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SHORT COMMUNICATION

Absence of *Mycoplasma* spp. in nightingales (*Luscinia megarhynchos*) and blue (*Cyanistes caeruleus*) and great tits (*Parus major*) in Germany and its potential implication for evolutionary studies in birds

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Abstract

Mycoplasma spp. are important pathogens in poultry and cause high economic losses for poultry industry worldwide. In other bird species (e.g. white storks, birds of prey, and several waterfowl species), *Mycoplasma* spp. are regularly found in healthy individuals, hence, considered apathogenic or part of the microbiota of the upper respiratory tract. However, as *Mycoplasma* spp. are absent in healthy individuals of some wild bird species, they might play a role as respiratory pathogen in these bird species, e.g. *Mycoplasma gallisepticum* in house finches. The knowledge on the occurrence of *Mycoplasma* spp. in wild birds is limited. To evaluate the relevance of *Mycoplasma* spp. in free-ranging nightingales and tits, 172 wild caught birds were screened for the presence of mycoplasmas. The birds were sampled via choanal swabs and examined via molecular methods ($n = 172$) and, when possible, via culture ($n = 142$). The *Mycoplasma* sp. was determined by sequencing the 16S rRNA gene and 16S-23S Intergenic Transcribed Spacer Region. All birds were tested negative for mycoplasmas via PCR and/or mycoplasmal culture. Hence, free-ranging nightingales and tits do not show any mycoplasma in their microbial flora of the respiratory tract. Therefore, these songbird species may suffer from clinical mycoplasmosis when being infected. We hypothesize that birds relying on their vocal ability for reproduction have excluded mycoplasmas from their respiratory flora compared to other bird species.

Keywords Apathogenic mycoplasmas · *Cyanistes caeruleus* · *Luscinia megarhynchos* · *Parus major* · Songbirds · Song voice

In domestic poultry *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis*, and *Mycoplasma iowae* are the most relevant pathogens, causing high economic losses. Furthermore, Nolan et al. (1998) estimated that 100 million House Finches (*Carpodacus mexicanus*) died in an outbreak of disease caused by *Mycoplasma gallisepticum* in

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the US because of severe conjunctivitis and consequential loss of eyesight. Several other passerine species have been affected by *Mycoplasma gallisepticum* infections (Mikaelian et al. 2001) too. However, in certain avian species, mycoplasmas are known as apathogenic bacteria of the respiratory tract showing a commensal character. For example, commonly isolated *Mycoplasma* spp. in Galliformes include *Mycoplasma gallinarum* (Poveda et al. 1990) and *Mycoplasma gallinaceum* (Jordan et al. 1982). In healthy birds of prey, *Mycoplasma buteonis*, *Mycoplasma falconis*, and *Mycoplasma gypis* were isolated from up to 100% of the sampled free-ranging birds (Lierz et al. 2008). *Mycoplasma ciconiae* was descripted as a commensal *Mycoplasma* sp. as it was detected in 99.1% of healthy, free-ranging white storks (*Ciconia ciconia*) (Möller Palau-Ribes et al. 2016). In studies on clinically healthy, free-ranging pelicans (*Pelecanus onocrotalus*) 98% were tested positive for mycoplasmas, however, there was no species differentiation performed (Assunção et al. 2007). In contrast, no mycoplasmas were found in healthy individuals belonging to other bird species e.g., psittacines or passerines (Lierz and Hafez 2009; Deem et al. 2012). In corvids, mycoplasmas were isolated from diseased birds (Pennycott et al. 2005), but also from 7% of clinically healthy individuals (Ziegler et al. 2017). Therefore, corvids seem to occupy an intermediate position, so that mycoplasmas may play a role in respiratory disease.

These findings lead to the hypothesis that species using sexually selected song traits as mating signals such as many passerines have a lower tolerance towards mycoplasmal infections of the respiratory tract, as respiratory disease would imply disadvantages in their mating behavior. Though not for *Mycoplasma gallisepticum*, it has been shown for other parasites and pathogens that they may reduce song performance measures and thus have an effect on mating success (Buchanan et al. 1999; Garamszegi et al. 2004; Spencer et al. 2005; Owen-Ashley et al. 2006; Gilman et al. 2007; York et al. 2016). These studies suggest that parasites may have played an important role in the evolution of sexually selected traits (Garamszegi et al. 2004). According to this suggestion, song may serve as an honest indicator of a strong immune system, which in turn might be a mate choice criterion for females to increase offspring fitness. Species not relying predominantly on vocalizations in mate choice decisions likely have not faced the same evolutionary pressure on the maintenance of a well-functioning vocal tract. To prove this thesis and to obtain more information on the occurrence of mycoplasmas in free-ranging, healthy passerine bird species using vocalizations in many communicative contexts and in particular in mating behavior, free-ranging nightingales and blue and great tits were sampled.

Samples were obtained in the context of different projects investigating functions of song during mating (in nightingales) in Golm (near Potsdam) and Berlin-Treptow or effects of supplemental feeding (in tits) in Berlin-Tempelhof. All adult birds were caught randomly and tested at the beginning of the breeding season, when they are territorial and live solitary (or in pairs). Chicks were tested in the nest. All study areas comprised several square kilometers and the majority of birds in these areas were tested, making sampling biases rather unlikely.

Birds were caught using mist nets to ring them and to obtain body measures, data on condition, and physiological samples (Landgraf et al. 2017).

Swabs were obtained from the pharyngeal region due to the small bird size. If birds would have shown any clinical signs of a mycoplasmosis e.g., conjunctivitis, respiratory signs, or arthritis, they would have been excluded from the study. However, this never occurred. Overall, 97 nightingales (*Luscinia megarhynchos*), 40 great tits (*Parus major*), and 35 blue tits (*Cyanistes caeruleus*) were screened for the occurrence of mycoplasmas prior to being ringed. In total, 31 were juvenile and 111 adult birds. In 30 cases, the bird's age was not documented. DNA extraction of all swabs was performed as described by Ziegler et al. (2017). All samples ($n = 172$) were screened via *Mycoplasma* genus-specific PCR (target sequence: 16S rRNA gene) as described by van Kuppeveld et al. (1992) modified by Lierz et al. (2007). One hundred forty-two samples were additionally cultured using SP4 liquid and agar media as described by Bradbury (1998) to isolate mycoplasmas.

Within this study, all birds were tested negative for mycoplasmas via *Mycoplasma* genus-specific PCR. Furthermore, it was not possible to isolate any mycoplasmas via culture.

The results of the present study indicate that the investigated populations of passerine species (nightingales, great and blue tits) do not show any mycoplasmas as part of their physiological respiratory flora. However, prior studies indicated that some songbirds do show detectable mycoplasmas in their respiratory tract, as there were 7% of healthy free-ranging corvids tested positive for *Mycoplasma sturni* (Ziegler et al. 2017). As all birds under study were tested negative for mycoplasmas and the prevalence of *Mycoplasma* spp. in corvids was rather low, it seems plausible to suggest that most bird species of the order Passeriformes may not have mycoplasmas in their physiological microbial flora of the respiratory tract. Especially the lack of mycoplasmas in several highly vocal passerine species (e.g., nightingales, blue and great tits) in which song is an important signal to demonstrate male quality to females (Bartsch et al. 2015; Poesel et al. 2001; Rivera-Gutierrez et al. 2010; Weiss et al. 2012), back the hypothesis that these species may not tolerate latent infections of the respiratory tract. This theory may be supported by the fact that healthy psittacines, also highly vocalizing, did not show mycoplasmas in their respiratory tract as well (Lierz and Hafez 2009). This is additionally underlined

by the presence of mycoplasmas in the respiratory tract of healthy birds of prey and white storks (Lierz et al. 2008; Möller Palau-Ribes et al. 2016) which do not rely predominantly on vocalizations in communication in general and in the mating context in particular. In passerines living closely to poultry flocks, poultry pathogenic mycoplasmas can occasionally be found in the upper respiratory tract (Hussein 2016; Stallknecht et al. 1982). However, as seen in the last decades, a single successful host jump of *Mycoplasma gallisepticum* can lead to an epidemic of mycoplasma-induced conjunctivitis in passerine birds, in this case in House finches (*Haemorhous mexicanus*) in the United States (Hochachka et al. 2013). Therefore, we speculate that one predisposing factor for a bird species to develop severe symptoms after an infection may be the lack of commensal mycoplasmas in the respiratory tract. Thus, every mycoplasma-host-combination needs careful evaluation to assess its significance. While case reports on mycoplasmas from single or low numbers of diseased birds give important insights, systematic studies are essential to provide a baseline for the occurrence on *Mycoplasma* spp. in free-ranging birds and evaluate exposure and host ranges (Dhondt et al. 2014).

In conclusion, literature and results of the present study provide the first hints that in bird species relying on their vocal ability in mate choice, and thus for reproduction, respiratory infections may lead to a strong evolutionary pressure towards the exclusion of pathogens from their respiratory tract. Species using also other signals than vocalizations for display and mating behavior might be able to compensate for such infections and still be able to succeed in partner selection. Mycoplasmas seem to be an ideal pathogen to investigate this hypothesis, as they are commensals in the respiratory tract of many bird species but may also lead to severe clinical signs in others. Certainly, this hypothesis needs to be confirmed by further studies in bird species completely relying on their song voice in mating behavior compared to those using other displays.

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Declarations

Ethical approval Capturing, ringing, and probing were conducted in the framework of research projects on the behavioral ecology of free-ranging birds: communication, mate choice, and breeding- and population ecology in nightingales, Reg. 23–2347-4–2009 (Landesamt für Verbraucherschutz, Landwirtschaft und Flurneuordnung Frankfurt (Oder)); effects of supplementary feeding in great tits and blue tits—an experiment using stable isotopes,' Reg. G 0060/11; and how hormones regulate vocal plasticity: the effects of melatonin and testosterone on song and mating success in nightingales,' Reg. 00078/12 (Landesamt für Gesundheit und Soziales, Berlin).

Consent to participate All authors contributed to data acquisition and writing of the manuscript

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