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CLOACAL MICROBES IN HOUSE SPARROWS¹

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Abstract. We examined the communities of bacteria and fungi associated with the cloaca of adult House Sparrows (*Passer domesticus*) to investigate whether microbes could be transferred during copulation and thus represent a cost to mating. The levels of microbes in the cloacae of the male and female of eight breeding pairs were significantly correlated. The levels of microbes on the rim of the cloacal protuberance, which comes into direct contact with another bird during copulation, were similar to those inside the cloaca. These findings are consistent with microbes being transferred during copulation. Females could also receive non-cloacal pathogens during copulation, given that two of five males sacrificed had microbes within their testes, which could be incorporated into the ejaculate. Undeveloped eggs were screened for the presence of microbes, although only a low proportion (18%) was contaminated. It seems unlikely that micro-

bial contamination is a general cause of egg failure in this species.

Key words: *cloaca, copulation, egg failure, House Sparrow, microbes, Passer domesticus.*

In many avian species, a proportion of individuals seek copulations outside of their social bond (Birkhead and Møller 1992). Males which can achieve successful extra-pair copulations (EPCs) benefit by siring more offspring without incurring additional rearing costs. Females which can achieve successful EPCs might benefit in several ways, such as increasing the genetic diversity or quality of their offspring (Petrie and Kempenaers 1998).

Extra-pair copulations might not always be beneficial, however. A frequently cited cost of EPCs, which could be incurred by both sexes, is the risk of acquiring a sexually transmitted disease (STD) (Birkhead and Møller 1992). Unfortunately, despite the burgeoning theoretical interest which STDs have attracted (Hamilton 1990, Graves and Duvall 1995, Thrall et al. 1997), there are few empirical data regarding their prevalence and transmission in wild birds (Sheldon 1993, Petrie and Kempenaers 1998).

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This deficiency is partly attributable to the practical difficulties of monitoring STDs. Because most are microbes (Sheldon 1993), specialist techniques are often required for their culture and identification. Recently, however, a relatively simple protocol was used to assess the microbial communities present in the cloacae of Tree Swallow (*Tachycineta bicolor*) families (Lombardo et al. 1996). We used this protocol to perform a similar assessment during a study of the breeding ecology of the House Sparrow (*Passer domesticus*).

The main objective of our work was to determine whether cloacal microbes are likely to be transmitted during within-pair copulations, and thus, by deduction, represent a potential cost to EPCs. We tested this possibility in three indirect ways.

The first test arose from the fact that House Sparrow pairs copulate frequently (around 30 times per day, Møller 1990; R. Stewart, unpubl. data). If cloacal microbes are exchanged during these copulations, then their incidence between the male and female of a pair should be similar. If microbes are not sexually transmitted, then the microbial levels of pair members should be independent.

A second test was then required, because during copulation, only the rims of the male and female cloacae come into direct contact (but see Briskie and Montgomerie 1997 for exceptions). Although the female everts her oviduct into her cloaca during copulation, and the cloacae of either sex are everted so that semen can be deposited directly onto the vaginal opening (Birkhead 1998), the inner walls of the male and female cloaca may never actually touch. We therefore assessed whether microbes were present on the protuberance rim of breeding adults as well as within their cloaca, and compared the incidence of microbes between each of the two sites.

Our third test addressed whether cloacal microbes could also be transmitted during copulation, albeit unidirectionally, if they became incorporated into an ejaculate (Sheldon 1993). This possibility arises because the cloaca lies between the vas deferens and the joint external opening of the digestive and reproductive tracts (King 1981). Unfortunately, we were unable to obtain ejaculates from live males. We therefore considered whether ejaculates could at least be contaminated at their source by examining the testes of five male House Sparrows taken from outside our study colony. We examined the ovaries of five female House Sparrows, also taken from outside our study colony, to gain comparable data on gonadal infection in this species.

A lesser objective of the work was to link the presence of microbes to egg failure. Egg failure is a regular occurrence in House Sparrows (Summers-Smith 1963, Seel 1968). At our field site, over 60% of clutches contain at least one unhatched egg, and the majority of these eggs (79%) appear to be infertile because they show no visible signs of embryonic development (R. Stewart, unpubl. data). However, microscopic examination of apparently infertile eggs collected from another House Sparrow colony revealed that most had actually been fertilized (73%), but for unknown reasons, had suffered very early embryonic mortality (Birkhead et al. 1995). Because Pinowski et al. (1994)

had isolated several genera of microbes from a comparably high proportion of unhatched House Sparrow eggs in Poland (70%), we hypothesized that contamination with pathogenic microbes was the general cause of infertility/early embryonic mortality in this species. Consequently, we predicted that any undeveloped sparrow eggs collected from our field site would be infected with microbes.

METHODS

Fieldwork was carried out between April and August 1996, at a nest-box population of House Sparrows distributed over several barns at the University of Kentucky's Agricultural Research Station near Lexington, Kentucky (35°7'N, 83°2'W). House Sparrows are multi-brooded at our site, and all pairs used in this study had already reared at least one brood that season. Adults were caught in Potter traps baited with seed, or trapped within their nests while feeding young nestlings.

A sterile cotton swab designed for urethral sampling (10 mm long × 2 mm wide, obtained from Grogan's Medical Supplies, Lexington, Kentucky) was inserted into the cloaca for 5 sec. The swab was placed into an eppendorf tube containing 1 ml of sterile thioglycollate broth, a liquid agar, which was kept at 5–10°C in a coolbox in the field before being transported to the laboratory and refrigerated at 4°C (always within 5 hr of the swab being obtained). These temperatures are sufficiently low to inhibit further microbial reproduction but are not considered cold enough to cause mortality (Lombardo et al. 1996).

Swabs were obtained from both the cloaca and the cloacal protuberance rim of 17 birds (8 males and 9 females). The protuberance rim was swabbed before the cloacal sample was obtained, in order to minimize the risk of contamination.

We collected 10 "field blanks" to determine whether swabs were being incidentally contaminated by airborne microbes (Lombardo et al. 1996). Sterile swabs were held beside randomly chosen nest boxes for 5 sec, and processed as if they were cloacal samples.

Five House Sparrows of each sex were collected (under permit) from a Lexington suburb outside of our study population, sacrificed, and transferred to the laboratory. The ovary of each female and the left testis of each male was aseptically sliced open lengthways and a swab taken from the inner surface. The swab was then placed in thioglycollate broth and processed as if it were a cloacal sample.

All eggs which were still present in the nest two days after the remainder of the clutch had hatched were taken to the laboratory. The shell surface was sterilized by wiping with 100% ethanol, the eggs were opened using sterile forceps, and a micropipette was used to extract approximately 200 µl of yolk from any which were undeveloped ($n = 38$). The yolk sample was placed into 1 ml of sterile thioglycollate broth and shaken briefly to disrupt its glutinous texture and thus dissociate any microbes.

To culture any microbes present, the tubes containing swabs or yolks were centrifuged for 1 min, and 100 µl of supernatant was plated out onto each of eight growth media for presumptive identification. All sam-

TABLE 1. The prevalence, average colony count, and within-pair similarity of cloacal microbes among eight breeding pairs of House Sparrows (= 16 adults).

Agar	Microbe detected	Percent positive	Average count \pm SD	Correlation between members of a pair (r_s)
BA	Anaerobic bacteria	100	3.1 \pm 0.6	0.33
CSM	<i>Staphylococcus</i> sp.	81	1.3 \pm 1.3	0.52
EMB	Gram negative enterics (dark lactose fermentors)	50	1.0 \pm 1.2	0.87
MCA	Gram negative enterics (red lactose fermentors)	50	1.0 \pm 1.2	0.62
SAB	Fungi	100	2.9 \pm 1.0	-0.08
SS	<i>Salmonella</i> sp.	60	1.3 \pm 1.2	0.69
TJA	<i>Lactobacillus</i> sp.	88	2.8 \pm 1.3	0.06
Y	<i>Yersinia</i> sp.	31	0.9 \pm 1.4	0.74

ples were plated within three days of the swab or yolk being obtained.

Microbes were cultured on eight growth media, which were obtained from a commercial source (Sigma[®], St Louis, Missouri) and prepared in the laboratory. Chapman stone agar (CHAP) was used to detect *Staphylococcus* spp., tomato juice agar (TJA) was used to detect lactobacilli, and sabouraud dextrose agar (SAB) was used to detect fungi. *Salmonella Shigella* agar (SS) was used to detect *Salmonella* spp., and cef-soludin irgasan agar (CIN) was used to detect *Yersinia* spp. MacConkey agar (MAK) was used to detect gram negative enterics; lactose fermentors are red on these plates. Eosin methylene blue (EMB) was used to detect gram negative enterics; lactose fermentors are dark on these plates. Although these two both detect lactose fermentors, the selective agents differ, and consequently somewhat different groups of microbes are detected (M. Lombardo, pers. comm.). Finally, blood agar (BA), containing 5% defibrinated horse blood, was used to detect anaerobic microbes.

The inoculated media were incubated at 37°C for 24 hr, with the exception of SAB cultures, which were maintained for 48 hr. Inoculates on BA plates were incubated under anaerobic conditions by placing them inside GasPak[®] envelopes saturated with gaseous carbon dioxide. Once incubation was complete, the number of colonies on each plate was assigned a count as follows; 0 = 0 colonies, 1 = 1–10 colonies, 2 = 11–100 colonies, 3 = 101–1,000 colonies, and 4 = > 1,000 colonies (Lombardo et al. 1996). Because the counts did not conform to the normal distribution, all statistical tests were nonparametric.

RESULTS

Cloacal microbes were cultured from eight breeding pairs (i.e., 16 adults) using eight different growth media. The prevalence and average colony count of each microbial type varied (Table 1). For example, anaerobic microbes were detected in all 16 adults and had the highest average colony count, whereas *Yersinia* spp. was relatively scarce and also had the lowest average colony count.

To determine whether the levels of microbes were similar between pair members, we first correlated the counts of the microbial types in each of the eight pairs.

The correlation coefficient was positive in seven of the eight pairs ($\bar{x} \pm$ SD = 0.63 \pm 0.14). The average coefficient was significantly higher than zero (Mann-Whitney *U*-test, $n_1 = 8$, $n_2 = 8$, $P < 0.01$), and hence the bacterial counts of pair members were significantly and positively correlated.

However, because an individual's immunocompetence will affect the levels of all potentially pathogenic microbes it harbors, the counts of each microbe within a particular bird may not be independent. Consequently, we performed a further analysis to control for pseudoreplication. We correlated the counts of each of the eight microbial types separately (i.e., the counts of *Salmonella* spp. between the males and females of each pair, the counts of *Yersinia* spp., etc) (Table 1). The correlation coefficient was positive for seven of the eight types ($\bar{x} \pm$ SD = 0.47 \pm 0.12), and the average coefficient was significantly greater than zero (Mann-Whitney *U*-test, $n_1 = 8$, $n_2 = 8$, $P < 0.01$). Hence, even when using this microbe-wise approach, there was still a significant, positive correlation between the microbial counts of pair members.

Swabs were obtained from both the cloaca and protuberance rim of 17 birds, which included two males and three females used in the pair-wise comparison described above. Fifty-two comparisons of cloaca/rim microbes were made, of which 14 were made on SAB, 10 on BA, 9 on MCA, 7 on SS, 7 on EMB, and 5 on TJA.

However, because several individuals had been assayed for more than one microbial type, some of the counts may not have been independent. We therefore compared the cloaca/rim counts of each of the six microbial types assayed using a Wilcoxon signed-rank test (Sokal and Rohlf 1981), and used Fisher's method, which calculates quantities distributed as χ^2 using the formula $\chi^2 = -2 \sum \ln P$ (Sokal and Rohlf 1981), to combine the probabilities associated with the individual *Z*-values. The microbial counts on the rim of the protuberance were not significantly different from those within the cloaca ($\chi^2_{12} = 7.3$, $P > 0.5$).

No microbes were cultured from the 10 field blanks, and we therefore assumed that our results were unaffected by environmental contamination.

Two of the five males surveyed had contaminated testes, which produced over 1,000 colonies of *Salmo-*

nella spp. and gram-negative enterics (detected using both EMB and MAK agars). The testis swab from one of these males also produced over 1,000 colonies of fungi.

Ovarian swabs from three of the five females produced over 1,000 colonies of *Yersinia* spp. and gram-negative enterics (detected on both EMB and MAK agars). Two of these females had concurrent ovarian contamination with over 1,000 colonies of *Salmonella* spp. and fungi.

Microbes were isolated from 7 (18.4%) of the 38 egg yolks sampled. Four yolks were contaminated with *Salmonella* spp., two were contaminated with *Yersinia* spp., and one was contaminated with both *Salmonella* spp. and fungi. Five of these contaminations were represented by fewer than 100 colonies, and the remaining three produced between 100 and 1,000 colonies.

Cloacal swabs were only taken from a proportion of the females in the study population, and hence relatively few eggs were obtained from females which had been swabbed. However, the cloacal levels of *Salmonella* spp. were known for three females which produced eggs contaminated with this pathogen. These were not significantly higher than the levels of four females which produced uncontaminated eggs (Mann-Whitney *U*-test, $n_1 = 3$, $n_2 = 3$, $P > 0.5$).

DISCUSSION

CLOACAE

Microbial counts were significantly and positively correlated between members of a pair, which is consistent with the supposition that microbes are traded during within-pair copulations. Extrapolating from this result implies that cloacal microbes could be transmitted during an EPC.

This extrapolation is admittedly circumstantial. The House Sparrow pairs used in our study had probably already copulated over 100 times that season, which would have provided a greater opportunity for microbial flux than that likely to be encountered during a single EPC. Moreover, other scenarios could explain this within-pair similarity, such as joint exposure to an environmental source of microbes, or assortative mating (Lombardo et al. 1996). The first scenario seems unlikely, because House Sparrows at our field site are not territorial and nest at high densities (between 12–20 pairs at each study barn), with birds gathering to feed at communal areas close to the barns. It is possible, however, that both members of a pair are exposed to microbes present on the surface of the nest material, which eventually colonize their cloaca. In the second scenario, if microbial infections were influenced by an individual's immunocompetence, and this in turn was dependent upon quality, then assortative mating with respect to quality would also generate a positive correlation between the microbial levels of a pair (Lombardo et al. 1996).

Microbial levels within the cloaca were similar to those present on the rim of the cloacal protuberance. Consequently, cloacal microbes are theoretically transferable, because they are present on the part of the reproductive anatomy which comes into contact during copulation.

GONADS

Some individuals of either sex had heavy gonadal infections, while others were apparently uninfected. The infected House Sparrows probably originally acquired microbes by ingestion (Barrow and Lovell 1991). This can lead to rapid colonization of the reproductive organs, given that Keller et al. (1995) detected *Salmonella enteritidis* in the ovary and oviduct of laying hens within two days of oral inoculation with infective organisms. Gonadal infection presumably results from a subsequent haematogenous spread of microbes, because ovarian infection of *S. enteritidis* in laying hens could also be produced by intravenous inoculation (Barrow and Lovell 1991, Miyamoto et al. 1997).

Although several of the House Sparrows surveyed from our study population had high levels of microbes within their cloaca, this is unlikely to be the direct source of gonadal infection (Miyamoto et al. 1997). The unidirectional flow of material through the vas deferens makes it unlikely that any pathogens present in the cloaca would spread naturally into the gonads (G. Duke, pers. comm.).

Nevertheless, EPCs could be costly for females if microbes which were usually resident within the cloaca were carried into the vagina along with the ejaculate, or if testicular microbes became incorporated into semen. Several genera of microbes have been isolated from the semen of both domestic and wild birds (Perek et al. 1969, Sheldon 1993, Westneat and Rambo 2000), although it is currently unclear whether these were derived from testicular infection or were merely acquired on passage through the cloaca. The presence of testicular microbes in two of the five House Sparrows examined here confirms that the former scenario is at least plausible.

Because we have no data on the breeding success of the birds used in the dissection, we can only speculate upon the costs of gonadal infection for either sex. Intuitively, large numbers of microbes subsisting within the gonads are unlikely to be insignificant, particularly for male birds. The interior of each testis is an immunologically privileged site, because haploid sperm surface proteins would be recognized as non-self by host antibodies and destroyed (Folstad and Skarstein 1996). Consequently, any microbes present within the testes are theoretically invulnerable.

Because spermatozoa and testosterone are both produced within the testes (Lake 1981), the consequences of testicular infection are potentially dramatic. Morphologically deformed sperm have been documented in both wild and domestic birds (Birkhead et al. 1997, and references therein), although the cause of the deformity is unclear. If the presence of testicular microbes impaired spermatogenesis, however, then a clear cost of gonadal infection would emerge, because the proportion of deformed sperm in male birds is inversely correlated with their fertility and sperm competitive ability (Birkhead et al. 1997).

Furthermore, the expression of the male secondary sexual character in some birds is dependent upon the level of circulating testosterone (Owens and Short 1995, Zuk et al. 1995). If the presence of testicular microbes simultaneously impaired both testosterone production and spermatogenesis, then males which

were able to express a large secondary sexual character would be advertising their freedom from testicular infection, and hence, their lack of deformed sperm. If this three-way interplay exists, then functional fertility would vary with male phenotype (*sensu* Sheldon 1994), and thus provide a mechanism for female choice for fertility benefits.

Recently, however, Lombardo et al. (1999) have suggested that because certain gastro-intestinal microbes have beneficial effects upon their host (Fuller 1989), female birds may copulate repeatedly with their social partner, or seek EPCs with other males, in order to receive cloacal inoculations of beneficial microbes. Although this is an intriguing possibility, we are unable to consider it here because we do not know whether the microbes we assayed from House Sparrows were pathogenic, beneficial, or neutral.

EGGS

Because only a small proportion of the undeveloped eggs collected from our site was infected with microbes, it seems unlikely that microbial contamination was the sole cause of egg failure. Contamination may merely be one of a suite of circumstances under which eggs fail to develop, including the possession of lethal alleles, a decline in viability due to delayed incubation, or genuine infertility (Potti and Merino 1996).

We do not know how microbes colonized the seven infected eggs. The eggs may have become contaminated when passing through an infected cloaca, given that suction caused by the cooling of freshly laid eggs can draw pathogens inside via the shell pores (Board 1966). Indeed, Barrow and Lovell (1991) concluded that most infected eggs laid by *Salmonella enteritidis*-infected hens are surface contaminated. In our study, females which laid infected eggs did not have higher levels of cloacal microbes than those which laid uninfected eggs, although our sample sizes were small.

Contamination may have occurred during ovulation, given that both *Yersinia* spp. and *Salmonella* spp. were isolated from undeveloped eggs as well as ovaries. Transovarian transmission is an important route by which *S. enteritidis* colonizes eggs of laying hens (Thiagrajan et al. 1993, Keller et al. 1995). It is also possible that environmental microbes colonized the eggs after they had failed, although it is unclear why this would only have affected a small proportion of the undeveloped eggs.

Because our study does not contain a satisfactory control, we do not know whether microbes were also present in the eggs which did develop. Further work is needed to distinguish whether microbial contamination is a cause or a consequence of egg failure in wild birds.

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EFFECTS OF ECTOPARASITES ON NESTLING BODY MASS IN THE HOUSE SPARROW¹

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Abstract. House Sparrow (*Passer domesticus*) nestlings were screened for ectoparasites; the most common ectoparasite was *Pellonyssus reedi*, a haematophagous mite. Parasite load was used to determine whether: ectoparasites have an effect on chick body mass prior to fledging, relative chick body mass is a within-brood predictor of relative parasite load, and parasite load per brood correlates with brood size. There was a negative correlation between parasite load and chick body mass, indicating that ectoparasites can reduce the quality of host offspring. Within broods, a chick's body mass was not related to its parasite load relative to its siblings' loads, suggesting that these ectoparasites do not preferentially target particular nestlings based on size. No relationship was found between brood size and total parasite load; thus, there was no evidence that within-nests, mite population size is limited by brood size.

Key words: ectoparasites, haematophagous, House Sparrow, mites, parasites, *Passer domesticus*, *Pellonyssus*.

Parasites have many diverse and wide-ranging effects on the physiology, morphology, and behavior of their hosts (Forbes 1993, Christie et al. 1994, Poulin 1994). Ectoparasites in particular have been shown to influence both the quality and quantity (viability) of host offspring in several bird species, such as the Great Tit, *Parus major* (Christie et al. 1996) and Cliff Swallow, *Hirundo pyrrhonota* (Brown and Brown 1986, 1996, Chapman and George 1991).

The purpose of this study was to examine the host-parasite relationship between House Sparrows, *Passer domesticus*, and their most common endemic ectoparasite, a haematophagous mite. House Sparrows usually lay 3–5 eggs per clutch, with the hatching to fledging interval averaging 13–15 days (Hegner and Wingfield 1987). Haematophagous mites typically have a 5–7 day life cycle (Richner and Heeb 1995). Thus, two to three generations of mites could potentially be produced per nestling cycle of this host.

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