J. Avian Biol. 41: 678–680, 2010
doi: 10.1111/j.1600-048X.2010.05144.x

Received 5 February 2010, accepted 7 July 2010

No detected effect of moult on feather bacterial loads in mallards *Anas platyrhynchos*

Mathieu Giraudeau, Gábor Á. Czirják, Camille Duval, Claude Guiterrez, Vincent Bretagnolle and Philipp Heeb

M. Giraudeau (giraudeau.mathieu@gmail.com), G. Á. Czirják, C. Duval and P. Heeb, Univ. de Toulouse III Paul Sabatier, Lab. Évol. et Diversité Biol. (EDB), UMR 5174, 118 Route de Narbonne, F-31062 Toulouse, France, and Centre Natl. de la Recherche Sci. (CNRS), EDB, F-31062, France. – C. Guiterrez, Lab. de Microbiol. et Génétique Mol. (LMGM), UMR 5100, UPS, F-31000 Toulouse, France. – V. Bretagnolle, Centre d'Etudes Biol. de Chize, CNRS UPR 1934, F-79360 Beauvoir-sur-Niort, France.

Feathers play an important role in many aspects of avian life histories, for exampling acting in thermoregulation, communication and flight. Damaged feathers may reduce the ability to perform these functions, so there are likely fitness costs for individuals possessing damaged plumage. Recently, descriptive studies provided evidence that birds carry feather-degrading bacteria on their plumage. These bacteria have the ability to degrade feathers, rapidly under laboratory conditions, more slowly under field conditions. If such feather-degrading bacteria reduce avian fitness, natural selection should favour the evolution of anti-bacterial defences. Moult has been suggested as one such defence. Here we test this prediction with mallards, *Anas platyrhynchos*, and the use of repeated measures of total cultivable and feather-degrading bacterial loads on the same birds before, during and after moult. We found that moult had no significant effect on total cultivable and feather-degrading bacterial loads on feathers. Our results show that the bacterial contamination that takes place after moult overrides the potential role of moult as a mechanism to reduce feather bacterial loads.

Diverse groups of animals including birds, mammals, and reptiles regularly moult. It has been proposed that in addition to replacing worn feathers, hair, or skin, moult reduces parasite infestations (Marshall 1981, Kim 1985, Lehane 1991, Clayton 1999, Moyer et al. 2002, Gunderson 2008). Most of the studies that have examined the effects of avian moult on parasite infestation have focused on ectoparasite loads (Markov 1940, Baum 1968, Moyer et al. 2002). These correlative studies reported a drop in the abundance of ectoparasites on moulting birds. In contrast, an experimental study by Moyer et al. (2002) counted louse abundance and concluded that moult had no significant effect on these ectoparasites. Results of Moyer's experiment could be explained by escape behaviour of ectoparasites from moulting feathers (Jovani and Serrano 2001).

In this study, we assessed the effect of avian moult on plumage bacteria with particular emphasis on feather-degrading bacilli. Recently, Burtt and Ichida (1999) demonstrated that birds carry bacteria on their feathers and that some of them, the keratinolytic bacteria, can rapidly degrade feathers, at least under laboratory conditions. Numerous studies have isolated feather-degrading bacteria from the plumage of wild birds (Shawkey et al. 2003, 2007, 2008, Lucas et al. 2005, Gunderson et al. 2009). The feather-degrading bacterial group is phylogenetically and physiologically highly diverse and is functionally defined by the ability to degrade β-keratin, a protein which composes more than 90% of feather mass (Onifade et al. 1998, Lucas et al. 2003, Gunderson 2008). The feather damage they cause could alter the insulative efficiency of the plumage causing thermoregulatory stress and a consequent reduction in body mass and survival (Clayton 1999). Other fitness consequences of feather damage might be reduction of aerodynamic efficiency (Clayton 1999), or coloration modifications (Shawkey et al. 2007, 2008, Gunderson et al. 2009). Assuming that feather-degrading bacteria reduce avian fitness under natural conditions, natural selection should have favoured the evolution of bird anti-bacterial defences (Clayton 1999, Gunderson 2008). The goal of our study was to test whether moult acts as a defence mechanism against bacteria as suggested by the observational study of Burtt and Ichida (1999) who found lower frequency of individuals with feather-degrading microbes after moult than before. We carried out this study with mallards *Anas platyrhynchos* where we quantified total-cultivable and feather-degrading bacterial loads. We sampled feathers before, during and after moult on the same birds and then compared bacterial loads on the birds' plumage between the three moult stages. If, as predicted, moult is acting to reduce plumage bacterial load and particularly feather-degrading bacterial infestation, we expected a drop in the abundance of total cultivable and feather-degrading bacterial loads after the moult.

Material and methods

The experiment was carried out between April and August 2008 at the Centre d'Etudes Biologiques de Chizé (CEBC, western France) using adult mallards descended from individuals caught in the wild. The 3-5 year-old birds were held, for the duration of the study, in a large open aviary (900 m^2, 30 by 30m) with access to a pool (25 m^2). Birds had free ad libitum access to water and food (mixture of crushed corn, wheat and commercial duck food). Experiments and handling were carried out in accordance with French veterinary services.

Eighteen birds (9 males and 9 females) were captured at given dates before (April), during (June), and after (August) the post-nuptial moult (Ginn and Melville 1983). Directly upon capture, one feather from a standardized position on the back was collected from each individual and placed in a sterile eppendorf in order to avoid further contaminations. New sterile materials (gloves, tweezers and scissors) were used for each sample. Eppendorfs were subsequently kept in a dark envelope at –20° C until further analyses.

Microbiological analyses were performed under sterile conditions on the outermost two cm of each feather. For details on microbiological techniques, see Møller et al. (2009). Briefly, to obtain both the free-living and attached micro-organisms, feathers were sonicated and vortexed in sterile physiological saline solution (Lucas et al. 2005). Afterwards, the feathers were dried for 24 h at 70° C and weighed to the nearest 1 µg on an analytical balance (AT1 Comparator, Mettler Toledo).

To quantify separately cultivable bacteria and keratinolytic bacteria loads we used two different growth medium. Firstly, Trypticase Soy Agar (TSA, #22091, Fluka) is a rich medium on which many heterotrophic bacteria grow, thus enabling assessment of the cultivable micro-organism loads of the feathers. Secondly, we quantified the feather-degrading bacterial loads using Feather Meal Agar (FMA) (Sangali and Brandelli 2000, Shawkey et al. 2003, 2007, Møller et al. 2009). Fungal growth was inhibited by adding 100 mg mL^{-1} of cycloheximide to the mediums.

To measure bacterial counts, we spread 100 µl of the microbial solution on each of the different growth media and the plates were incubated at 25° C, for 3 days in the case of TSA, and for 14 days in the case of FMA (Shawkey et al. 2003, 2007). After incubation, the numbers of visible colony forming units (CFU) on each plate were counted. Counts were then corrected for the initial volume of the suspension and feather mass. The microbial concentration for each medium type was expressed as CFU per mg feather (CFU mg^{-1}). We used only one dilution because preliminary tests showed that the counts were always between 0 and 150 CFU per plate. All counts were performed by one of us (MG) without knowledge of the sampling period.

Statistical analyses

We log transformed the microbiological data to normalise them. Then, we performed repeated measure ANOVAs on total cultivable and feather-degrading bacterial loads with sex, moult stage and sex × moult stage interaction as factors. Statistical analyses were conducted using STATISTICA 6.0.

Results and discussion

Preliminary work by our group indicates that sampling only a single feather from each bird at each time period provides suitable data to investigate the effects of moult on feather bacterial loads. This study revealed significant correlations between back, wing-flash and head samples obtained from the same individuals, indicating that within individuals, bacterial loads on back feathers are indicative of bacteria intensity in other plumage areas (Back/head: $F_{1,25} = 13.91$, $p < 0.001$, $R^2 = 0.36$; back/wing flash: $F_{1,28} = 4.4$, $p < 0.05$, $R^2 = 0.14$); as it was already shown for passerine species (Shawkey et al. 2008, Gunderson et al. 2009). Our results suggest that this pattern may be widespread. Moreover, our preliminary study revealed a high repeatability from triplicate data for plate counts (repeatability = 0.97) calculated using the method of Lessels and Boag (1987), indicating that our method of bacteria quantification is repeatable.

We also found no significant differences for total cultivable ($F_{2,15} = 0{,}07$; $P = 0{,}93$) and feather-degrading bacterial loads ($F_{2,15} = 0{,}32$; $p = 0.73$) between our three sampling dates (Fig. 1). We did not find any significant effect of sex for total cultivable ($F_{1,15} = 0{,}69$; $p = 0.42$), or feather-degrading bacterial loads ($F_{1,15} = 1{,}53$; $p = 0.23$). Finally, the interaction sex*moult stage was not significant on total cultivable ($F_{2,15} = 1{,}27$; $p = 0.29$), and feather-degrading bacterial load ($F_{1,15} = 1{,}10$; $p = 0.34$).

To summarize, we found that moult had no lasting detectable effects on feather bacterial loads in mallards. Our results are not consistent with the hypothesis that a function of moult is to decrease feather-degrading bacteria loads. In their observational study, Burtt and Ichida (1999) found fewer incidences of passerines with feather-degrading microbes after prebasic moult. Here, we used a repeated measures approach with a non-passerine, which showed that on the same bird, bacterial loads did not differ significantly before, during and after moult.

In mallards, moult is a slow process which can take several weeks (Ginn and Melville 1983) and during which new and old feathers can be in contact (Payne 1972, Welty

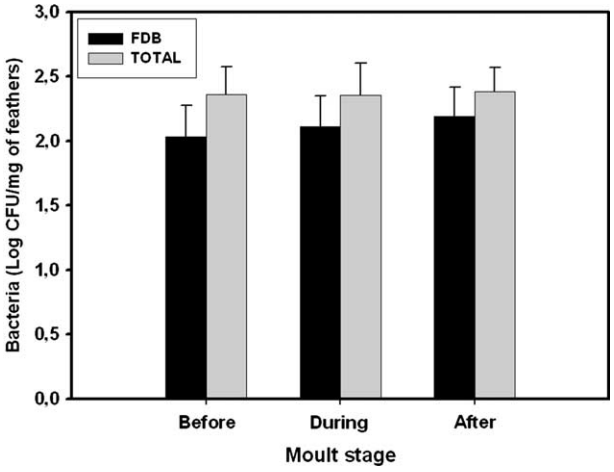

Figure 1. Mean (+SE) total cultivable and feather-degrading bacteria (FDB) abundance on mallard back feathers before, during and after moult. The two sexes were pooled in the figure.

and Baptista 1988). The time intervals between samplings in this study were apparently enough for the colonization of the new feathers by bacteria. It is probable that new feathers have few bacteria when they emerge from their follicules but become rapidly contaminated by contact with old feathers and the environment, thus leading to the establishment of similar bacterial loads as on old feathers. In our study, we sampled feathers several days after moult was finished and this could explain why we did not detect any significant differences of bacterial loads between the sampling times. In the future, in vitro experiments could be used to estimate the bacterial recolonisation rate of a newly emerged feather.

Another explanation for our non-significant results could be the use of semi-captive birds for this experiment. In our study, mallards were fed ad libitum and probably faced lower predation risks than wild birds and this could lead to a greater investment in sanitation behaviours (Lucas et al. 2005), with an overall reduction and a homogenization of bacterial loads among contiguous feathers.

We found high loads of feather-degrading bacteria compared to total cultivable bacteria for the three sampling events like Shawkey et al. (2008) found on carotenoïd based plumage of wild house finches *Carpodacus mexicanus*. Those results suggest that feather-degrading bacteria represent a high proportion of plumage bacterial load. However, as the growth medium used to quantify total cultivable and feather-degrading bacterial loads were different, further studies are needed in order to confirm our observation.

Our study is the first to examine in vivo the effect of a mechanism proposed to control feather-degrading bacteria. Further studies with this approach are now required to determine whether other plumage maintenance mechanisms (i.e preening, sunbathing; Clayton 1999, Gunderson 2008) evolved in birds to limit the potential detrimental effect of feather-degrading bacteria. For example, it could be interesting to examine the effects of a preen gland surgical removal on feather-degrading bacterial load. Moreover, an examination of the impact of avian moult on feather-degrading bacteria diversity could also constitute a future way of research.

Acknowledgements – We are grateful to Nadine and Noël Guillon, Jerome Fort and Matthew D. Shawkey for their help. This project was partly supported by a French research grant from the ANR to Philipp Heeb (ANR-05, NT05-3_42075). MG was supported by a PhD scholarship from the French Government and GAC by an Eiffel grant from the French Ministry of Foreign Affairs.

References

Baum, H. 1968. Biology and ecology of the feather lice of blackbirds. – Angew Parasitol. 9: 129–175.

Burtt, E. H. Jr. and Ichida, J. M. 1999. Occurrence of feather-degrading bacilli in the plumage of birds. – Auk 116: 364–372.

Clayton, D. H. 1999. Feather busting bacteria. – Auk 116: 302–304.

Ginn, H. B. and Melville, D. S. 1983. Moult in birds. p. 40. London. – BTO, UK.

Gunderson, A. R. 2008. Feather–degrading bacteria: a new frontier in avian and host-parasite research. – Auk 125: 1–8.

Gunderson, A. R., Forsyth, M. H. and Swaddle, J. P. 2009. Evidence that plumage bacteria influence feather coloration and body condition of eastern bluebirds *Sialia sialis*. – J. Avian Biol. 40: 440–447.

Jovani, R. and Serrano, D. 2001. Feather mites (Astigmata) avoid moulting wing feathers of passerine birds. – Anim. Behav. 62: 723–727.

Kim, K. C. 1985. Coevolution of parasitic arthropods and mammals. – Wiley, New York.

Lehane, M. J. 1991. Biology of blood-sucking insects. – Harper Collins, London.

Lessells, C. M. and Boag, P. T. 1987. Unrepeatable repeatabilities: a common mistake. – Auk 104: 116–121.

Lucas, F. S., Broennimann, O., Febbraro, I. and Heeb, P. 2003. High diversity among feather-degrading bacteria from a dry meadow soil. – Microb. Ecol. 45: 282–290.

Lucas, F. S., Moureau B., Jourdie, V. and Heeb, P. 2005. Brood size modifications affect plumage bacterial assemblages of European starlings. – Mol. Ecol. 14: 639–646.

Markov, G. C. 1940. Seasonal and annual variations in parasitofauna of starlings in connection with changes in meteorological factors. – Zool Zhurn. 19: 741–749.

Marshall, A. G. 1981. The ecology of ectoparasitic insects. Academic Press, London.

Moller, A. P., Czirjak, G. A. and Heeb, P. 2009. Feather microorganisms and uropygial anti-microbial defenses in a colonial passerine bird. – Funct. Ecol. 23: 1097–1102.

Moyer, B. R., Gardiner, D. W. and Clayton, D. H. 2002. Impact of feather molt on ectoparasites: looks can be deceiving. – Oecologia 131: 203–210.

Onifade, A. A., Al-Sane, N. A., Al-Musallam, A. A. and Al-Zarban, S. 1998. A review: Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. – Bioresource Technol. 66: 1–11.

Payne, R. B. 1972. Mechanisms and control of molt. – In: Farner, D. S. and King, J. R. (eds), Avian biology vol II., pp. 103–155. Academic Press, New York.

Sangali, S. and Brandelli, A. 2000. Feather keratin hydrolysis by a *Vibrio* sp. strain kr2. – J. Appl. Microbiol. 89: 735–743.

Shawkey, M. D., Pillai, S. R. and Hill, G. E. 2003. Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. – J. Avian Biol. 34: 345–349.

Shawkey, M. D., Pillai, S. R. and Hill, G. E. 2008. Do feather-degrading bacteria affect sexually selected plumage color? – Naturwiss. 96: 123–128.

Shawkey, M. D., Pillai, S. R., Hill, G.E., Siefferman, L. M. and Roberts, S. R. 2007. Bacteria as an agent for change in structural plumage color: correlational and experimental evidence. – Am. Nat. 169 (Suppl.): S112–S121.

Welty, J. C. and Baptista, L. 1988. The life of birds, 4th edn. – Saunders, New York.