

Goshawk prey have more bacteria than non-prey

A. P. Møller^{1*}, J. M. Peralta-Sánchez², J. T. Nielsen³, E. López-Hernández² and J. J. Soler²

¹Laboratoire d'Ecologie, Systématique et Evolution, CNRS UMR 8079, Université Paris-Sud, Bâtiment 362, F-91405 Orsay Cedex, France; ²Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas, Crta. Sacramento s/n, Cañada de San Urbano, E-04001 Almería, Spain; and ³Espedal 4, Tolne, DK-9870 Sindal, Denmark

Summary

1. Predators often prey on individuals that are sick or otherwise weakened. Although previous studies have shown higher abundance of parasites in prey, whether prey have elevated loads of micro-organisms remains to be determined.
2. We quantified the abundance of bacteria and fungi on feathers of woodpigeons *Columba palumbus* L., jays *Garrulus glandarius* L. and blackbirds *Turdus merula* L. that either fell prey to goshawks *Accipiter gentilis* L. or were not depredated.
3. We found an almost three-fold increase in bacterial load of prey compared with non-prey, while there was no significant difference between prey and non-prey in level of fungal infection of the plumage.
4. The results were not confounded by differences in size or mass of feathers, date of collection of feathers, or date of analysis of feathers for micro-organisms.
5. These findings suggest a previously unknown contribution of bacteria to risk of predation, with important implications for behaviour, population ecology and community ecology.

Key-words: *Accipiter gentilis*, bacteria, feather micro-organisms, fungi, goshawk

Introduction

Predation is a powerful selective force with important implications for the evolution of behaviour, life history, and morphology of prey (Curio 1976; Endler 1986; Caro 2005). Given the intensity of predation on many different species of prey, there is ample opportunity for evolutionary changes in prey feeding back to changes in predators, which in turn may select for further changes in prey (e.g. Vermeij 1987). Predator–prey interactions may be affected by parasite–host interactions or other interspecific interactions that may facilitate or compromise co-evolution (Møller & Nielsen 2007). Here, we test for such interactions between ecological interactions by determining whether birds with high loads of micro-organisms on their feathers were more likely to fall prey to a common predator than individuals with few micro-organisms.

While prey may be perfectly healthy individuals, it is more likely that predators differentially succeed in capturing sub-standard individuals that are suffering from parasitism and disease. In a ground-breaking paper, Temple (1987) compared rates of parasitism in mammalian prey such as chipmunks, rabbits and squirrels captured by a red-tailed hawk *Buteo jamaicensis* with random potential prey collected with a shotgun, finding dramatic differences in prevalence and intensity of parasite infections. Later studies have subse-

quently shown similar elevated levels of parasitism in prey (e.g. Hudson 1986; Murray, Cary & Keith 1997). Similarly, studies of immune defence have indicated that prey have lower levels of defence than non-prey (Møller & Erritzøe 2000). Presence of predators may also increase the level of stress hormones such as corticosterone in prey, and such hormones can be maternally transferred to offspring that therefore are stunted in their growth (Saino *et al.* 2005). Interestingly, because predators may have strong indirect effects on prey (e.g. Abrams 1991; Lima 1998; Lima & Dill 1990), the mere presence of a predator can lower levels of immunity and subsequently lead to an increase in prevalence and intensity of parasitism (Navarro *et al.* 2004), which in turn may increase the risk of predation. Thus, predator effects on prey populations may be both direct and indirect, and they may interact with other interspecific interactions (Møller 2008).

The role of predation in eliminating sick individuals is supposedly large (Packer *et al.* 2003). Predators may either attack severely sick animals or they may attack mildly affected animals that are impaired in their escape ability just sufficiently to make them easier to capture than non-infected conspecifics. Evolution of anti-parasite strategies may differ between these two extreme kinds of predators with birds of prey detecting infected prey, and scavengers such as vultures relying on better immune defences (Blount *et al.* 2003). However, even experienced field workers spending months in the

*Correspondence author. E-mail: anders.moller@u-psud.fr

field every year may not see a single sick individual per year, a fact that may arise as a consequence of differential predation of sick prey rather than an absence of sick animals. Perhaps this apparent absence of sick animals is the reason why Lack (1954) in his book on population regulation dismissed parasites as being unlikely to have any significance.

Micro-organisms may have strong negative effects on health and fitness of their hosts. In humans and domestic animals, micro-organisms are a common cause of disease or death (e.g. Beaver & Jung 1985; Evans & Brachman 1998; Strauss & Strauss 2002), and several defence mechanisms have evolved to cope with such infection. Møller *et al.* (2009c) reviewed the literature on causes of parasite-induced nestling mortality in 115 studies of birds but were only able to find a handful of studies of micro-organisms. When it comes to effects of micro-organisms on predation, only a single study showed that species of birds differentially depredated by two species of *Accipiter* hawks had higher prevalence of blood parasites (Møller & Nielsen 2007). Møller, Couderc & Nielsen (2009) showed for the barn swallow *Hirundo rustica* L. that the abundance of cultivable bacteria living on feathers was higher in larger colonies and that the abundance of bacteria decreased with increasing size of the uropygial gland, while that was not the case for fungi. This gland produces antimicrobial substances that have a negative effect on abundance of micro-organisms (Jacob & Ziswiler 1982; Martín-Vivaldi *et al.* 2010) that protect against feather-degrading bacteria (Shawkey, Pillai & Hill 2003; Ruiz-Rodriguez *et al.* 2009). Interestingly, the predatory goshawk *Accipiter gentilis* L. prefers prey with small uropygial glands (Møller, Erritzøe & Nielsen 2010a). By inference, this finding implies that species of prey with a low priority of self-maintenance and hence investment in antimicrobial defences are disproportionately depredated by the raptor. Antimicrobial defences produced by the uropygial gland are associated with a greater diversity of other parasites such as chewing lice of the sub-order Amblycera and a larger abundance of feather mites in different species of birds (Møller, Erritzøe & Rózsa 2010b). Thus, it is likely that the detected relationship between size of uropygial gland and probability of predation was mediated by the effect of uropygial secretion preventing feather degradation by bacteria and parasites (Moreno-Rueda 2011), which would affect feather quality and flight performance. Thus, finding a relationship between feather bacterial density and probability of predation would complete the argumentation and suggest direct effects of feather-degrading bacteria as selective agents on bird populations.

The objective of this study was to test whether micro-organisms were more abundant on the plumage of prey than on the plumage of individuals that did not suffer from predation. To this end, we used samples of feathers from wood pigeons *Columba palumbus* L., jays *Garrulus glandarius* L. and blackbirds *Turdus merula* L. that are preferred prey species for the goshawk in our study site in Denmark. Wood pigeon and blackbird were three times as common as prey as expected from their abundance, while jays were 43 times as common as expected from their abundance (Møller &

Nielsen 2007). We sampled feathers from prey brought to the vicinity of nests of the goshawk but also sampled feathers that were moulted at the same time in the same forests as those where the feathers of the prey were found. Because the moulting birds were alive, this sample constitutes a suitable control group for our tests. Because the load of bacteria on feathers is relatively high just prior to moult, and because feathers that drop to the ground will have picked up bacteria from the ground, any difference in bacterial load between moulted and prey feathers will probably be greater in life. This study provides a test for interactions between host–parasite and predator–prey interactions by determining whether birds with high loads of micro-organisms on their feathers were more likely to fall prey to a predator than individuals with few micro-organisms.

Materials and methods

STUDY SITES AND FEATHER COLLECTION

Jan Tøttrup Nielsen (JTN) collected primary, secondary and rectrix feathers from wood pigeon, jay and blackbird prey found in or near 42 nests of goshawks in Northern Vendsyssel (57°10′–57°40′N, 9°50′–10°50′E), Denmark during April–August 2009. Dates of collection ranged from 19 April to 12 August, mean 16 May (SE = 2). Each nest was visited at least three times during the breeding season, and the nest and its surroundings were searched systematically for prey items. All feathers collected were only from recent prey not more than a couple of days old as reflected by their soft structure because feathers rapidly become stiff with rain and exposure to weather. We avoided problems of contamination of feathers by nest contents by only including feathers found on the ground, as were the samples of feathers from live individual prey.

As a sample of feathers from live individuals, JTN also collected moulted feathers from the same forests. Dates of collection ranged from 24 April to 12 August, mean 28 May (SE = 4). Feathers are moulted sequentially, and they are dropped at regular intervals (Ginn & Melville 1983), causing an even distribution of moulted feathers across habitats frequented by the three species of prey. JTN searched for feathers during extensive surveys of forests in his capacity as a forester, and all sites were visited at weekly intervals allowing for feathers not to have been contaminated from the ground for longer than feathers from prey. All feathers were marked with information on locality and date before storage. There is no possibility of confounding feathers from prey with moulted feathers from live birds because feathers from prey were found near nests, while moulted feathers were found away from nests. None of the feathers were fouled by blood or tissue because the goshawk rips out feathers before starting to eat its prey (Cramp 1980). See Møller & Nielsen (2007) for a description of the study areas and methods of study of prey remains.

Feathers were placed in zip-lock plastic bags, numbered and provided with information on date and locality. Feathers were stored frozen (–20 °C) until microbiological analyses. Thus, we only cultivated bacteria that could resist frost, although this should not affect the conclusions because all samples were treated similarly.

ACCUMULATION OF BACTERIA IN THE FIELD OVER TIME

We quantified the rate of accumulation of micro-organisms over time by placing in random order at a distance of 1 m 10 primaries from 10

different individuals on the ground in an oak forest. We divided the feather into five sections of similar length, and we removed a randomly chosen section of 20% at the start of the experiment and after 1 day, 2 days, 4 days and 8 days. These feather samples were subsequently marked without any information on identity or date of sampling to allow for blind tests of the accumulation of micro-organisms from the ground over time. The samples were subsequently cultivated and micro-organisms quantified as described later. All feathers were handled with sterile gloves during sampling, and samples were analysed blindly without information on their identity or time left in the field.

MEASUREMENT OF FEATHERS

Anders Pape Møller (APM) recorded four measures for all feathers (see Fig. 1 in Møller, Couderc & Nielsen 2009): (i) Diameter of the calamus, reflecting the sturdiness of the feather and its resistance to breakage. (ii) Length of the calamus that is partly inserted into the flight muscles of the wing and tail. (iii) Area of the feather estimated as the product of the length and the maximum width of the feather, constituting the part of the feather that generates lift. (iv) Feather mass that reflects the amount of material in the feather, but also the specific density of the feather, when feather mass is entered together with area in a single statistical model. Feather mass was recorded on a precision balance to the nearest 0.001 g, while all linear dimensions were recorded with a digital calliper with a precision of 0.01 mm. Repeatability (Becker 1984) of the five morphological characters was highly significant (Møller, Couderc & Nielsen 2009).

Anders Pape Møller identified the position of feathers in the wing and tail by using dried wings and tails from the three species as a reference, allowing unambiguous assignment of feathers to their position (see Møller, Couderc & Nielsen 2009).

Feathers were assigned to different individuals, thus ensuring statistical independence of data, by assuming that two feathers with the same position belonged to two different individuals. However, if we found primary 1 and primary 5 of a given species at a site, we only counted this as a single individual because we could not be sure whether more than one individual contributed these two feathers. We studied feathers from 42 goshawk nests. From the perspective of a prey individual, it does not matter if that individual died because of predation by a predator that has already consumed another prey individual, or if that was not the case. Any predator will by definition consume multiple prey during its lifetime, and through its activities, it will contribute to shaping total selection on the prey population.

All feathers from juveniles were discarded based on whether they were growing or had a paler colour than those of adults (only 11 individuals). This ensured that all feathers were from adult wood pigeons.

CULTIVATION OF MICRO-ORGANISMS

We cultured micro-organisms (bacteria and fungi) from two feathers from each individual bird whenever possible using two plates, thus allowing for estimates of repeatability. All feathers were cultured during October–November 2010. The order of analysis was done blindly with respect to the order of collection and whether feathers belonged to prey or live birds. Thus, there was no possibility of the interval between date of collection and date of analysis to have influenced the findings. If only one feather was available, we cultured micro-organisms from only that feather. In every case, we ensured that two samples of each feather were used. All microbiological analyses were performed in sterile conditions and with sterile material (Petri dishes, forceps and scissors) in a flow chamber. In order of

standardise micro-organism collection from feathers, we discarded 5 mm from the tip of the feather. Afterwards, we cut two pieces of 100 mm² from the tip, and we placed each piece in an individual 1.5-mL micro-centrifuge tube with 1.0 mL of phosphate buffer. We shook the micro-centrifuge tubes in a vortex for three periods of 10 s. Then, we cultured 100 µL of each micro-centrifuge tube in a Petri dish, containing sterile Tryptic Soja Agar (TSA; Scharlau Chemie, S.A. Barcelona, Spain). The use of feather samples from the tip ensured that the part of the feather that the goshawk pulled to remove the feather from the carcass (as evidenced from marks on the feather) was not used for analyses. This sampling procedure ensured that differences in bacterial load were not caused by contamination from the predator. Excluding samples from the tip of feathers also means that we were sampling the part of feathers with the lowest bacterial load (Muza, Burt & Ichida 2000).

Tryptic Soja Agar is the lowest restrictive medium for culturing aerobic bacteria. Restrictive media for culturing feather-degrading micro-organisms will select for bacteria with the highest keratinolytic activity in artificial media and conditions, which may result in an underestimate of bacterial density and reduce the importance of bacteria at high density that degrade feathers under natural conditions. Bacteria with keratinolytic activity (feather-degrading bacteria) are the most common micro-organisms detected on feathers (Shawkey, Pillai & Hill 2003; Gunderson 2008). Thus, because we are not interested in identifying bacteria living on the collected feathers, but in quantifying bacterial density, we used the non-restrictive media for our estimates of bacterial density.

After culturing, dishes were incubated at 30 °C during 21 days. Bacterial and fungal counts were therefore expressed as number of colonies per mm² of individual feather. Bacterial and fungal colonies (CFUs – colonies forming units) were counted every day during this period, with counts ranging from 0 to a maximum of 2064, with 'a very large number' being when colonies were so large that they overlapped each other and hence preventing colonies from being counted individually. We assigned a value of 5000 to these cases, although alternative numbers provided qualitatively similar conclusions. Within the same plate, bacteria were distinguished from fungi by morphological traits including the presence of hyphae following Brown (2001).

STATISTICAL ANALYSES

The number of colonies of micro-organisms was $\log_{10}(x + 1)$ transformed to achieve approximately normal distributions.

The possible effect of the accumulation of micro-organisms over time was explored in a field experiment. The analysis was performed by means of repeated-measures ANOVAS with days in the field as the first within factor (five levels; three levels in the case of fungi because it was only recorded in samples collected on days 1, 2 and 8) and days of bacterial growth in the plates as second within factor (four levels). Feather section was also included in the models. The experimental set-up exploring the association between depredation and feather bacterial load had a repeated-measures design with micro-organisms being recorded on days 2, 8, 14 and 20 as a within factor with four levels, with feather identity being nested within individual identity, individual identity being nested within goshawk prey and goshawk prey (yes or no) being between factors. Prey species and the interaction between prey species and the other factors were also included as predictors. We used type III decomposition of variance to calculate least mean squares of abundance of micro-organisms on different days for goshawk prey and control individuals.

Date of collection of feathers from both prey and non-prey did not explain abundance of bacteria. A repeated-measures ANOVA with

Table 1. Means, medians and standard errors (SE) of \log_{10} -transformed counts of colony forming units of bacteria and fungi from 1 mm² of feathers after 2, 8, 14 and 20 days of growing in heterotrophic medium. Values from feathers from individual *Columba palumbus* (feathers: $N_{\text{prey}} = 50$, $N_{\text{non-prey}} = 72$; individuals: $N_{\text{prey}} = 25$, $N_{\text{non-prey}} = 13$), *Garrulus glandarius* (feathers: $N_{\text{prey}} = 26$, $N_{\text{non-prey}} = 40$; individuals: $N_{\text{prey}} = 13$, $N_{\text{non-prey}} = 10$) and *Turdus merula* (feathers: $N_{\text{prey}} = 4$, $N_{\text{non-prey}} = 72$; individuals: $N_{\text{prey}} = 2$, $N_{\text{non-prey}} = 18$) that were preyed or not preyed upon by goshawks

	Species	Bacteria				Fungi			
		Non-prey		Prey		Non-prey		Prey	
		Median	Mean (SE)	Median	Mean (SE)	Median	Mean (SE)	Median	Mean (SE)
Day 2	<i>C. palumbus</i>	0.301	0.691 (0.122)	0.874	1.398 (0.146)	0.000	0.163 (0.076)	0.000	0.268 (0.086)
	<i>G. glandarius</i>	0.845	1.096 (0.208)	0.923	1.368 (0.204)	0.000	0.157 (0.105)	0.000	0.164 (0.096)
	<i>T. merula</i>	0.874	0.768 (0.277)	0.812	1.275 (0.145)	0.000	0.175 (0.175)	0.000	0.338 (0.096)
Day 8	<i>C. palumbus</i>	0.602	1.167 (0.181)	1.312	1.802 (0.158)	0.000	0.686 (0.152)	0.000	0.888 (0.139)
	<i>G. glandarius</i>	1.142	1.339 (0.215)	1.263	1.641 (0.226)	0.000	0.423 (0.149)	0.000	0.735 (0.167)
	<i>T. merula</i>	1.161	1.160 (0.498)	1.230	1.584 (0.153)	0.906	0.901 (0.376)	0.301	0.740 (0.121)
Day 14	<i>C. palumbus</i>	0.699	1.261 (0.188)	1.312	1.862 (0.157)	0.301	0.822 (0.160)	0.301	0.954 (0.139)
	<i>G. glandarius</i>	1.172	1.378 (0.211)	1.255	1.641 (0.226)	0.151	0.467 (0.147)	0.477	0.815 (0.160)
	<i>T. merula</i>	1.188	1.174 (0.494)	1.290	1.657 (0.154)	1.199	1.092 (0.455)	0.301	0.764 (0.124)
Day 20	<i>C. palumbus</i>	0.739	1.299 (0.185)	1.332	1.881 (0.155)	0.151	0.806 (0.162)	0.477	0.991 (0.137)
	<i>G. glandarius</i>	1.172	1.456 (0.230)	1.204	1.646 (0.225)	0.151	0.494 (0.147)	0.477	0.849 (0.157)
	<i>T. merula</i>	1.188	1.174 (0.494)	1.322	1.661 (0.154)	1.199	1.092 (0.455)	0.301	0.768 (0.124)

micro-organisms being recorded on days 2, 8, 14 and 20 as a within factor with four levels, and feather identity being nested within individual identity ($F = 1.49$, d.f. = 46,131, $P = 0.043$), individual identity being nested within species identity ($F = 6.00$, d.f. = 83,131, $P < 0.001$), species identity ($F = 0.35$, d.f. = 2,131, $P = 0.71$) revealed no effect of date of collection ($F = 0.001$, d.f. = 1,131, $P = 0.97$). A similar conclusion was reached for fungi (identical statistical model; feather identity: $F = 0.72$, d.f. = 46,131, $P = 0.90$; individual identity: $F = 1.80$, d.f. = 83,131, $P = 0.001$; species identity: $F = 0.01$, d.f. = 2,131, $P = 0.99$; and date of collection: $F = 0.02$, d.f. = 1,131, $P = 0.89$). Because the field experiment did not demonstrate any accumulation of micro-organisms over time independent of whether feathers were moulted or derived from prey, and because date of collection of feathers was not related to load of micro-organisms, we eliminated date of feather collection from the models testing for an association between goshawk predation and loads of micro-organisms on feathers.

Results

Accumulation of micro-organisms was not affected by time since feathers were deposited on the ground in our experiment. We found no evidence of time since feathers were placed on the ground on subsequent count of bacteria and fungi. In contrast, there was a significant effect of incubation time on abundance of bacteria (repeated-measures ANOVA with feather section as a covariate ($F = 0.71$, d.f. = 4,32, $P = 0.79$) and time since collection and incubation time of samples as repeated-measures factors, time since collection: bacteria: $F = 0.42$, d.f. = 4,32, $P = 0.79$; incubation time: $F = 13.52$, d.f. = 3,24, $P = 0.0002$; fungi: identical model, time since collection: $F = 0.34$, d.f. = 24,16, $P = 0.72$; incubation time: $F = 0.30$, d.f. = 3,24, $P = 0.82$). Thus, there was no evidence of accumulation of micro-organisms over time in the field experiment.

The number of bacteria and fungi on feather samples is shown in Table 1. There were much smaller numbers of fungi than bacteria (Table 2). There was a positive relationship between abundance of bacteria and abundance of fungi among samples [$F = 82.91$, d.f. = 1,144, $r^2 = 0.37$, $P < 0.0001$, slope (SE) = 0.57 (0.06)].

Most of the variance in abundance of bacteria on feathers was explained by a repeated-measures model that included feather and individual as nested factors (Table 2). There was a significant effect of whether individual birds were prey or

Table 2. Repeated-measures analysis of the abundance of bacteria on feathers of birds in relation to whether they were goshawk prey or not prey, individual to account for the non-independence of multiple feathers from a single individual, time for bacterial growth and their interactions. Feather identity was nested within individual identity, which was nested with the factor goshawk prey

Effect	Sum of squares	d.f.	F	P
Between factors				
Intercept	1123.19	1	498.20	<0.0001
Species	1.29	2	0.29	0.75
Goshawk prey	26.46	1	11.74	0.00082
Species \times goshawk prey	5.77	2	1.28	0.28
Individual	1132.43	80	6.28	<0.0001
Feather	155.33	46	1.50	0.040
Error	297.60	132		
Within factors				
Time	16.74	3	53.15	<0.0001
Time \times species	1.54	6	2.45	0.024
Time \times goshawk prey	0.11	3	0.34	0.80
Time \times species \times goshawk prey	0.11	6	0.17	0.98
Time \times individual	37.06	240	1.47	0.00035
Time \times feather	12.20	138	0.84	0.88
Error	41.57	396		

not on abundance of bacteria (Fig. 1a). The effect of species was not statistically significant nor was the interaction between species and whether an individual was prey or not (Table 2). The mean abundance of bacteria after back-transformation was almost three times higher in prey than in non-prey. In addition, there were significant differences in abundance of bacteria among individual birds and less so among feathers within individuals (Table 2), indicating that our estimates were repeatable for samples from the same feather ($R = 19.8$) and for feathers from the same individual ($R = 60.2$). The abundance of bacteria increased over time during cultivation, and this temporal increase differed among individuals, but not significantly among feathers within individuals, or among prey and non-prey over time (Table 2, Fig. 1a).

In contrast to bacteria, the abundance of fungi did not differ significantly between prey and non-prey (Table 3; Fig. 1b). There was no statistically significant effect of prey species and nor in the interaction between species and whether an individual was prey or not (Table 3). The abundance of fungi differed significantly among individuals,

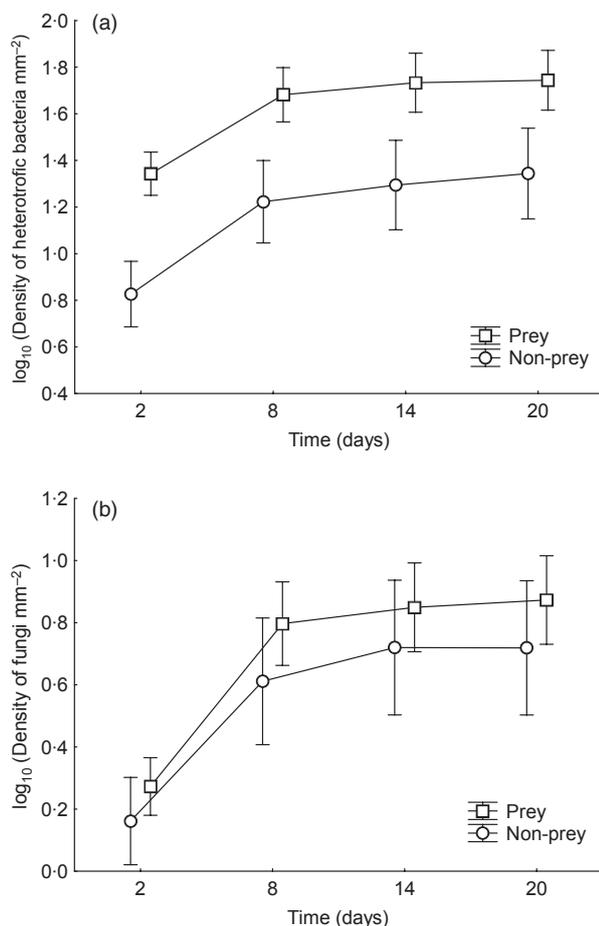


Fig. 1. Abundance of bacteria (a) and fungi (b) on feathers of birds in relation to time since start of cultivation (days). Values are means ($\pm 95\%$ confidence intervals) for prey and non-prey.

Table 3. Repeated-measures analysis of the abundance of fungi on feathers of birds in relation to whether they were goshawk prey, individual, time for bacterial growth and their interactions. Feather identity was nested within individual identity, which was nested with the factor goshawk prey

Effect	Sum of squares	d.f.	F	P
Between factors				
Intercept	230.98	1	89.52	< 0.0001
Species	5.90	2	1.14	0.32
Goshawk prey	1.45	1	0.56	0.45
Species \times goshawk prey	2.15	2	0.42	0.66
Individual	386.27	80	1.87	0.0007
Feather	87.35	46	0.74	0.88
Error	340.59	132		
Within factors				
Time	34.61	3	58.64	< 0.0001
Time \times species	0.95	6	0.80	0.57
Time \times goshawk prey	0.07	3	0.12	0.95
Time \times species \times goshawk prey	1.57	6	1.33	0.24
Time \times individual	88.72	240	1.88	< 0.0001
Time \times feather	19.62	138	0.72	0.99
Error	77.92	396		

abundance increased over time, and this temporal increase differed among individuals (Table 3, Fig. 1b).

The range in abundance of micro-organisms on prey and non-prey was the same for bacteria (0–3.7 in all six samples) and for fungi (0–3.7) in prey and non-prey of the wood pigeon and in prey and non-prey of the jay and the blackbird (0.3–3.7).

The abundance of bacteria and fungi was not significantly related to feather position or feather morphology, with the exception of a weak relationship for abundance of fungi and the length of the calamus (Table 4). Therefore, the difference in abundance of bacteria between prey and non-prey was not because of heterogeneity in feathers that were sampled or in size of such feathers.

Discussion

Feathers of wood pigeons, jays and blackbirds differed in abundance of bacteria between prey of the goshawk and live individuals that had moulted feathers in the same forests, while that was not the case for fungi. This difference in abundance of bacteria amounted to a factor of 3. We found little or no evidence that these differences in abundance of micro-organisms on feathers could be accounted for by alternative explanations such as position, mass, size or shape of feathers. These findings are consistent with the hypothesis that goshawks are differentially successful in their pursuit of prey when prey individuals harbour large amounts of bacteria on their plumage.

Feather micro-organisms are generally thought to be benign (Gundersen, Forsyth & Swaddle 2009; Shawkey, Pillai & Hill 2009) and only affect breakage of feather barbs many before the annual moult. Thus, the abundance of

Table 4. Relationship between abundance of bacteria and fungi after 20 days of growth and feather position, morphology and mass in models that also included species as a factor. The two models had the statistics $F = 1.49$, d.f. = 18,233, $r^2 = 0.10$, $P = 0.10$ and $F = 1.77$, d.f. = 18,233, $r^2 = 0.19$, $P = 0.035$

	Bacteria				Fungi			
	Sum of squares	d.f.	F	P	Sum of squares	d.f.	F	P
Feather position	33.83	12	1.65	0.08	38.99	12	1.54	0.12
Diameter of calamus	0.05	1	0.03	0.86	0.06	1	0.03	0.86
Length of calamus	0.55	1	0.32	0.57	9.24	1	4.38	0.038
Area of feather	0.36	1	0.21	0.65	1.75	1	0.83	0.36
Feather mass	0.36	1	0.21	0.65	1.75	1	0.83	0.37
Species	2.78	2	0.82	0.44	10.10	2	2.39	0.10
Error	397.01	233			282.90	233		

feather micro-organisms is unlikely to be a main risk factor associated with susceptibility to predation. Pathogenic micro-organisms are also common in wild birds (Hubálek & Halouzka 1996; Hubálek 2004), but their effects on wild animals remain poorly understood. Intuitively, predators should differentially capture prey with pathogenic infections because it is only small margins that determine whether an individual survives or dies during predatory pursuit. Here, we have shown a difference by a factor of 3 in abundance of bacteria on feathers of prey of goshawks and non-prey. Goshawks are known to differentially prey on birds with small uropygial glands that produce antimicrobial substances (Møller, Erritzøe & Nielsen 2010a), independent of a number of potentially confounding variables such as foraging (Burt & Ichida 1999) and habitat (Burt & Ichida 1999; Peele *et al.* 2009). This pattern of interspecific variation in susceptibility to predation can only come about through differential predation on individuals with low levels of antimicrobial defences and hence high levels of microbial infections of their plumage. We found differences in effects between bacteria and fungi, and that seems to be a common pattern also in other studies of micro-organisms on birds (Møller, Couderc & Nielsen 2009; Czirják *et al.* 2010). We interpreted the correlation between predation risk and amount of bacteria as implying that prey individuals infested with bacteria were less able to escape a predation attempt. We cannot exclude the possibility that predation risk affected bacterial loads. Indeed, indirect effects of predation are commonplace (e.g. Abrams 1991; Lima 1998; Lima & Dill 1990) and could potentially have produced the main result. In this case, we expect that high activity levels of predators prevent or reduce the preening activity of potential prey with secretions from the uropygial gland, allowing micro-organisms on the plumage to proliferate. If that were the case, we would predict potential prey living near goshawk nests to be differentially affected by micro-organisms. Some diseases or infections may also directly affect preening activity and thus bacterial loads of feathers (Møller 1991). In this case, bacteria on feathers would not be the cause of differential predation, but an indirect indicator of susceptibility to predation. These alternatives can only be assessed in an experimental setting.

Feathers harbour a complex bacterial community that commonly includes feather-degrading bacteria (Shawkey, Pillai & Hill 2003; Bisson *et al.* 2007, 2009; Gunderson 2008). These bacteria greatly vary in their keratinolytic activity, those belonging to the genus *Bacillus* being the most abundant and pathogenic bacteria for avian feathers. Uropygial secretions are known to inhibit growth of this genus of bacteria either on heterotrophic media (Shawkey, Pillai & Hill 2003) or on feathers (Ruiz-Rodriguez *et al.* 2009). Thus, it is likely that birds with lower bacterial density on their feathers are those with a higher capacity of feather preening (Møller, Couderc & Nielsen 2009; Møller, Erritzøe & Rózsa 2010b).

We found significant consistency in estimates of both kinds of micro-organisms among individuals both among samples within and among feathers. Micro-organisms on feathers may derive from different sources: bacteria with keratinolytic activity are abundant and ubiquitous in nature (e.g. Gunderson 2008; Shawkey, Pillai & Hill 2003), and, thus, they would grow on feathers that are not frequently cleaned and smeared with preen secretion. The bird itself may have acquired these bacteria from its food, drinking or bathing water, the ground when foraging or dust bathing or the air, and from conspecifics such as its parents, competitors and mates. The detected bacteria may also come from heterospecifics such as predators during failed predation attempts. Thus, independent of the source of feather bacteria, it is likely that their abundance strongly depends on the frequency of feather preening.

Alternatively, feathers from sampled individual prey may have acquired micro-organisms through contamination from the predator during the actual predation event. However, this explanation seems less likely because high consistency in abundance of micro-organisms on different feathers would not be expected. Furthermore, goshawks pull the feathers out by holding onto the base of the feathers with the beak, as revealed by marks left on feathers, while we assessed the abundance of micro-organisms on the tip of the feathers. Thus, there was little or no opportunity of direct transmission of bacteria from goshawks to prey. Finally, the range in abundance of micro-organisms on the feathers of prey would be expected to be outside the range found on non-prey if depredation increased abundance of micro-organisms. If the range in abundance of micro-organisms on prey represented

a subset of the distribution of abundance on live birds, this would suggest that micro-organisms were the cause of predation. The high degree of overall in the distribution of the abundance of micro-organisms on prey and non-prey is consistent with the latter hypothesis.

We envisage that two alternative mechanisms may potentially account for our findings. First, feather-degrading bacteria may degrade some of the feather keratin thereby reducing the flexibility of the feather, the air flow across a scarred surface becomes less efficient, the cortex of the barb is degraded, and the barb eventually breaks. This process of feather degradation peaks during the breeding season when hot and humid weather creates optimal conditions for bacteria. Birds may respond to this degradation by increasing the rate of preening and eventually by moulting. Individual differences in response to feather degradation may arise as a consequence of individuals being sick, stressed because of reproductive effort, or being young and inexperienced in terms of preening efficiency. Møller, Couderc & Nielsen (2009) have shown that feathers of prey do not differ from feathers of non-prey in terms of feather breakage, rendering this mechanism less likely. Second, birds may have pathogenic bacteria on their plumage because of horizontal transfer during interactions with conspecifics or heterospecifics, or because sick individuals with diarrhoea smear their plumage during preening. Such accidental smearing of the plumage may also result in ingestion of these bacteria because the beak is used both for preening and ingestion, thereby potentially eliciting an immune response if the bacteria penetrate the wall of the digestive system or the skin. Such bacterial infection may render birds susceptible to predation as reported here.

The findings that we report here have implications for disease control (Packer *et al.* 2003) and for the evolution of virulence (Møller & Nielsen 2007). Predators play an important role in maintaining prevalence and intensity of the infection of prey at a low level if they differentially capture sick prey. Such differential predation can also have implications for the evolution of virulence defined as the impact of a parasite on the fitness of its host. If predators eliminate hosts with high intensity of parasite infection, this can potentially reduce the transmission rate of parasites, especially if successful predation occurs early during infection, before infectious stages of the parasite have developed or multiplied. Alternatively, if predators avoid overly sick individuals then virulence will not be decreased as a consequence of predation.

Møller, Couderc & Nielsen (2009) have previously shown that wood pigeons with specific feather morphology are disproportionately likely to suffer from predation by goshawks. Here, we have shown that feather position, morphology and mass are not significant predictors of micro-organisms on feathers. This implies that these factors cannot have caused the relationships that we report here.

In conclusion, we have shown that birds that fell prey to goshawks had almost three times as many bacteria on their plumage as survivors. These findings are consistent with the hypothesis that predators differentially succeed in attacking potential prey that suffer from bacterial infections.

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