involvement in nesting, feeding or raising the chicks (Robinson & Frith 1981).

Lyrebirds are a sexually dimorphic species. Males, females and juveniles differ in size and plumage (Higgins et al. 2001). The two striking features males use in courtship are their spectacular tail plumage (e.g. Smith 1965, 1999, Higgins et al. 2001), as well as their beautiful song, much of which is accurate mimicry of many other bird species (Powys 1995, Robinson & Curtis 1996). These two features are essential to the male lyrebird courting display. The vocal displays are also used by males in territorial defence (Powys 1995, Robinson & Curtis 1996).

Recent research suggest that a factor that may be influencing the decline of some superb lyre-

bird populations may be *Chlamydia*/*Chlamydophila* infection (Holz et al. 2003).

Chlamydiosis, also known as psittacosis and ornithosis, is a ubiquitous disease of birds and is caused by the gram negative bacterium *Chlamydia*/*Chlamydophila* (e.g. *Chlamydophila psittaci*) (Weisburg et al. 1986). The disease affects the eye as well as the respiratory, genital and gastrointestinal tracts (Mardh et al. 1989). The former genus *Chlamydia* has recently been split into two: *Chlamydia* and *Chlamydophila* (Everett 2000).

Ch. psittaci is known to cause disease in a wide range of bird species (Holz et al. 2003), especially psittaciforms (e.g. Crimson Rosella *Platycercus elegans*). Holz et al. (2003) suggested that the Crimson Rosella might be involved in the transmission of *Chlamydophila*/*Chlamydia* in the lyrebird population. However, Archbold (2003) did not detect the bacterium in a sample of 25 Crimson Rosellas.

To date, *Escherichia coli* infection has not been studied in the Superb Lyrebird. However, this may also be a bacterium worthwhile studying in this species as birds have been found to be easily infected by the microorganism (Best et al. 2003, Gordon & Cowling 2003). Pathogenic *E. coli* are usually associated with poultry (Gross 1991) but they may also be found in wild passerines (Gordon & Cowling 2003). The pathogen has adapted very well to surviving in faeces, and has been known to also persist for months in soil and grass (Duffy 2003). There is no account of the diseases *E. coli* causes in wild Australian birds, but experiments conducted by Gomis et al. (1997) found that diseases which resulted from *E. coli* inoculations in chickens were primarily avian cellulitis and septicaemia, along with other lesions. In a recent work, Gordon & Cowling (2003) studied *E. coli* infection in 27 Australian bird species concluding that major variables such as living in close association with humans, body size and diet that account for about 65% of variation in *E. coli* prevalence in mammals, explained only 21% of *E. coli* prevalence variation in birds. This suggests that other variables (e.g. mating system) may affect prevalence of *E. coli* in birds; for instance, Gordon & Cowling (2003) report that one of the highest prevalence values of *E. coli* in their sample was found in *Malurus cyaneus*, a very small (10 g) but highly promiscuous passerine. In birds, they detected strains in the four A, B1, B2 and D *E. coli* groups, all of which are known to be pathogenic in vertebrates (Gordon & Cowling 2003). Although some *E. coli* strains can be pathogenic to vertebrates not all of them are. Given the ability of *E. coli* to survive outside its host, it can potentially be transmitted between

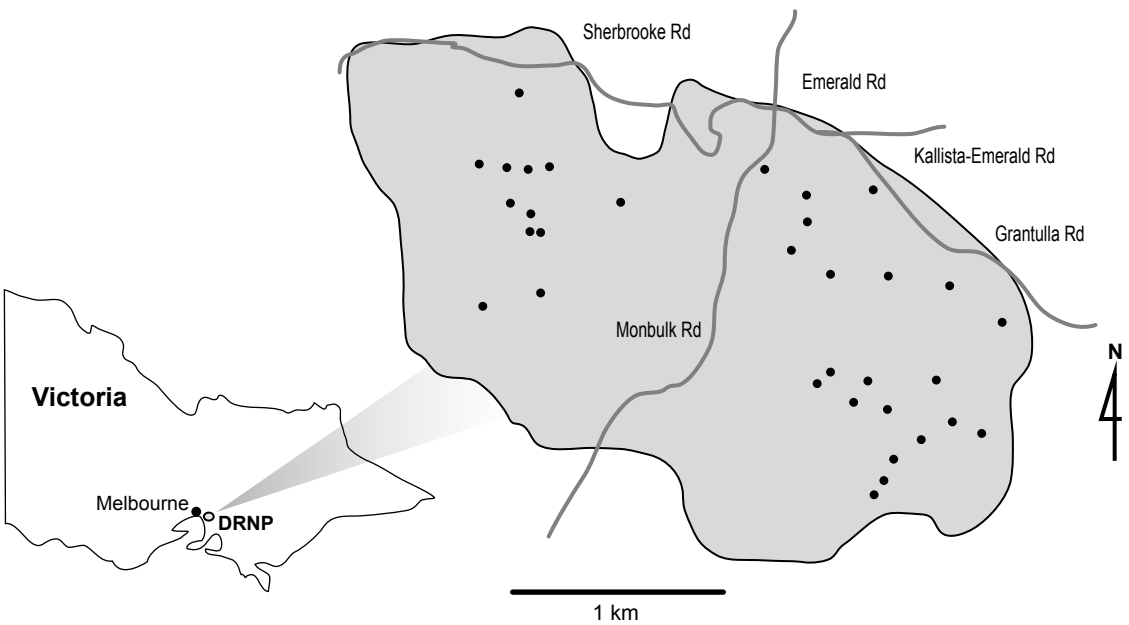

Fig. 1. Location of the Dandenong Ranges National Park (DRNP) in the state of Victoria (Australia) and Sherbrooke Forest map (in the DRNP) showing sampling location of *M. novaehollandiae* faeces.

hosts by at least two routes: directly through sexual transmission and indirectly through soil contamination.

The study was conducted in the Sherbrooke Forest, in the Dandenong Ranges National Park (DRNP) (38°55'S, 145°13'E; see Fig. 1) from July to September 2003. This ensured sampling was carried out during the breeding season. All sampling was conducted from 7.30 a.m. until 1.00 p.m. The estimated population size of lyrebirds in the DRNP is 120 individuals (Lyrebird Survey Group, unpublished data).

Data Collection

For each lyrebird studied a faecal sample was collected and video footage recorded. Faecal samples, rather than cloacal swabs, were taken as lyrebirds are not easily caught. Video footage was obtained in order to carry out behavioural analyses. No two birds of the same class (male, female or juvenile) were sampled in the same location unless banded, and sites were chosen as far away as possible (Fig. 1). To determine the probability of pseudoreplication, an average of male lyrebird home ranges was taken from the literature and found to be just under 300 meters in radius (Smith 1968, Robinson & Frith 1981, Reilly 1988). Thus unbanded, same sex birds found at a distance smaller than 300 m apart where considered likely to be the same individual. Two pairs of adult male birds were found to be within this distance of each other. The same approach was used for female birds. We do not have precise information on size of female territories, but they are smaller than males', thus by taking the 300 m threshold to be valid for females as well we are using a conservative approach. No female birds were found within this distance of each other. Of the five juvenile birds sampled, three of them were banded and could thus be identified as different individuals. Two juveniles were not banded, but were sampled on opposite sides of the park (greater than 3 km apart). Thus, of the 33 birds sampled only two birds were within the possibility of being duplicate samples and so the probability of pseudo replication in the study was only 6%.

A total of 13 male, 15 female and 5 juvenile birds were sampled. Once a bird was located, filming began using a Sony digital handy cam (DCR-VX1000E). If the bird defecated during filming, faeces location was marked by a volunteer standing by, once the bird had moved far enough from the faeces that recording would not be disrupted. Filming continued for 10 minutes or until the bird was out of sight. Then the faeces were collected. If the bird did not defecate within the 10 minute interval, filming was stopped at the end of the 10 minutes period and the bird was watched until it did defecate.

Collection of faecal samples follows Archbold's (2003) procedure. A standard amount of faeces (approximately 0.2 ml) was scooped up using a clean plastic spoon and stored in a 1 ml cryotube. In addition to this, an associated soil sample was also taken for each faecal sample obtained. Soil samples were at least two metres from the faecal sample and were not less than two metres from any other faeces. Soil samples were at least equal in volume to the faecal samples and stored and labelled consistently. All samples were stored in a container with ice for the duration of the rest of the sampling trip. The lyrebird's age, sex, and colour band combinations were recorded, as well as the time the faecal sample was collected. At the end of each field trip faecal samples were delivered to the Victorian Institute of Animal Science (VIAS) in Atwood for analysis and soil samples stored in a freezer at -70°C. Five soil samples associated with positively tested faecal samples were later tested at VIAS. We could only analyse 5 soil samples due to budgetary constraints and we chose soil samples associated with *E. coli*-positive faecal samples in order to maximise the probability of detecting *E. coli* in the soil, thus making this a conservative test of the potential for *E. coli* to be transmitted to the birds through soil contamination. Soil samples were also tested for *Ch. psittaci*.

Microbiological analyses

All faecal samples were processed within two to four hours following their arrival into the laboratory.

Test for chlamydial antigen. Faecal and soil samples were tested for chlamydial antigen using a commercial direct immunofluorescence test (Imagen™ Chlamydia) according to recommendations indicated by the manufacturer (DakoCytomation Ltd, UK). The kit used includes a positive control. Specimens were incubated with the fluorescent isothicyanate (FITC) conjugated reagent for 15 minutes. The excess reagent was then removed by washing with phosphate buffered saline (PBS). Stained areas were then mounted and viewed using epifluorescence illumination to determine if *Chlamydia* was present.

Culture and identification of *E. coli*. A swab of each sample was taken. Samples were then

cultured onto Sheep Blood Agar (SBA) (Oxoid Australia) and incubated aerobically for 24 hours at 37°C. Colonies which were morphologically consistent with *E. coli* were selected for confirmation. Confirmation consisted of culturing on MacConkey Agar No. 3 (Oxoid Australia) and performing the following tests: lysine decarboxylase test, urea broth test, indole test, motility by culture, citrate utilisation, methyl red test and Vogues-Proskauer (VP) test (Quinn et al. 1994).

Colony Forming Units (CFUs); i.e. the number of colonies counted on the growth plate, were expressed as categorical variables in the analyses due to the difficulty of producing exact counts of number of colonies, especially when growth was intense. The categories are as follows: N — no colony growth, L — light colony growth ($0 < L \leq 20$ CFU), M — moderate colony growth (20 CFU $< M \leq 200$ CFU) and H — heavy growth (200 CFU $< H$).

Transcription of video data

Transcription was carried out by RJM. At the time of transcription, she was not aware of the outcome of the microbiological analyses, therefore the behavioural data was transcribed blind.

Time budget. The recording of each bird from videotapes was timed using a stop watch. The section for a given bird was then rewound and played again. This time a stop watch was used to time the periods when the bird was not visible on the tape, and this time was subtracted from the total time to give an exact time that the bird was visible on tape. This was done as behavioural activities sometimes overlapped. The tape was then rewound again and each behavioural activity was timed. The activities were categorised as follows: foraging — scratching the ground and litter for invertebrates; preening — fluffing feathers, shaking tail (not associated with the tail shimmying of courting displays), scratching, and ruffling feathers with the beak; sexual courting behaviour — male lyrebird displaying and performing its traditional courting ritual; sexual fighting behaviour — males or females aggressively attacking and/or squawking at each other; vigilance — head is up, and the bird is looking around; vocalising — singing and other vocalisations not associated with the courting display; and moving — walking, running or flying from one area to another. Time spent in each activity was calculated as a proportion of the total time the lyrebird was visible on the tape.

Foraging. Each footage of foraging bouts was replayed and assessed. Any sections showing a good, clear view of the bird foraging (i.e. both feet and head were clearly visible) were timed again. For each section the number of prey items obtained (counted by the bird's head dipping down to the soil) and scratches were counted. Separate sections of tape for any one bird contributed to the data set for that bird (n = 13 birds). Three variables were analysed: scratches per second, prey items per second and scratches per prey item.

Data analyses

Sex and age differences between infected and non-infected birds were tested using Fisher's exact tests and Kolmogorov-Smirnov two sample tests. For behavioural analyses, all proportions of time allocated to each behaviour were $\arcsin\sqrt{(x+2)}$ transformed and two-way Analyses of Variance (ANOVA) were used to determine differences for each category of behaviour.

Data for the sub-categories of foraging behaviour were log-transformed to obtain stability of variance. All three sub-categories were analysed using a two-way ANOVA. For all ANOVAs, the critical value α was not corrected to account for the number of tests, as sample sizes, although larger than those used by Archbold (2003), were still relatively small given the large variances, thus affording low power to each test. Summary statistics will be reported as mean ± standard deviation, sample size.

RESULTS

A total of 33 lyrebirds (representing ca. 27.5% of population in study area) were tested for *Ch. psittaci* and *E. coli* infection. None of the samples tested positive for *Ch. psittaci*. Although we collected 33 soil samples (one for each faecal sample) only 5 of them could be analysed due to budgetary constraints. We analysed soil samples associated with positive faecal samples in order to maximise the probability of detecting faecal soil contamination, however all of the five soil samples analysed tested negative for both *Ch. psittaci* and *E. coli*.

Sex and age class effects on *E. coli* infection

For *E. coli* infection each bird was assigned a categorical value of N, L, M or H based on CFU counts as described in the Methods section (frequency of infection — see Fig. 2). In an initial analysis all categories of positive infection (L, M and H) were grouped together as one single category of "infected birds". There were 69.23 % (9/13)

of males infected with *E. coli* compared to 66.67% (10/15) females. This difference was not significant (Fisher's exact test: p = 1.00). A more detailed analysis that compares the frequency distributions of males and females across the four infection categories indicates that males were not significantly different from females (Kolmogorov-Smirnov two sample test: $D_{13,15} = 0.11$, P > 0.10, Fig. 2).

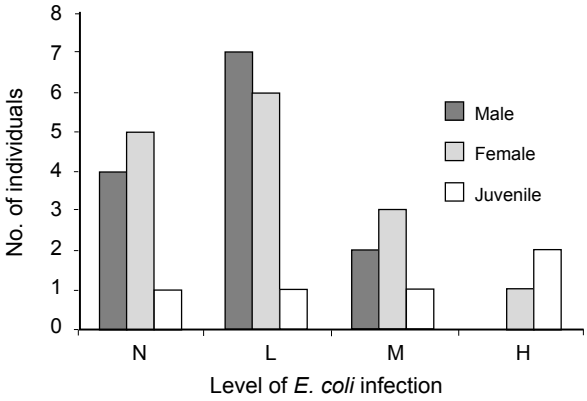

Fig. 2. Frequency of *M. novaehollandiae* adult male, adult female, and juvenile faeces infected at various intensities of *E. coli.* N — no colony growth, L — light colony growth, M — moderate colony growth, H — heavy growth (see text for details).

There were 80% (4/5) of juveniles infected with *E. coli* compared with 67.86% (19/28) of adults. However, this trend was not significant (Fisher's exact test: p = 1.00). Adults and juveniles were not significantly different across the range of levels of *E. coli* infection (Kolmogorov-Smirnov two sample test: $\chi^2_2 = 2.52$, p > 0.20).

Differences in behaviour between *E. coli* infected and non-infected lyrebirds

Foraging. The results show that *E. coli* infected lyrebirds do not allocate more of their time to foraging (proportion of time spent foraging: 0.49 ± 0.07, n = 23) than non infected birds (0.45 ± 0.12, n = 10) (two-way ANOVA: $F_{1,27} = 0.05$, p = 0.82). Nor was there any difference in allocation to time spent foraging between males (0.57 ± 0.09, n = 13), females (0.34 ± 0.09, n = 15) and juveniles (0.67 ± 0.16, n = 5) (two-way ANOVA: $F_{2,27} = 1.75$, p = 0.19). The interaction was also not significant ($F_{2,27} = 0.42$, p = 0.66).

Sexual behaviour: fighting. There was no significant difference, in regard to the time allocated to fighting , between infected (proportion of time spent fighting: 0.04 ± 0.04, n = 23) and non infected birds (0, n = 10) (two-way ANOVA: $F_{1,27} = 0.15$, p = 0.69). Nor was there a significant difference between males (0, n = 13), females (0.06 ± 0.06, n = 15) and juveniles (0, n = 5) (two-way ANOVA: $F_{2,27} = 0.25$, p = 0.77). The interaction between individuals and infection was also not significant ($F_{2,27} = 0.25$, p = 0.77).

Sexual behaviour: displaying. There was no significant difference in time allocated to sexual displaying behaviour between infected (proportion of time spent displaying: 0.06 ± 0.04, n = 23) and non infected (0, n = 10) lyrebirds (two-way ANOVA: $F_{1,27} = 1.190$, p = 0.28). There was also no significant difference between females (0, n = 15), males (0.04 ± 0.04, n = 13) and juveniles (0.16 ± 0.16, n = 5) (two-way ANOVA: $F_{2,27} = 0.41$, p = 0.66). The interaction between individuals and infection was also not significant ($F_{2,27} = 0.49$, p= 0.66).

Vigilance. Female birds were significantly more vigilant (proportion of time spent vigilant: 0.20 ± 0.07, n = 15) than male birds ($8.33\times10^{-4} \pm 8.32\times10^{-4}$, n = 13) and juvenile birds (0.02 ± 0.01, n = 5), (two-way ANOVA: $F_{2,27} = 4.59$, p = 0.01). However, there was no significant difference between infected (0.07 ± 0.03, n = 23) and non infected birds (0.10 ± 0.03, n = 10) (two way ANOVA: $F_{1,27} = 0.11$, p = 0.73). There was also a no significant interaction between individuals and infection ($F_{2,27} = 0.16$, p = 0.85).

Vocalising. Uninfected lyrebirds did not spend significantly more time vocalising (proportion of time spent vocalising: 0.19 ± 0.09, n = 10) than infected lyrebirds (0.07 ± 0.03, n = 23) (two-way ANOVA: $F_{1,27} = 0.50$, p = 0.48). Nor was there a significant difference between adult male (0.22 ± 0.09, n = 13), adult female (0.06 ± 0.03, n = 15), or juvenile birds (0, n = 5) (two-way ANOVA: $F_{2,27} = 2.16$, p = 0.13). There was also no significant interaction between the two variables ($F_{2,27} = 0.21$, p = 0.81).

Preening. *E. coli* infected birds did not allocate more time to preening (proportion of time spent preening: 0.07 ± 0.03, n = 23) than uninfected birds (0.004 ± 0.002, n = 10) (two-way ANOVA: $F_{1,27} = 0.76$, p = 0.39). There was also no difference in the time allocated to preening between males (0.02 ± 0.01, n = 13), females (0.09 ± 0.05, n = 15) and juveniles (0.002 ± 0.002, n = 5) (two-way ANOVA: $F_{2,27} = 0.63$, p = 0.53). The interaction was not significant ($F_{2,27} = 0.37$, p = 0.69).

Moving. Time allocated to moving between one location and another between infected (proportion of time spent moving: 0.06 ± 0.01, n = 23)

and uninfected birds (0.13 ± 0.04, n = 10) was not significantly different (two-way ANOVA: $F_{1,27} = 0.88$, p = 0.35). However, adult female birds (0.12 ± 0.02, n = 15) tended to spend more time moving than adult male birds (0.05 ± 0.02, n = 13) or juvenile birds (0.04 ± 0.01, n = 5), but this trend was marginally not significant (two-way ANOVA: $F_{2,27} = 2.53$, p = 0.09). There was no significant interaction between individual and infection variables ($F_{2,27} = 0.31$, p = 0.73).

Foraging efficiency

Scratches per second. Lyrebirds not infected with *E. coli* tended to scratch more frequently (1.50 ± 0.26 scratches per second, n = 4) than infected birds (1.30 ± 0.06, n = 9) but this result was marginally non significant (two-way ANOVA: $F_{1,9} = 3.58$, p = 0.09). Adults (1.37 ± 0.07, n = 10) and juveniles (1.35 ± 0.16, n = 3) did not differ in scratching frequencies (two-way ANOVA: $F_{1,9} = 0.03$, p = 0.84). The interaction between age and infection was also not significant ($F_{1,9} = 1.25$, p = 0.29).

Prey items per second. No significant difference for rate of prey items caught was found between infected (0.24 ± 0.05 prey items caught per second, n = 9) and non infected (0.17 ± 0.03, n = 4) lyrebirds (two-way ANOVA: $F_{1,9} = 0.85$, p = 0.38). Nor was there a difference between adults (0.23 ± 0.03, n = 10) and juveniles (0.19 ± 0.02, n = 3) (two-way ANOVA: $F_{1,9} = 0.30$, p = 0.59). The interaction was also not significant ($F_{1,9} = 0.009$, p = 0.92).

Scratches per prey item. Non infected birds did not scratch more frequently for each prey item caught (9.49 ± 1.51 scratches made per prey item caught, n = 4) than infected birds (6.78 ± 1.63, n = 9)(two-way ANOVA: $F_{1,9} = 1.79$, p = 0.21). There was also a not significant difference for the number of scratches made per prey item caught for adults (7.62 ± 1.55, n = 10) and juveniles (7.59 ± 1.87, n = 3) (two-way ANOVA: $F_{1,9} = 0.14$, p = 0.71). There was no significant interaction between age and infection ($F_{1,9} = 0.14$, p = 0.71).

DISCUSSION

***Ch. psittaci* infection in the Superb Lyrebird**

None of the samples tested positive for *Ch. psittaci*, a result consistent with the work of Archbold (2003). Archbold (2003) listed a couple of reasons as to why her study did not detect *Ch. psittaci* in lyrebirds. One of them was that the faecal material collected produced high level of PCR inhibition. In our study an immunohistochemical technique was used, which overcame the problem of PCR inhibition. Cloacal swabs were also taken from Crimson Rosellas in the DRNP during Archbold's (2003) study and all tested negative for *Ch. psittaci*; in Crimson Rosellas DNA extracted from cloacal swabs showed very low PCR inhibition (Archbold 2003). Thus although Crimson Rosellas are suspected to play a role in transmission of the bacterium to lyrebirds (Holz et al. 2003) there are no reasons to believe that virulent *Ch. psittaci* strains are endemic in avian populations of the DRNP. If this is so, then superb lyrebirds of the DRNP may lack immune defences against pathogenic *Ch. psittaci,* and their health may be severely affected by the occasional introduction of virulent strains of the bacterium in the Park by, for instance, feral or domestic mammals or native birds such as psittaciforms. In cases where the introduction may have occurred, and the *Ch. psittaci* strain was virulent enough, any infected birds, lacking immune defences against the pathogen, may have died before transmission to other lyrebirds was possible. This would explain both the occasional finding of *Ch. psittaci*-positive dead lyrebirds (n = 3, Archbold 2003), the lack of immunity against *Ch. psittaci* and the undetectable levels of prevalence of *Ch. psittaci* in the host population.

***E. coli* infection in adult males and females**

It was expected that males would be more infected than females due to, for instance, the immunosuppressive effects of androgens, a pattern described in previous studies of parasitism in bird populations (e.g. Borgia & Collis 1989). Although our results show that adult males are slightly more infected than adult females, the difference in *E. coli* prevalence between sexes is not significant. One potential explanation for this pattern may reside in the mating system of the lyrebird. It has been suggested that the lyrebird is polygynous, however there are arguments in favour of the possibility that the species is promiscuous (Kenyon 1972, Robinson & Frith 1981, Robinson 1991). In promiscuous species both sexes copulate repeatedly with several partners, a pattern that may favour (everything else being equal) a more even probability of infection between sexes. Incidentally, mating system effects could also explain why major variables such as body mass, vicinity to humans and diet can only explain 21% of variability in *E. coli* prevalence across avian species, whereas in mammals it can explain up to 65% of variability (Gordon &

Cowling 2003). The presence of a cloaca in birds can favour sexual transmission of *E. coli* which will be affected by the species' mating system.

E. coli infection in juveniles and adults

There was a slight trend for juveniles to be more infected than adults, a trend expected from the hypothesis that juveniles may have a less developed immune system. However, the difference was not significant. The immunosuppressive effect of stress hormones such as corticosterone and perhaps sexual hormones such as testosterone in adult males might be a factor decreasing the differences between adults and juveniles during the breeding season. If this is the case, the difference between adults and juveniles should increase during the non-breeding season, when gonads are regressed and less testosterone should appear in circulation in adults, and they are not under the stress of reproductive activities such as displays and agonistic behaviours (Hillgarth & Wingfield 1997). During the non-breeding season the main differences between adults and juveniles may be explained by differences in the relative maturation of their immune system. Differential exposure to the parasite may also explain differences in infection rates among hosts (e.g. Getty 2001, Staszewski & Boulinier 2004), but this would require habitat segregation between adults and juveniles, especially during the breeding season. Although adult males are territorial during the breeding season, juveniles do wander through territories and sometimes they are joined by the adults, contacts between juveniles and adults are even more frequent during the non-breeding season (Reilly 1988).

E. coli infection and lyrebird behaviour

Time budget analyses. This study was conducted during the lyrebird breeding season, and many of the females sampled were encountered near their nests where they had either a chick or an egg to attend to. This would explain why the results show that females are significantly more vigilant than males and juveniles. Females are the sole carers and providers for young in lyrebirds, and so their parental activities entail nest building and maintenance, incubating, feeding of the young and protection against predators (Higgins et al. 2001).

Increased antipredator vigilance is very common in vertebrates during the period of parental care (e.g. Colagross & Cockburn 1993, Woodard & Murphy 1999, Childress & Lung 2003).

There was a slight trend for females to move more than the other age/sex classes. This trend may be associated with that found for vigilance, as females with young may increase their level of vigilance around the nest area by moving frequently.

As no differences were detected between infected and non-infected lyrebirds across all behavioural categories: foraging, fighting, displaying, vigilance, vocalising, preening and moving, this could suggest that the *E. coli* strains circulating in the lyrebird population at the DRNP during our study may not be pathogenic. No bird sampled during the study showed any visible signs of being unwell (e.g. anorexia, lethargy). Parasites however can sometimes have subtle effects on the animal, such as an effect on metabolic rate and hence energy needs, that can progress slowly and would not be detected in a short-term field study such as this one (Booth et al. 1993). Booth et al. (1993) claim that only experimental manipulations are successful in documenting the more subtle effects parasites might be having on hosts. Experimental infection of lyrebirds would not be recommended due to the protected status of the species; however, subtle, cumulative effects of parasites on bird's viability and reproductive success may be detected in long-term studies of colour banded individuals. Not all cloacal microorganisms, however, are expected to be pathogenic, mildly or otherwise. Indeed some cloacal bacteria can have beneficial effects on their hosts through, for instance, their ability of outcompeting more pathogenic strains (Lombardo et al. 1999, Hupton et al. 2003).

A similar explanation might apply for the lack of differences detected between infected and non infected lyrebirds in relation to their frequency of scratching, prey items caught and number of scratches per prey item. Lack of differences can be explained by *E. coli* strains currently circulating in the lyrebird population of the DRNP being relatively benign.

In conclusion, *Ch. psittaci* do not seem to be endemic in the Superb Lyrebird population of the DRNP, whereas *E. coli*, although detected may not be represented by pathogenic strains.

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REFERENCES

Archbold M. K. 2003. Sexual selection and ecological aspects of cloacal microorganism infection in some Australian birds. Honours Thesis, University of Melbourne.

Baker G. B., Gales R., Hamilton S., Wilkinson V. 2002. Albatrosses and petrels in Australia: a review of their conservation and management. Emu 102: 71–97.

Banko P. C., David R. E., Jacobi J. D., Banko, W. E. 2001. Conservation status and recovery strategies for endemic Hawaiian birds. Studies in Avian Biol. 22: 359–376.

Belthoff J. R., Duffy A. M. J. 1998. Corticosterone, body condition and locomotor activity: A model for dispersal in screech owls. Anim. Behav. 55: 405–415.

Best A., La Ragione R. M., Cooley W. A., O'Connor C. D., Velge P., Woodward M. J. 2003. Interaction with avian cells and colonisation of specific pathogen free chicks by Shiga-toxin negative *Escherichia coli* O157:h7 (NCTC 12900). Vet. Microbiol. 93: 207–222.

Biggins D. E., Kosoy M. Y. 2001. Influences of introduced plague on North American mammals: implications from ecology of plague in Asia. J. Mammal. 82: 906–916.

Booth D. T., Clayton D. H., Block B. 1993. Experimental demonstration of the energetic cost of parasitism, in free-ranging hosts. Proc. R. Soc. London B 253: 125–129.

Borgia G., Collis K. 1989. Female choice for parasite-free male satin bowerbirds and the evolution of bright male plumage. Behav. Ecol. Sociobiol. 25: 445–454.

Cassinello J., Gomendio M., Roldan E. R. S. 2001. Relationship between coefficient of inbreeding and parasite burden in endangered gazelles. Conserv. Biol. 15: 1171–1174.

Childress M. J., Lung M. A. 2003. Predation risk, gender and the group size effect: does elk vigilance depend on the behaviour of conspecifics? Anim. Behav. 66: 389–398.

Christe P., Richner H., Oppliger A. 1996. Of great tits and fleas: Sleep baby sleep. Anim. Behav. 52: 1087–1092.

Clayton D. H. 1990. Mate choice in experimentally parasitized Rock Doves: lousy males lose. Am. Zool. 30: 251–262.

Clayton D. H. 1991. Coevolution of avian grooming and ectoparasite avoidance. In: Loye J. E., Zuk M. (eds). Bird-parasite interactions. Oxford Univ. Press, pp. 258–289.

Clayton D. H., Moore J. (eds). 1997. Host parasite evolution: general principles and avian models. Oxford Univ. Pres.

Colagross A. M. L., Cockburn A. 1993. Vigilance and grouping in the eastern grey kangaroo, *Macropus giganteus*. Aust. J. Zool. 41: 325–334.

Dobson A., Hudson P. 1995. The interaction between the parasites and predators of red grouse *Lapogus lapogus scoticus*. Ibis 137: 87–96.

Duffy G. 2003. Verocytoxigenic *Escherichia coli* in animal faeces, manures and slurries. J. Appl. Microbiol. 94: 94–106.

Dwyer G., Levin S. A., Buttell L. 1990. A simulation model of the population dynamics and evolution of myxomatosis. Ecol. Monog. 60: 423–447.

Everett K. D. E. 2000. *Chlamydia* and *Chlamydiales*: more than meets the eye. Vet. Microbiol. 75: 109–126.

Fenner F., Kerr P. J. 1994. Evolution of the pox viruses, including the coevolution of virus and host in myxomatosis. In: Moorse S. S. (ed.). The evolutionary biology of viruses. Raven Press, New York, pp. 273–292.

Getty T. 2001. Signaling health versus parasites. Am. Nat. 159: 363–371.

Gomis S. M., Watts T., Riddell C., Potter A. A., Allan B. J. 1997. Experimental reproduction of *Escherichia coli* cellulitis and septicemia in broiler chickens. Avian Dis. 41: 234–240.

Gordon D. M., Cowling A. 2003. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. Microbiol. 149: 3575–3586.

Gross W. B. 1991. Colibacillosis. In: Calnek B. W., Barnes H. J., Beard, C. W., Reid W. M., Yoder H. W. Jr (eds). Diseases of poultry. Iowa State Univ. Press: Ames, pp. 138–144.

Hamilton W. D., Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218: 384–387.

Higgins P. J., Peter J. M., Steele W. K. 2001. *Menura novaehollandiae* Superb Lyrebird. In: Higgins P. J., Peter J. M., Steele W. K. (eds). Handbook of Australian, New Zealand and Antarctic Birds. Oxford Univ. Press, pp. 142–173.

Hillgarth N., Wingfield J. C. 1997. Parasite-mediated sexual selection: Endocrine aspects. In: Clayton D. H., Moore J. (eds). Host parasite evolution: General principles and avian models. Oxford University Press, pp. 78–104.

Holz P. H., Middleton D. R., Slocombe R. F. 2003. Chlamydiophilosis in superb lyrebirds (*Menura novaehollandiae*). Aust. Vet. J. 81: 426–427.

Hupton G., Portocarrero S., Newman M., Westneat D. F. 2003. Bacteria in the reproductive tracts of red-winged blackbirds. Condor 105: 453–464.

John J. L. 1997. Seven comments on the theory of sosigonic selection. J. Theor. Biol. 187: 333–349.

Kenyon R. F. 1972. Polygyny among superb lyrebirds in Sherbrook Forest, Kallista, Victoria. Emu 72: 70–76.

Kirkpatrick M., Ryan M. J. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350: 33–38.

Latta S. C. 2003. Effects of scaley-leg mite infestations on body condition and site fidelity of migratory warblers in the Dominican Republic. Auk 120: 730–743.

Lee K. A., Franson J. C., Kinsella J. M., Hollmen T., Hansen S. P., Hollmen A. 2004. Intestinal helminths in mourning doves (*Zenaida macroura*) from Arizona, Pennsylvania, South Carolina, and Tennessee, U.S.A. Comp. Parasitol. 71: 81–85.

Lombardo M. P., Thorpe P. A., Power H. W. 1999. Inoculation of beneficial microbes as a potential direct benefit to female birds from participating in copulation, including extra-pair copulations. Behav. Ecol. 10: 333–337.

Loye J., Carrol S. 1995. Birds, bugs and blood: avian parasitism and conservation. Trends Ecol. Evol. 10: 232–235.

Loye J. E., Zuk M. 1991. Bird Parasite Interactions. Oxford Univ. Press.

Lung N. P., Thompson J. P., Kollias G. V. Jr, Olsen J. H., Zdziarski J. M., Klein P. A. 1996. Maternal immunoglobulin G antibody transfer and development of immunoglobulin G antibody responses in Blue and Gold Macaw (*Ara ararauna*) chicks. Am. J. Vet. Res. 57: 1162–1167.

Mardh P., Paavonen J., Puolakkainen M. 1989. Chlamydia. Plenum Publishing, New York.

Moore J. 1995. The behaviour of parasitized animals. Bioscience 45: 89–96.

Poiani A. 2002. Sperm competition promoted by sexually transmitted pathogens and female immune defences. Ethol. Ecol. Evol. 14: 327–340.

Poiani A., Gwozdz J. 2002. Cloacal microorganisms and mating systems of four Australian bird species. Emu 102: 291–296.
Poiani A., Wilks C. 2000a. Cloacal microparasites and sexual selection in three Australian passerine species. Ethol. Ecol. Evol. 12: 251–258.
Poiani A., Wilks C. 2000b. Sexually transmitted diseases: a possible cost of promiscuity. Auk 117: 1061–1065.
Potti J., Merino S. 1995. Louse loads of Pied Flycatchers: Effects of host's sex, age, condition and relatedness. J. Avian Biol. 26: 203–208.
Powys V. 1995. Regional variation in the territorial songs of superb lyrebirds in the central tablelands of New South Wales. Emu 95: 280–289.
Proctor H. C., Jones D. N. 2004. Geographical structuring of feather mite assemblages from the Australian brush-turkey (Aves: Megapodiidae). J. Parasitol. 90: 60–66.
Quinn P. J., Carter M. E., Markey B. 1994. Clinical veterinary microbiology. Wolfe Publishing, London.
Reilly P. N. 1988. The Lyrebird. University of New South Wales Press, Sydney.
Robinson F. N. 1991. Phatic communication in bird song. Emu 81: 145–157.
Robinson F. N., Curtis H. S. 1996. The vocal displays of the lyrebirds (Menuridae). Emu 96: 258–275.
Robinson F. N., Frith H. J. 1981. The Superb Lyrebird *Menura novaehollandiae* at Tidbinbilla, ACT. Emu 81: 145–157.
Rozsa L. 1993. An experimental test of the site specifity of preening to control lice in feral pigeons. J. Parasitol. 79: 968–970.
Rozsa L., Rkasi J., Reiczigel J. 1996. Relationship of host coloniality to the population ecology of avian lice (Insecta: Phthiraptera). J. Anim. Ecol. 65: 242–248.
Shutler D., Ankney C. D., Dennis D. G. 1996. Could the blood parasite *Leucocytozoon* deter mallard range expansion? J. Wildl. Manage. 60: 569–580.
Smith L. H. 1965. Changes in the tail feathers of the adolescent lyrebird. Science 147: 510–512.
Smith L. H. 1968. The Lyrebird. Lansdowne Press, Melbourne.
Smith L. H. 1982. Moulting sequences in the development of the tail plumage of the superb lyrebird, *Menura novaehollandiae*. Aust. Wildl. Res. 9: 311–330.
Smith L. H. 1990. A method for determining the curvature of natural forms and its application to certain tail feathers of the Superb Lyrebird *Menura novaehollandiae*. Emu 90: 231–240.
Smith L. H. 1999. Structural changes in the main rectrices of the Superb Lyrebird *Menura novaehollandiae* in the development of the filamentary feathers. Emu 99: 46–59.
Spalding M. G., Forrester D. J. 1993. Pathogenesis of *Eustrongylides ignotus* (Nematoda: Dioctophymatoidea) in ciconiiformes. J. Wildl. Dis. 29: 250–260.
Staszewski V., Boulinier T. 2004. Vaccination: a way to address questions in behavioral and population ecology? Trends Parasitol. 20: 17–22.
Suzuki S. 1994. Pathogenicity of *Salmonella enteritidis* in poultry. Int. J. Food Microbiol. 21: 89–105.
Toigo C. 1999. Vigilance behaviour in lactating female Alpine ibex. Can. J. Zool. 77: 1060–1063.
Van Riper C., Van Riper S. G., Goff M. L., Laird M. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecol. Monog. 56: 327–344.
Waldenstrom J., Broman T., Carlsson I., Hasselquist D., Achterberg R. P., Wagenaar J. A., Olsen B. 2002. Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. Appl. Environ. Microbiol. 68: 5911–5917.
Weisburg W. G., Hatch T. T., Woese C. R. 1986. Eubacterial origin of Chlamydiate. J. Bacteriol. 1986: 167.
Wheeler T. A., Threlfall W. 1986. Observations on the ectoparasites of some Newfoundland passerines (Aves: Passeriformes). Can. J. Zool. 64: 630–636.
Woodard J. D., Murphy M. T. 1999. Sex roles, parental experience and reproductive success of eastern kingbirds, *Tyrannus tyrannus*. Anim. Behav. 51: 105–115.
Zheng W. M., Yoshimura Y. 1999. Localization of macrophages in the chicken oviduct: effects of age and gonadal steroids. Poultry Sci. 78: 1014–1018.
Zheng W. M., Yoshimura Y., Tamura T. 1997. Effects of sexual maturation and gonadal steroids on the localization of IgG-, IgM- and IgA-positive cells in the chicken oviduct. J. Reprod. Fertil. 111: 277–284.
Zuk M. 1992. The role of parasites in sexual selection: current evidence and future directions. Adv. Study Behav. 21: 39–68.
Zuk M., McLean K. A. 1996. Sex differences in parasite infections: Patterns and processes. Int. J. Parasitol. 26: 1009–1024.

STRESZCZENIE

[Wpływ wieku i płci na zarażenie bakteriami *Escherichia coli* i *Chlamydophila psittaci* u lirogona wspaniałego]

Populacje lirogona zmniejszają swą liczebności od lat 1940-tych przede wszystkim w związku z postępującym procesem urbanizacji. Jednakże ostatnie badania wskazują, ze także infekcje chlamydiami mogą być zagrożeniem dla tego gatunku. Zbadano poziom infekcji populacji lirogona zamieszkującej las Sherbrooke (południowo-wschodnia Australia, Fig. 1) dwoma bakteriami: *E. coli* i *Ch. psittaci*. Próby kału zebrano dla 33 ptaków (15 samic, 13 samców i 5 młodych) stanowiących około 27.5% całej populacji zamieszkującej teren badań. W żadnej z prób nie stwierdzono *Ch. psittaci*. W 67.9% prób zebranych dla ptaków dorosłych i 80% od ptaków młodych stwierdzono obecność *E. coli*. Nie stwierdzono różnic w częstości ani poziomie zapasożycenia ptaków dorosłych i młodych jak również samców i samic tymi bakteriami (Fig. 2). Analizie poddano również nagrania wideo zachowania ptaków. Nie stwierdzono, aby poszczególne formy aktywności i zachowania związane z żerowaniem ptaków zainfekowanych E. coli różniły się od tych stwierdzanych dla ptaków nie zainfekowanych.