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Communities of Cloacal Bacteria in Tree Swallow Families

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Lifetime nesting area fidelity in male Cooper's Hawks, as evidenced by the population in Wisconsin, contrasts with the results of other studies of fidelity in congeners, especially the work by Newton (1986) and Newton and Wyllie (1992) on *A. nisus*. These studies did, however, show greater breeding site fidelity in males than in females.

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COMMUNITIES OF CLOACAL BACTERIA IN TREE SWALLOW FAMILIES¹

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Key words: *Bacteria; cloacal bacteria; disease; Tachycineta bicolor; Tree Swallow.*

Bacteria are well-known causes of disease and mortality. However, bacterial infections are not usually considered in studies of wild bird populations. This is surprising for two reasons. First, numerous pathogenic bacteria species have been isolated from the pharynxes and cloacae of domestic and wild birds (e.g., Petrak 1982, Brittingham et al. 1988, Calnek et al. 1991, Sheldon 1993). Second, infectious diseases can be important sources of mortality and reduced fitness in wild

bird populations (Anderson and May 1979, Hudson and Dobson 1991). Thus, bacterial infections have the potential to be important selective forces in the evolution of many aspects of avian biology (Hamilton and Zuk 1982, Hamilton 1990, Zuk 1991, Sheldon 1993) yet we know little about the epidemiology and prevalence of these microparasites in wild birds (Zuk 1991, Sheldon 1993). Our lack of information about the prevalence, epidemiology, and pathogenicity of bacterial infections in wild bird species has hindered our understanding of the importance of bacteria as causes of natural selection and in the formulation of satisfactory theories that incorporate parasites into models of sexual selection (Zuk 1991, Sheldon 1993).

The pathogenicity of some bacteria are well known

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in domestic (Calnek et al. 1991) and caged bird species (Petrak 1982, Flammer and Drewes 1988). Outbreaks of avian cholera (*Pasteurella multocoda*) and botulism (*Clostridium botulinum* type C) resulting in epizootics that kill thousands of waterfowl are well known (Robinson and Bolen 1989). Cooper et al. (1980) isolated many species of bacteria from the pharynxes and cloacae of free-living Peregrine Falcons (*Falco peregrinus*) and Gyrfalcons (*Falco rusticolus*) in North America but concluded that the effect of these bacteria on general fitness and mortality was uncertain. Brittingham et al. (1988) surveyed the prevalence of selected bacteria in wild birds using cloacal swabs obtained from birds visiting winter feeding stations in Wisconsin. They found that 217 of 387 (56%) swabs from 364 birds were negative and that infected Black-capped Chickadees (*Parus atricapillus*) suffered higher mortality rates during 12 weeks post-swabbing than did non-infected chickadees, but the differences in mortality rates were not significant.

Our aim in this study was to survey the communities of bacteria found in the cloacae of adult and nestling Tree Swallows (*Tachycineta bicolor*), determine if there were familial patterns of prevalence, and determine if there were relationships between bacteria loads and nestling size when 12 days old and fledging success.

METHODS

In 1994, we studied the cloacal bacteria of Tree Swallows that nested in wooden nest boxes that are mounted on metal poles which are erected in grids in old fields on the campus of Grand Valley State University, Otawa County, Michigan, USA (42°57'N, 85°53'W).

Nestling swallows were measured and weighed on nestling day 12 (where the day the first egg in a clutch hatches is nestling day 1). On each nestling we measured the length of the left tarsus with an electronic digital caliper to the nearest 0.1 mm and left flattened wing chord to the nearest 1 mm with a ruler with a stop fixed to one end. Each nestling was weighed to the nearest 0.2 g with an Avinet spring scale. Each nestling was banded with a U.S. Fish & Wildlife Service numbered aluminum band.

To sample cloacal bacteria, adult swallows were captured at their nest boxes as they delivered food to their nestlings on nestling day 12. As part of another study, adult swallows were initially captured, measured and weighed during the egg-laying period at each nest. The sex of each captured swallow was determined by noting the presence of a well-developed brood patch in females or a cloacal protuberance in males. Each adult swallow was banded and given a unique color-mark on its breast, tail, throat, or wing feathers using waterproof marking pens and acrylic paints to facilitate individual identification. Based on their iridescent blue-green dorsal plumages, all of the breeding female Tree Swallows in our study were after second year (ASY) females (Hussell 1983).

Cloacal swabs were taken from adults and nestlings on nestling day 12. Cloacal swabs were obtained by inserting a sterile Dacron swab (5 mm long × 2 mm wide, Dacrostik® MW151) into the cloaca for 10 seconds. After removing the shaft, the swab was then transferred to 3 ml of sterile thioglycollate broth. Sam-

ples were held for 90 minutes at ambient temperature in the field and during transport to the laboratory and then held at 4°C until plated. Samples were plated within 5.5 hours of collection. Immediately before plating, sample tubes were vortexed for 10 seconds with the swab in the tube and then the swabs were removed. Dilution series were done in sterile thioglycollate broth.

Samples were plated on a variety of growth media. Blood agar (BA) was used to make plate counts of anaerobic bacteria and to detect hemolysis. Blood infusion agar (BHI) was used to make plate counts of aerobic bacteria. Campylobacter (CAMP) was used to detect *Campylobacter* spp. Champman stone agar (CHAP) was used to detect *Staphylococcus* spp. Eosin methylene blue agar (EMB) was used to detect gram negative enterics; lactose fermentors are dark on these plates. MacConkey agar (MAK) was used to detect gram negative enterics, lactose fermentors are red on these plates. Tomato juice agar (TJA) was used to detect lactobacilli and other acidophilic microorganisms. Sabouraud dextrose agar (SAB) was used to detect fungi. Salmonella Shigella agar (SS) was used to detect *Salmonella* and *Shigella* spp. BA (5 percent sheep blood) and CAMP (containing five antimicrobes and 10 percent sheep blood) plates were obtained from commercial sources. Single plates were plated for 0.1 ml of the thioglycollate resuspension and for 0.1 ml of a 10⁻¹ dilution of all media except BHI with which a plate for 0.1 ml of a 10⁻² dilution was also made. All samples were incubated at 35°C for 24 hours. BA and CAMP plates were incubated in BBL® GasPak jars under CO₂ conditions and microaerophilic conditions in BBL® CampyPak envelopes. SAB plates were incubated for an additional 24 hours. After incubation, colonies on plates were counted and the plates given the following scores; 0 = 0 colonies, 1 = 1–10 colonies, 2 = 11–100 colonies, 3 = 101–1,000 colonies, and 4 = more than 1,001 colonies. Total plate scores were calculated as the sum of individual plate scores. The maximum possible total plate score was 36 (i.e., an individual plate score of 4 on each of 9 different media). It was not possible to plate each dilution in triplicate so to check the precision of plate scoring, 11 samples were plated in triplicate. The mean of the percent standard deviation for plates with 10 or more colonies was 11 percent and for all plates, 32 percent. Therefore, our plate scoring was precise.

Field blanks were collected by holding a sterile swab in the air for 10 seconds and then treating the swabs and plating the samples as previously described.

Data were analysed using SAS (SAS Institute 1985). Individual nestlings were treated as independent samples in the statistical analyses of the relationship between bacterial loads and size and weight because we assumed that nestlings varied genetically and because individuals are affected by the bacteria residing in their own bodies and not directly by the mean bacterial loads of their nest-mates.

RESULTS AND DISCUSSION

We surveyed the communities of cloacal bacteria from 11 adults ($n = 6$ females, $n = 5$ males, $n = 5$ mated pairs) and 22 nestlings ($n = 4$ broods). We isolated one or more types of bacteria from the cloacae of every

TABLE 1. The prevalence of cloacal bacteria in Tree Swallows in Michigan.

Bacteria	Adults		Nestlings	
	n	Percent positive	n	Percent positive
Gram negative enterics (dark lactose fermentors)	11	100	21	86
Gram negative enterics (red lactose fermentors)	11	100	22	73
Lactobacilli	11	100	22	86
<i>Staphylococcus</i> spp.	11	36	22	5
Fungi	11	100	21	81
<i>Campylobacter</i> spp.	8	100	13	38
Anaerobic	11	100	22	86
Aerobic	11	100	22	82
<i>Salmonella</i> spp., <i>Shigella</i> spp.	11	100	22	55

adult and nestling (Table 1). Among the nine types of bacteria that we tested for, only those plated for *Staphylococcus* spp. (Fisher Exact Test, $P = 0.033$), *Campylobacter* spp. (Fisher Exact Test, $P = 0.007$), and *Salmonella* and *Shigella* spp. (Fisher Exact Test, $P = 0.013$) plates were more prevalent in adults than in nestlings (Table 1). *Staphylococcus* spp., *Campylobacter* spp., and *Salmonella* and *Shigella* spp. were found in significantly fewer nestlings than were other types of bacteria (Log-Likelihood $G = 62.45$, $df = 8$, $P < 0.0001$) (Table 1). These differences in patterns of prevalence probably reflect differential exposure to the different types of bacteria we tested for. Nestlings probably acquire their intestinal bacteria from the regurgitated insects that their parents feed them and their parents' saliva (Kyle and Kyle 1993). The diversity of cloacal bacteria probably increases with age as nestlings become exposed to more food sources. This hypothesis awaits testing.

All of the swallows that we sampled were positive for one or more types of bacteria. Cooper et al. (1980) surveyed pharyngeal and cloacal bacteria from 32 wild Peregrine Falcons and 13 Gyrfalcons but did not present their data in such a way that prevalence could be determined. None of the 36 Passeriformes (genera were not identified) sampled by Yogasundram et al. (1989) for *Campylobacter jejuni* were positive. In contrast, all of the adults and 38 percent of the nestlings we sampled for *Campylobacter* spp. were positive (Table 1). In a data set comparable to ours, Brittingham et al. (1988) found that 148/290 (51%) Black-capped Chickadees surveyed were negative for all six genera of bacteria (*Escheria* spp., *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Yersinia* spp.) tested.

Our results suggest that cloacal bacteria may be more prevalent in Tree Swallows than in Black-capped Chickadees. These differences may reflect differences in the feeding habits of swallows and chickadees. Tree Swallows feed almost exclusively on aerial insects and most likely acquire some of their intestinal, and consequently cloacal, bacteria from these food items. Parents then pass the bacteria residing in and on food items to their nestlings when they regurgitate food for them. In contrast, chickadees are omnivorous although they feed primarily on seeds during the winter when their

cloacal bacteria were sampled by Brittingham et al. (1988).

Brittingham et al. (1988) argued that the omnivorous birds in their sample had a greater prevalence of *Streptococcus* spp. than did the granivorous birds because the omnivorous birds sometimes fed on animal carcasses and horse manure. We are not aware of any studies that compare the prevalence of cloacal bacteria types between frugivorous, granivorous, insectivorous and omnivorous birds. Yogasundram et al. (1989) found in a study of 445 cases of domestic and wild birds from 13 orders submitted for necroscopy in Louisiana that *Campylobacter jejuni* was most prevalent in Galliformes (38/151, 25% of Galliformes samples) and Anseriformes (4/31, 13% of Anseriformes samples) and absent from another 59 birds from 8 orders. The low prevalence of *Campylobacter jejuni* in Yogasundram et al.'s (1989) study may be an artifact of small sample sizes because the negative samples make up only 13 percent of their total sample.

Sample sizes were too small to statistically examine the relationships between bacterial loads and adult size.

Total plate score had a significant positive effect on nestling wing length, a significant negative effect on nestling weight, but no significant effect on nestling tarsus length (Fig. 1). Bacterial loads of some types of bacteria were significantly correlated with nestling size on day 12 (Table 2). Note that some bacterial loads were negatively correlated with nestling weights and left tarsus length, while others were positively correlated with wing length (Table 2).

The relationships between bacteria and nestling growth are difficult to interpret for at least two reasons. First, because we did not identify bacteria to species. Second, ecological interactions between different species of bacteria may affect nestling growth. Plate scores for bacteria groups grown on all media except *Staphylococcus* spp. were significantly positively correlated with one or more other groups of bacteria. There were no negative correlations between any plate scores. Ecological interactions between different groups of symbiotic bacteria in wild birds deserve further study.

While the pathogenic effects of bacteria are well known, the positive effects of symbiotic bacteria are not often considered. Hutcheson et al. (1991) reviewed a variety of studies that showed that gastrointestinal

FIGURE 1. Left tarsus length, weight, and left wing length of 12-day-old Tree Swallow nestlings and total plate score. Total plate score equals the sum of individual plate scores of cloacal bacteria grown on nine different media. See text for scheme used to score individual plates. Maximum total plate score equals 36.

and urogenital microbes such as bacteria and fungi can have important physiological benefits to their hosts through the competitive exclusion of potentially pathogenic microbiotic species, enhanced host nutrition, and the enhanced development of host immunocompetence. Kyle and Kyle (1993) demonstrated the potential positive effects of symbiotic bacteria in wild birds by

showing by experiment that adult saliva containing a variety of microbes was absolutely required for the successful rearing of Chimney Swift (*Chaetura pelagica*) nestlings less than six-days old. The saliva was used to inoculate food used in the handrearing of nestlings and could come from any healthy adult swift (Kyle and Kyle 1993).

TABLE 2. Significant Spearman rank correlations between cloacal bacteria colony counts and nestling Tree Swallow size on nestling day 12 (day of hatching is nestling day 1).

Bacteria	Measure	n	r_s	P
Gram negative enterics (lactose fermentors dark)	weight (g)	22	-0.47	0.026
<i>Salmonella</i> spp., <i>Shigella</i> spp. S	weight	22	-0.44	0.039
Lactobacilli	left tarsus length (mm)	22	-0.48	0.025
Anaerobic	left tarsus length	22	-0.43	0.043
Gram negative enterics (lactose fermentors red)	left wing length (mm)	20	0.72	0.0004
Lactobacilli	left wing length	21	0.50	0.021
Fungi	left wing length	20	0.45	0.047
Anaerobics	left wing length	21	0.47	0.030

Bacterial loads had no effect on fledging success; all nestlings in our study fledged. Further study of the relationship between bacteria loads and nestling growth are justified since size at fledging is positively correlated with post-fledging survival (O'Connor 1984) and the association between bacterial loads and nestling growth, survival, and fledging success has not been previously studied in wild bird species.

In order to determine whether there were familial patterns in cloacal bacteria communities we performed a canonical discriminant analysis (SAS 1985) that compared individuals by both the identity and plate scores of their communities of cloacal bacteria and then grouped them with individuals with similar communities and plate scores of cloacal bacteria. The canonical discriminant analysis correctly classified 19/22 (86%) of nestlings to their correct brood based on cloacal bacteria. The composition and sizes of communities of cloacal bacteria was a better predictor of the nest where a nestling was reared than was nestling size. A canonical discriminant analysis correctly classified 16 of 22 nestlings (73%) based on measures of size. The four broods were significantly different in mean weight (Kruskal-Wallis ANOVA $\chi^2 = 13.66$, $df = 3$, $P = 0.003$), mean left tarsus length (Kruskal-Wallis ANOVA $\chi^2 = 8.30$, $df = 3$, $P = 0.04$), and mean left wing length (Kruskal-Wallis ANOVA $\chi^2 = 13.01$, $df = 3$, $P = 0.005$). These results suggest two non-mutually exclusive possibilities. First, nestlings in a nest have similar communities of cloacal bacteria because they are fed by the same parents (cf. Kyle and Kyle 1993). Second, there may be genetic influences on the communities of cloacal bacteria. Different broods have different communities of bacteria because they differ genetically and thus have different internal environments that are more or less favorable to the growth of different types of bacteria. Stern et al. (1990) found that some stocks of broiler chickens (*Gallus domesticus*) were more resistant to cecal colonization by *Campylobacter jejuni* than were others. Immune response genes have been found in chickens (Pevzner et al. 1975) and have been implicated in the control of infections of bacterial (Karakoz et al. 1974, Krejci et al. 1974, Pevzner et al. 1975) and viral (Crittenden et al. 1974) pathogens. Nestlings that were not matched with their nest-mates by the canonical discriminant analyses may have resulted from extrapair fertilization which are relatively common in Tree Swallows (Lifjeld et al. 1993).

A canonical discriminant analysis correctly paired nine of 10 (90%) mates based on both the identity and plate scores of their communities of cloacal bacteria. This pattern suggests that mates trade cloacal bacteria during copulation. The avian cloaca serves the dual functions of excretion and gamete transfer. Thus, bacteria may be horizontally transmitted from males to females during copulation because intestinal bacteria could become incorporated into an ejaculate (Sheldon 1993). If bacteria and virus are easily transmitted from males to females during sex in birds, then sexually transmitted diseases may have a previously unconsidered role on the evolution of avian mating systems (cf. Sheldon 1993).

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THE EFFECT OF MALE PRESENCE ON NESTLING GROWTH AND FLUCTUATING ASYMMETRY IN THE BLUE TIT¹

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Key words: Blue tit; growth; fluctuating asymmetry; brood reduction; paternal care.

Generally, lack of paternal effort in temperate passerine bird species manifests itself in terms of either fewer fledged young, and/or lighter young (Björklund and Westman 1986, Sasvári 1986, Kempenaers 1994). However, the effect of male absence on the growth of young is poorly known (Sasvári 1986). Recent interests in fluctuating asymmetry (defined as random fluctuations from perfect symmetry of bilateral traits) have suggested that developmental stress caused by inadequate nutrition, for example caused by limited paternal care, can lead to an increased level of asymmetry (Palmer and Strobeck 1986, Watson and Thornhill 1994). A high level of asymmetry can be an indicator of poor feeding conditions during growth or the inability of certain individuals to cope with poor growing conditions, and thus serve as a cue for genetically-based mate choice (Møller and Pomiankowski 1993, Watson and Thornhill 1994). This suggests that chicks without paternal care might suffer not only in terms of being lighter at fledging but also by a higher degree of asymmetry, leading to reduced reproductive success.

In this paper I will analyse the effect of male absence on a number of breeding parameters in the Blue Tit (*Parus caeruleus*). In this species male paternal effort is extensive and normally at the same rate as female effort (Perrins 1979). In particular I concentrate on clutch size and number of fledged young, as well as growth of individual chicks in biparentally attended as

opposed to uniparentally attended nests. Furthermore, I examine the degree of fluctuating asymmetry in chicks in the two classes of nests with the expectation that asymmetry will be higher in broods without male attendance.

METHODS

The field work was carried out in the surroundings of Uppsala, central Sweden, during May and June 1994. All the Blue Tit pairs were found breeding in luxuriant deciduous forests. Nest boxes were checked regularly and the date of first egg, clutch size and the day of hatching were recorded. At two days of age, one young in each nest was randomly picked and marked. This chick was then measured at 2, 5, 8, 11 and 14 days of age. At age 14 days, all young in the nest were counted and weighed. I measured wing length, tarsus length, tail length, bill length, skull length and body mass. However, since all these traits are heavily intercorrelated (Björklund, unpubl.) only tarsus, wing and mass measures will be used in the present analysis. Tarsus length was measured to the nearest 0.1 mm, wing length to the nearest 1.0 mm, and mass to the nearest 0.5 g. To estimate fluctuating asymmetry (FA) wing length was measured on both sides from day 8 and after (i.e., when feathers start to grow), since it is known that growth of feathers is condition dependent (e.g., Grubb 1989). To be able to separate measurement error from estimates of FA each chick was measured twice (see below). When chicks were eight days or older, adults were captured.

Normally, when nest boxes were opened, the male behaved very aggressively especially when young approached fledging (14 days). However, at some boxes

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