Fault bars and bacterial infection

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Abstract

Fault bars are conspicuous malformations on bird feathers produced during feather growth. The causes behind fault bars are poorly understood. Here we used the presence of *Campylobacter jejuni* infections in 302 urban feral pigeons (*Columba livia*) as a proxy of physiological stress, and correlated it with fault bar abundance. Overall, we found a prevalence of 24.5% of these bacteria. Bacterial infection was equally prevalent in young and adults, but males showed a slightly higher prevalence than females. Fault bars were more abundant in young than in adults, particularly among young males. Pigeons with *Campylobacter* infection had more fault bars than uninfected birds. These results suggest that the physiological state of the individuals could be as important as external stressors to understand the occurrence of fault bars. Also, it suggests that parasites could have a role in fault bar formation.

Keywords: bacteria, feather, physiology, stress bars, stress marks.

Introduction

Fault bars are conspicuous malformations on bird feathers produced during feather growth (Riddle 1908). They can be from a slight notch on the feather surface to a clear discontinuity on keratin deposition (Sarasola and Jovani 2006). This creates a perpendicular bar to the feather rachis where barbules are thinner or completely missing, thus weakening the feather and often causing feather breakage (Riddle 1908, Sarasola and Jovani 2006). This could be particularly harmful if flight feathers are involved. In fact, even slight reductions in wing surface area have been experimentally shown to reduce bird fitness (Velando 2002). Note also that broken feathers are not replaced until the next programmed moult, hence the costs of a fault bar weakening or breaking a given feather can last from months (e.g. in some passerine species) to several years (e.g. in many raptors; Rohwer et al. 2009).

The causes of fault bars is an open question. Malnutrition, handling, and parasites have been proposed as important stressors producing fault bars (Sebright 1826, Riddle 1908, King and Murphy 1984, Murphy et al. 1988, 1989, Negro et al. 1994). Whatever the cause of fault bars, it appears to be something difficult to overcome by birds because they are still produced in all bird species after millions of years of evolution of bird feathers (Riddle 1908). This makes fault bars interesting because they may be telling us something important occurring during the life of individual birds (e.g. life-history trade-offs; Møller 1989, Møller et al. 1996, Bortolotti et al. 2002, Pap et al. 2007, Romano et al. 2011, Vágási et al. 2012), or in their habitats (e.g. contaminants, parasites, land uses, habitat fragmentation; Sodhi 2002, Freed et al. 2005, 2008). Therefore, understanding the causes behind fault bars has an intrinsic interest, but also applied relevance. This is especially true because recording fault bars is easy and nonintrusive, and can be recorded from moulted feathers (Jovani and

Diaz-Real 2012), living birds (Jovani and Blas 2004; Jovani and Tella 2004), and historical series of museum specimens.

Here we bring a new piece to this puzzle by exploring the relationship between fault bar occurrence in urban feral pigeons (*Columba livia*) and infection by the bacteria *Campylobacter jejuni*. This enteric bacteria is a major cause of human gastroenteritis worldwide (Moore et al. 2005). It is highly widespread in wild birds and poultry (Kapperud and Rosef 1983, Waldenstrom et al. 2002) where it largely has a commensal relationship (Newell 2001, Bull et al. 2008), but detrimental effects on bird health have been also reported (Moore et al. 2005). Moreover, their presence is highly related with a myriad of other health problems and stressors in birds (Cogan et al. 2007, Bull et al. 2008). In fact, studies in commercially housed broilers show that stresses alter gut flora composition and/or host immune responses which may increase their susceptibility to *Campylobacter* and other infections (Bull et al. 2008). Thus we used *Campylobacter* infection as a proxy of general health state in pigeons because of its direct effects on birds and its relationship with stressors and diseases affecting them.

Methods

Pigeons were captured using compressed air-propelled nets during the ongoing monitoring and control programme of urban birds carried out by the Monitoring and Control of Urban Pests Service (Barcelona City Council) in different localities within Barcelona city from November 10th, 2009 to February 16th, 2010.

One cloacal swab was collected from each pigeon just after capture (see below), and then birds were aged following Uribe et al. (1985). Sex was determined through sexual gonads inspection during necropsy (pigeons were euthanized in a tight container with CO₂). The number of fault bars was recorded for each of the 23 remiges of the

right wing and the 12 rectrices of the tail of each bird (only birds without missing or moulting feathers were studied). For each fault bar, it was also recorded its intensity following Sarasola and Jovani (2006). In brief, the intensity of fault bars could be from "light" (discontinuity on the structure of the feather created by the absence of some barbules), "medium" (a <1 mm translucent line across the feather), to "strong" (≥ 1 mm translucent line across the feather; see Sarasola and Jovani for pictures and further details).

Swabs were preserved at 2°C- 8°C and were analysed within 24 hours at the Laboratori de l'Agència de Salut Pública de Barcelona. *Campylobacter spp* infection was assessed according to Norm ISO 10272-1:2006. In brief, each swab was inoculated into a modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid) for 44h $\pm$ 4h at 41.5 °C in microaerobic atmosphere. After that, potential *Campylobacter* colonies were detected through their typical greyish colour, often with a metallic sheen, appearing as flat and moist, with a tendency to spread. These colonies were then subcultured on two plates of non-selective Columbia blood agar (Oxoid): one of them was incubated in an aerobic atmosphere and the other one in a microaerobic atmosphere, both at 37°C during 24h-48h. Finally, candidate colonies were identified as *Campylobacter spp.* using the oxidasa reaction (Merck), triple iron sugar reaction (Merck) and Dryspot *Campylobacter* latex agglutination test (Oxoid). Hydrolysis of hippurate reaction (Sigma-Aldrich) was used to determine if the *Campylobacter* colonies isolated were *Campylobacter jejuni*.

The number of fault bars was modelled with a generalized linear model using PROC GENMOD of SAS 9.2 software with a Poisson distribution and log link function. The dependant variable was the number of fault bars (either all fault bars together or the number of each fault bar intensity separately), and independent variables were the

occurrence of *Campylobacter* infection in each individual (either 0 or 1), and the age and sex of the individual. Type 3 errors were used in all analyses; i.e. results show the net effect of a given variable (e.g. *Campylobacter* infection) once including the rest of variables fitted in the model.

Results

A total of 302 pigeons were sampled; 51 young females, 18 young males, 81 adult females and 152 adult males. 24.5% were positive for *Campylobacter*, and 92.7% had at least one fault bar. Pigeons with fault bars had a median (range) = 30 (1-161) fault bars.

Fault bars and *Campylobacter* vs. age and sex

Campylobacter infections were equally distributed across ages (young=27.5%, n=69; adults=23.6%, n=233; age: $\chi^2_1 = 0.445$, p=0.505), but males showed a higher prevalence than females (males=29.4%, n=170; females=18.2%, n=132; sex: $\chi^2_1 = 5.065$, p=0.024). There was not a statistically significant interaction between age and sex (age*sex: $\chi^2_1 = 0.30$, p=0.58).

The total number of fault bars was higher in young than in adult pigeons (age: $F_{1,299} = 6.83$, p=0.009), and higher in males than in females (sex: $F_{1,299} = 20.86$, p<0.001; Fig. 1a). There was not a statistically significant interaction between age and sex (age*sex: $F_{1,298} = 2.05$, p=0.153; Fig. 1a).

Campylobacter vs. fault bars

Fault bar abundance (all intensities together; ages and sexes clumped) was more than two times higher in pigeons positive for *Campylobacter* (median, range=53.5, 0-161) than for *Campylobacter*-negative pigeons (median, range=24, 0-96; *Campylobacter*: $F_{1,300}=101.19$, $p<0.001$; Fig. 2a,b). Same was found when statistically controlling for the effect of age and sex upon fault bar abundance (*Campylobacter*: $F_{1,297}=88.98$, $p<0.001$). Similar results were found considering light (*Campylobacter*: $F_{1,297}=54.20$, $p<0.001$), medium (*Campylobacter*: $F_{1,297}=82.12$, $p<0.001$), or strong fault bars separately (*Campylobacter*: $F_{1,297}=5.74$, $p=0.017$; Fig. 2c).

Discussion

We found a clear positive correlation between *Campylobacter jejuni* infection and the abundance of fault bars in the flight feathers of pigeons. Also, we found a higher abundance of fault bars in young pigeons. These two results suggest that individuals with their general physiological state more compromised (Sol et al. 1998, 2000, Moore et al. 2005, Cogan et al. 2007, Bull et al. 2008) had a higher propensity to develop fault bars. This result adds to the idea that not all individuals are equally prone to develop fault bars. Thus, that while some particular kind of stress (e.g. experimental handling; Murphy et al. 1989, Negro et al. 1994) may be necessary to produce a fault bar, the actual production of a fault bar depends on the physiological state of the individual. Thus, our results add to the currently low understanding of the causes of fault bars by showing that it may be wrong searching for "the cause" of fault bars, because whatever is the specific stress causing a particular fault bar, the general physiology of the individual seems to be playing a role, likely mediated by glucocorticoids released by the hypothalamic-pituitary-adrenal axis (Romero et al. 2005, Bortolotti et al. 2009). Also, our study supports the few previous studies (Møller et al. 1996, Freed et al. 2005, 2008)

showing that parasites and the immune system of birds may have an important role on
 fault bar formation.

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Figure Legends

Fig. 1 Box-plot of the abundance of fault bars (counted on 23 remiges of the right wing and the 12 rectrices of the tail) in pigeons according to age and sex. Lower and upper box boundaries indicate 25th and 75th percentiles, respectively. The line inside the box indicates the median. Lower and upper error lines indicate the 10th and 90th percentiles, respectively. Dots indicate data outside 10th and 90th percentiles.

Fig. 2 (a) Box-plot (see details in Fig. 1) comparing the total number of fault bars (counted on 23 remiges of the right wing and the 12 rectrices of the tail) for infected and uninfected pigeons by *Campylobacter*. (b) Histogram showing the abundance of fault bars in infected (black bars) and uninfected pigeons (grey bars) by *Campylobacter*. (c) Box-plot comparing the number of light (left), medium (middle) and strong (right) fault bars for infected and uninfected pigeons by *Campylobacter*.

Fig. 1

283 **Fig. 2**

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