

Microorganisms Associated with Feathers of Barn Swallows in Radioactively Contaminated Areas Around Chernobyl

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Abstract The Chernobyl catastrophe provides a rare opportunity to study the ecological and evolutionary consequences of low-level, environmental radiation on living organisms. Despite some recent studies about negative effects of environmental radiation on macroorganisms, there is little knowledge about the effect of radioactive contamination on diversity and abundance of microorganisms. We examined abundance patterns of total cultivable bacteria and fungi and the abundance of feather-degrading bacterial subset present on feathers of barn swallows (*Hirundo rustica*), a colonial migratory passerine, around Chernobyl in relation to levels of ground level environmental radiation. After controlling for confounding variables, total cultivable bacterial loads were negatively correlated with environmental radioactivity, whereas abundance of fungi and feather-degrading bacteria was not significantly related to contamination

levels. Abundance of both total and feather-degrading bacteria increased with barn swallow colony size, showing a potential cost of sociality. Males had lower abundance of feather-degrading bacteria than females. Our results show the detrimental effects of low-level environmental radiation on total cultivable bacterial assemblage on feathers, while the abundance of other microorganism groups living on barn swallow feathers, such as feather-degrading bacteria, are shaped by other factors like host sociality or host sex. These data lead us to conclude that the ecological effects of Chernobyl may be more general than previously assumed and may have long-term implications for host–microbe interactions and overall ecosystem functioning.

Keywords Bacteria · Radiation · Chernobyl · Fungi · Sociality

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Introduction

The natural levels of radiation vary considerably on the surface of the Earth, implying that living organisms can experience some levels of radiation. Besides the naturally occurring high levels of radiation (e.g. in Evolution Canyon, Israel or in the Andean Altiplano) [11], another very important sources of anthropogenic radiation, with both long- and short-term effects on living organisms, are the radiation resulting from the nuclear disaster at Chernobyl, Ukraine or the testing and contamination from nuclear weapons [27]. The negative effect of both low- and high-level radiation is well known [40, 41], but information is lacking on the ecological or evolutionary consequences of human-induced and naturally occurring radiation [34].

The meltdown and subsequent explosion of one of the reactors at Chernobyl Nuclear Power Plant on 26 April 1986 released >80 PBq of radioactive materials affecting 200,000 km² area in Europe [34]. The nuclear accident at Chernobyl provides a rare experimental framework to study long-term ecological and evolutionary effects of low-level radiation on different organisms. The detrimental effects of background radiation on genetics, ecology and evolution have been found for a wide range of organisms [34, 56]. There have been several studies showing genetic and phenotypic modifications in plants [23], insects [29] or different vertebrates [14, 33, 54] associated with higher doses of environmental radiation due to the Chernobyl accident. It has been suggested that these modifications in environmental radiation affect reproduction and survival of the organisms [34, 38]. Accordingly, population sizes and diversity of several taxonomic and ecological groups decreased [35–37] with increasing levels of radiation with the Chernobyl population acting as an ecological sink [32].

However, to date, there is little knowledge about the effects of radioactive contamination on diversity and abundance of microorganisms. Around Chernobyl, filamentous fungal species diversity has declined with increasing radioactive contamination [13]. Highly resistant melanized [58] and genetically adapted [27] species are favoured in radioactive areas, and it has been suggested that some fungal lineages may be able to use ionizing radiation as an energy in their life cycles [10, 11]. Using cultivable soil bacteria, Romanovskaya et al. [46, 47] showed that anthropogenic radiation had a negative effect on abundance and diversity of bacteria. Numerous functional bacterial groups (nitrifying, sulphate-reducing, nitrogen-fixing, cellulose-fermenting bacteria) disappeared or reduced significantly in contaminated soils, severely affecting soil's ecological balance [46]. The most abundant isolated species from contaminated soils were found to be resistant to different stress factors (γ -radiation, desiccation, UV, H₂O₂, X-rays). Therefore, radioactive contamination was considered

to be acting as a selective force on the bacterial soil community [44, 45, 57].

Avian plumage is a diverse microbial ecosystem harbouring a variety of fungi [19, 42] and bacteria [2, 6, 49], sharing at least one important functional group, feather-degrading bacteria, with the soil [24], which is considered to be an important source in acquisition and colonization of feathers by microorganisms [2, 6]. It has been shown that host behaviour and ecology such as social structure [31], reproductive investment [25], migration [3] or foraging-strategies [6] affect the bacterial assemblage on feathers. Besides host-associated traits, geographic location and local environmental factors (humidity, temperature, radiation level) may drive the composition of the plumage-associated microbial communities [2, 7]. Therefore, the objective of our study was to investigate if the effects of radioactive contamination in areas around Chernobyl on soil microbiota can be detected via changes in the abundance of cultivable microorganisms on feathers of barn swallows, *Hirundo rustica*, a colonial migratory passerine.

Methods

Study Area and Populations

As part of an ongoing long-term project on barn swallows breeding in Ukraine and Belarus, feathers from randomly chosen individuals captured in 2007 and 2008 from ten farms were included in this study (Fig. 1). Our study farms are surrounded by open farmland and scattered trees and plantations, presenting similar climate and habitat type, but different environmental radiation levels [38]. In these populations, environmental radiation is the main factor affecting different life-history traits of the barn swallows [38]. We took care to have equilibrated sex ratios and we worked with the following sample sizes: $N_{2007}=31$ females, 29 males; $N_{2008}=58$ females, 56 males.

Field Procedures

We measured radiation at each farm and cross-validated with measurements by the Ukrainian Ministry of Emergencies [52], recording α , β and γ radiation at ground level using a hand-held dosimeter (Model: Inspector, SE International, Inc., Summertown, TN, USA); both measures were strongly correlated with each other (linear regression on log–log-transformed data: $F=165.9$, $r^2=0.49$, $P<0.0001$, slope (SE)=0.445 (0.034)).

Barn swallows were captured at windows and doors of farm buildings using mist nets. This method has been shown to be unbiased and highly efficient during the breeding seasons [39]. Birds were captured after finishing their nest construction, and most were incubating eggs during capture sessions.

Figure 1 Location of the study sites and levels of radioactive contamination in Ukraine, Russia and Belarus. Adapted from the European Union, 1998 [15]



Wearing sterile gloves immediately after capture a small number of feathers (five to ten) from the centre of the back, the red throat patch and the centre of the breast were removed and placed in a sealed plastic bag before being stored ~6 months in a freezer in the dark until microbiological analysis. Storage type and duration can affect bacterial viability and diversity [1]. Our samples were stored at -20°C without any cryoprotectant, but previous studies showed that this does not lead to significant changes in community composition [1]. Furthermore, since all samples were stored under the same condition, there is no objective reason to believe that our storage would have biased our results.

Birds were then ringed; morphological characters were measured, including tarsus and length of outermost tail feathers (with a ruler to the nearest millimetres), and body mass was recorded (with a Pesola spring balance to the nearest 0.1 g). Previous studies of barn swallows in Chernobyl have shown that capture probability of adults is very high [38]. We used the total number of occupied nests at each site as an estimate of colony size.

Microbiological Analyses

Microbiological analyses were performed under sterile conditions on five randomly selected body feathers, using

the method described by Møller et al. [31]. Briefly, in order to obtain both free-living and attached microorganisms, feathers were sonicated for 15 min in three repeats (5 min each with 5 min pause between the repeats) in 0.8 ml of sterile (0.90%, w/v) saline solution. After sonication, samples were vortexed for 20 s and bacterial suspensions were transferred to a sterile 1.5 ml Eppendorf tube. Feathers were re-suspended in 0.5 ml sterile saline solution and vortexed again for 20 s. The supernatant was transferred to a similar sterile Eppendorf, resulting in a ~1.3-ml solution. After obtaining the bacterial solution, the ‘clean’ feathers from individual birds were placed in sterile Eppendorf, dried for 24 h at 70°C and weighed to the nearest $1\ \mu\text{g}$ on an analytical balance (AT1 Comparator, Mettler Toledo).

To quantify different groups of microorganisms, we used three different growth media. First, tryptic soy agar (TSA; #22091, Fluka) is a rich medium on which both heterotrophic bacteria and fungi grow, thus enabling assessment of the cultivable microorganism load of feathers. Second, we inhibited fungal growth and quantified overall bacterial load, including most of the feather-degrading bacteria and other bacterial biota, by adding $100\ \text{mgml}^{-1}$ of cycloheximide (#01810, Fluka) to TSA (TSAcy) [12, 53]. Third, for the samples from 2008, we quantified feather-degrading bacterial load using feather

meal agar (FMA) containing 15 g l⁻¹ feather meal, 0.5 g l⁻¹ NaCl, 0.30 g l⁻¹ K₂HPO₄, 0.40 g l⁻¹ KH₂PO₄, 15 g l⁻¹ agar and 0.1 mg l⁻¹ cycloheximide [31, 48, 55]. FMA was used specifically to estimate abundance of the feather-degrading bacterial subset, as it contains feather keratin as the only carbon source [48, 55]. Therefore, any bacterium growing on it must be able to secrete keratinases in order to digest feather and thus could be considered ‘feather-degraders’ [51]. Previous studies of bird–bacteria interactions using FMA for feather-degrading bacterial load quantification have shown that FMA is a selective medium for bacterial species able to use keratin as unique carbon source [17, 31, 51].

To measure bacterial counts, we spread 100 µl of the microbial solution on different growth media, and the plates were incubated at 25°C, for 3 days in the case of TSA and TSAcy and for 14 days in the case of FMA [51]. After incubation, the numbers of visible colony-forming units (CFU) on each plate were counted. Counts were then corrected for initial volume of the suspension and feather mass. The microbial concentration for each medium type was expressed as CFU per milligram feather. We used only one dilution because an earlier study on another barn swallow population showed that counts were always between 0 and 150 CFU per plate [31]. All counts were performed blindly by one of us (GAC). The fungal load of feathers was estimated indirectly by calculating the difference between total microorganism counts and total bacterial counts (TSA–TSAcy).

Since the total cultivable bacterial load is composed in part of feather-degrading bacteria and in part of other functional bacterial groups [51], for the samples from 2008, we calculated the abundance of cultivable bacteria ‘other than feather-degrading bacteria’ as the total abundance of bacteria minus the abundance of feather-degrading bacteria (TSAcy–FMA) [31]. In this paper, we limit our analyses and discussion to the results for the total cultivable bacteria because we had data for 2 years for this bacterial group and we found the same results and effects when using the

counts for the total cultivable and the ‘other than feather-degrading bacteria’ (see Table 3).

Statistical Analyses

The abundance of total and other cultivable bacteria, radiation levels, and colony size was log₁₀-transformed in order to normalize the data. Because we were unable to obtain normally distributed data for the abundance of fungi and feather-degrading bacteria, we used non-parametric Spearman rank correlation tests when examining these variables. The relationship between total cultivable bacterial abundance and radiation was examined in a model which included year and colony size as potentially confounding variables and host-related parameters (sex, body mass, tarsus length and tail length) as covariates. The nonsignificant ($P > 0.05$) terms (sex, body weight, tarsus, tail length and interactions) were removed from the final model. For the non-parametric analyses, an index of condition was calculated using residuals from the linear regression of body mass on tarsus length [18, 21, 51]. The condition index was not correlated with wing length ($r_s = -0.02$, $P = 0.87$), assuring that our estimate was free of allometric effects [16]. Except for one recapture, all the birds were measured once. Sampling sites were classified as low (background radiation range 0.02–0.11 µSv/h), intermediate (background radiation range 0.45–0.46 µSv/h) or high level (background radiation 2.9 µSv/h) [4], and Tukey–Kramer post hoc test was made to determine differences in bacterial abundances between different radiation levels. All analyses were performed using the software JMP 5.0.1 [22].

Results

The abundance of microorganisms associated with feathers of barn swallows from radioactively contaminated areas around Chernobyl was highly variable (Table 1). Most

Table 1 Summary statistics for abundance of different groups of microorganism isolated from feathers of adult barn swallows from areas around Chernobyl

Microorganism	Mean	SE	Median	Range	<i>N</i>
Total cultivable bacteria	50.09	5.67	25.98	0–550.63	60; 114
Fungi	14.03	1.03	10.25	0–65.85	60; 114
Feather-degrading bacteria	15.09	1.66	9.93	0–87.54	–; 114
Cultivable bacteria ‘other than feather-degrading bacteria’	42.85	7.49	16.9	0–505.3	–; 113

Values were back-transformed from log₁₀-transformed values in the case of total cultivable bacteria and other cultivable bacteria, while values are CFUs for fungi and feather-degrading bacteria. *N* represents the sample sizes for the two sampling years (2007, 2008)

surface microorganisms are harmless or even beneficial, and thus, host individuals with lower levels of total bacteria or fungi do not necessarily imply better condition. In contrast, increased feather-degrading bacterial load appears to have detrimental effects on host fitness [17]. There were positive correlations between the four categories of microorganisms (Table 2).

After removing the nonsignificant host-related parameters (sex, body weight, tarsus, tail length and interactions) from the model and controlling for the effect of colony size, the abundance of total cultivable bacteria of barn swallow feathers was negatively correlated with the level of background radiation (Table 3; Fig. 2). Total cultivable bacterial load of barn swallows living in areas with low and intermediate environmental radiation did not differ significantly (Tukey–Kramer post hoc test, $P>0.05$), while there was a significant difference between areas with lower radiation levels and highly contaminated areas (Tukey–Kramer post hoc test, all $P<0.05$). In contrast with results on total bacterial loads, the abundances of feather-degrading bacteria and fungi were not significantly correlated with background radiation (feather-degrading bacteria: $r_s=-0.11$, $P=0.26$; fungi: $r_s=-0.08$, $P=0.30$).

Swallows breeding in larger colonies had higher loads of total cultivable bacteria than swallows breeding in smaller colonies (Table 3). The interaction between background radiation and colony size was not significant for total cultivable bacteria (Table 3). Feather-degrading bacteria loads tended to increase with colony size ($r_s=0.18$, $P=0.055$), whereas the abundance of fungi was not significantly correlated with colony size ($r_s=0.11$, $P=0.15$).

Males had significantly fewer feather-degrading bacteria than females (feather-degrading bacteria on males: median (range)=6.91 (0–71.20); feather-degrading bacteria of females: median (range)=12.87 (0–87.55); $z=-2.00$, $P=0.045$). In contrast, there were no significant sex differences in abundance of total cultivable bacteria ($F_{1, 143}=0.019$, $P=0.887$) or fungi ($z=-0.41$, $P=0.68$). There were no significant correlations between abundance of total cultivable bacteria and body mass, tarsus length or

abundance of fungi, feather-degrading bacteria and condition index (all $P>0.40$).

Discussion

The main finding of this study was that the abundance of total cultivable bacteria on barn swallow feathers was lower in populations with higher levels of background radiation, while that was not the case for fungi or feather-degrading bacteria. Furthermore, the abundance of feather-degrading and total cultivable bacteria increased with colony size, reflecting a potential cost of sociality. Finally, male barn swallows breeding in the Chernobyl area had significantly fewer feather-degrading bacteria than females.

In accordance with previous studies on soil bacteria [46, 47], the abundance of total cultivable bacteria of barn swallow feathers was lower in the most contaminated areas. Besides differences in environmental radioactive contamination level, our study sites had similar microclimates [38]. Thus, we could expect similar microbial load since biogeographic factors and habitat quality are important factors shaping feather bacterial assemblages [2]. Romanovskaya et al. [46, 47] have reported reduced abundance of soil bacteria in highly contaminated areas around Chernobyl, and their findings could be due to the fact that soil bacteria are continuously exposed to background radiation. Our results suggest that bacteria present in radioactive environments contaminate barn swallow feathers either when at the nest and/or when occasionally landing on the ground to collect mud for nest construction.

Two mechanisms may explain why there are fewer cultivable bacteria on feathers of barn swallows in the most contaminated areas. Either direct effects of radiation on bacteria could account for the association or radiation may have an indirect effect on host defences. First, the dose-dependent detrimental effects of radiation on bacterial survival [8] may reduce the abundance of total cultivable bacteria. A test of this hypothesis will require that experimental exposure to radiation reduces the abundance of bacteria. Second, barn swallows breeding around

Table 2 Correlations between the four categories of microorganisms isolated from feathers of adult barn swallows from areas around Chernobyl

	r_s	P
Total cultivable bacteria and feather-degrading bacteria	0.64	<0.0001
Total cultivable bacteria and cultivable bacteria ‘other than feather-degrading bacteria’	0.94	<0.00001
Feather-degrading bacteria and cultivable bacteria ‘other than feather-degrading bacteria’	0.51	<0.0001
Fungi and total cultivable bacteria	0.35	<0.0001
Fungi and feather-degrading bacteria	0.21	0.02
Fungi and cultivable bacteria ‘other than feather-degrading bacteria’	0.23	0.02

r_s = Spearman

Table 3 Relationship between abundance of total cultivable bacteria and cultivable bacteria ‘other than feather-degrading bacteria’ with year, background radiation and colony size

Variable	Sum of squares	df	F	P	Slope (SE)
Abundance of total cultivable bacteria					
Year	0.31	1	1.32	0.25	−0.046 (0.040)
Radiation	1.22	1	5.17	0.024	−0.156 (0.068)
Colony size	1.40	1	5.90	0.016	0.666 (0.274)
Radiation × colony size	0.09	1	0.37	0.54	−0.311 (0.509)
Abundance of cultivable bacteria ‘other than feather-degrading bacteria’					
Radiation	1.53	1	5.45	0.021	−0.233 (0.099)
Colony size	1.29	1	4.62	0.033	0.866 (0.402)
Radiation × colony size	0.13	1	0.47	0.49	−0.733 (1.071)

Chernobyl have reduced reproductive success if they reproduce at all [38], and this may cause such individuals to allocate more time to self-maintenance and preening behaviours. Thus, reduced abundance of total and other cultivable bacteria may arise as a consequence of greater investment in defence. In starlings, parent birds that raise an experimentally increased number of nestlings have less time for feather maintenance than controls, causing an increase in the abundance of free living, but not of attached bacteria [25]. We believe that this mechanism is unlikely as Møller et al. have shown that investment in the uropygial gland and its anti-microbial secretions primarily reduces the abundance of feather-degrading bacteria, but not the abundance of other bacteria or fungi [31].

The abundance of cultivable fungi and feather-degrading bacteria was not affected by environmental radioactivity. There are several reasons that may account for this fact. Firstly, the stability of the microbial communities can be explained by the interactions between different bacterial and fungal species, as a result of microbial interference [5, 12, 31]. The lack of negative relationship between the abundance of our measured microbial groups suggests that the stability of microbial communities in Chernobyl may be

explained by other mechanisms and not by interference. However, further *in vitro* tests and bacterial identification are needed to test this hypothesis.

In addition, the abundance of microorganisms can be affected by anti-microbial defences of the hosts. Birds possess diverse behavioural and anatomical anti-microbial defence mechanisms that can affect the abundance of feather-associated microorganisms [9, 20, 31, 43, 50]. These mechanisms could act as a filter favouring establishment of beneficial microorganisms whilst excluding harmless ones or selecting for specific resistant bacterial and fungal species. In contaminated areas around Chernobyl, the additive effects of environmental radiation and host defences could have selected for resistant bacteria and fungi. We believe that this hypothesis would explain the stability of the fungal and feather-degrading bacterial loads. The fungal species associated with plumage are predominantly dark-spored melanized fungi [42] (Czirájk et al., personal observations). Several species of fungi isolated from the plumage of birds [19, 42] were found to be present within and around the Chernobyl nuclear reactor [13]. These species show genetic adaptations like melanin content of the cell wall that enable them to cope with and potentially exploit the radioactive environment [10, 27, 58]. Using culture-based surveys, spore-forming, feather-degrading bacterial species from the genus *Bacillus* predominate on feathers [49]. These species are present in the soil around Chernobyl and are highly resistant to different stressors [44, 45]. Such defence mechanisms could explain why the abundance of fungi and feather-degrading bacteria did not covary with levels of radioactive contamination.

We have shown that the abundance of total cultivable bacteria was positively related to colony size, while the abundance of feather-degrading bacteria was marginally positively related to colony size. These findings are consistent with predictions concerning parasitism as a cost of sociality [30] and with a previous study of barn swallows from Denmark which found the same positive correlation [31].

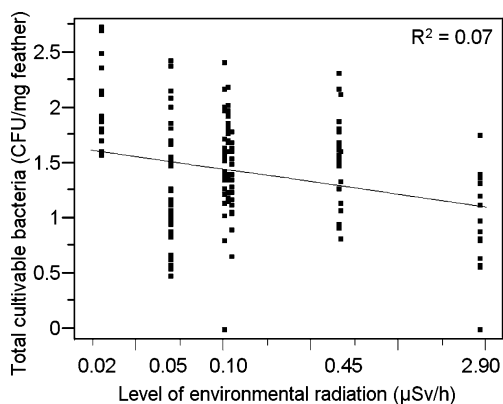


Figure 2 Abundance of total cultivable bacteria (CFU per milligram feather) in relation to environmental radiation (microsieverts per hour) around Chernobyl. The *line* is the linear regression line on log-transformed values

In our study, male barn swallows breeding in Chernobyl had significantly fewer feather-degrading bacteria than females, while there was no sex difference for the abundance of total cultivable bacteria or fungi. A similar result was also found by Møller et al. in Denmark [31] and could be explained by female barn swallows investing more in reproduction than males [28], as well as the likelihood that females are more exposed to feather-degrading bacteria from soil [24] when incubating and brooding.

In this study, we were only able to quantify the abundance of cultivable microorganisms, which imposes limitations on our interpretations. Extending our results to whole bacterial communities using culture-independent techniques [2, 26, 49] and identifying the bacterial and fungal species that are affected or are resistant to the environmental radiation would certainly be promising steps towards an understanding of the ecological and evolutionary effects of low-level radiation on microbiota.

Conclusion

In this study, we found that abundance of different cultivable microorganisms on feathers of barn swallows breeding around Chernobyl was differentially affected by both ecological factors and host-associated traits. Previous research has demonstrated significant negative effect of Chernobyl-related fallout on abundance and diversity of soil bacteria and fungi, with highly resistant and genetically adapted species being favoured in highly contaminated areas [13, 46]. Here, we generalize these findings, documenting similar patterns for feather-associated cultivable microorganisms of an aerial, migratory bird. Extensive reduction in the abundance of overall bacterial assemblage on feathers of barn swallows with increasing levels of radiation around Chernobyl contrasted with opportunistic pathogens, such as feather-degrading bacteria, which were unrelated to environmental radiation. These effects may have long-term implications for host–microbe interactions and overall ecosystem functioning and reinforce the notion that the Chernobyl region and its surroundings are a perturbed ecosystem.

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References

- Achá SJ, Kühn I, Mbazima G, Colque-Navarro P, Möllby (2005) Changes of viability and composition of the *Escherichia coli* flora in faecal samples during long time storage. *J Microbiol Meth* 63:229–238
- Bisson IA, Marra PP, Burt EH Jr, Sikaroodi M, Gillevet PM (2007) A molecular comparison of plumage and soil bacteria across biogeographic, ecological, and taxonomic scales. *Microb Ecol* 54:65–81
- Bisson IA, Marra PP, Burt EH Jr, Sikaroodi M, Gillevet PM (2009) Variation in plumage microbiota depends on season and migration. *Microb Ecol* 58:212–220
- Bonisolli-Alquati A, Mousseau TA, Møller AP, Caprioli M, Saino N (2010) Increased oxidative stress in barn swallows from the Chernobyl region. *Comp Biochem Physiol A* 155:205–210
- Brook I (1999) Bacterial interference. *Crit Rev Microbiol* 25:155–172
- Burt EH Jr, Ichida JM (1999) Occurrence of feather-degrading bacilli in the plumage of birds. *Auk* 116:364–372
- Burt EH Jr, Ichida JM (2004) Gloger's rule, feather-degrading bacteria, and color variation among song sparrows. *Condor* 106:681–686
- Caillet S, Millette M, Dussault D, Shareck F, Lacroix M (2008) Effect of gamma radiation on heat shock protein expression of four foodborne pathogens. *J Appl Microbiol* 105:1384–1391
- Clayton DH, Cotgreave P (1994) Relationship of bill morphology to grooming behaviour in birds. *Anim Behav* 47:195–201
- Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P, Nosanchuk JD, Casadevall A (2007) Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. *PLoS ONE* 5:e457
- Dadachova E, Casadevall A (2008) Ionizing radiation: how fungi cope, adapt and exploit with the help of melanin. *Curr Opin Microbiol* 11:525–531
- Dehorty BA, Tirabasso PA (2000) Antibiosis between ruminal bacteria and ruminal fungi. *Appl Environ Microbiol* 66:2921–2927
- Dighton J, Tugay T, Zhdanova N (2008) Fungi and ionizing radiation from radionuclides. *FEMS Microbiol Lett* 281:109–120
- Ellegren H, Lindgren G, Primmer CR, Møller AP (1997) Fitness loss and germline mutations in barn swallows breeding in Chernobyl. *Nature* 389:593–596
- European Union (1998) Atlas of caesium deposition on Europe after the Chernobyl accident. EU Publication EUR 16733, Bruxelles, Belgium
- Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* 82:1473–1483
- Gunderson AR, Forsyth MH, Swaddle JP (2009) Evidence that plumage bacteria influence feather coloration and body condition of eastern bluebirds *Sialis sialis*. *J Avian Biol* 40:440–447
- Horrocks N, Perrins C, Charmantier A (2009) Seasonal change in male and female bill knob size in the mute swan *Cygnus olor*. *J Avian Biol* 40:511–519
- Hubálek Z (1978) Coincidence of fungal species associated with birds. *Ecology* 59:438–442
- Jacob J, Eigener U, Hoppe U (1997) The structure of preen gland waxes from pelecaniform birds containing 3, 7-dimethyloctan-1-ol: an active ingredient against dermatophytes. *Zeit Naturforsch C52*:114–123
- Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices. *Oikos* 77:61–67
- JMP 5.0.1 (2002) JMP. SAS Institute, Cary
- Kovalchuk O, Dubrova YE, Arkhipov A, Hohn B, Kovalchuk I (2000) Wheat mutation rate after Chernobyl. *Nature* 407:583–584

24. Lucas FS, Broennimann O, Febbraro I, Heeb P (2003) High diversity among feather-degrading bacteria from a dry meadow soil. *Microb Ecol* 45:282–290
25. Lucas FS, Moureau B, Jourdie V, Heeb P (2005) Brood size modifications affects plumage bacterial assemblages of European starlings. *Mol Ecol* 14:639–646
26. Mennerat A, Mirleau P, Blondel J, Perret P, Lambrechts MM, Heeb P (2009) Aromatic plants in nests of the blue tit *Cyanistes caeruleus* protect chicks from bacteria. *Oecologia* 161:849–855
27. Mironenko NV, Alekhina IA, Zhdanova NN, Bulat SA (2000) Intraspecific variation in gamma-radiation resistance and genomic structure in the filamentous fungus *Alternaria alternata*: a case study of strains inhabiting Chernobyl reactor nr. 4. *Ecotoxicol Environ Saf* 45:177–187
28. Møller AP (1994) Sexual selection and the barn swallow. Oxford University Press, Oxford
29. Møller AP (2002) Developmental instability and sexual selection in stag beetles from Chernobyl and control area. *Ethology* 108:193–204
30. Møller AP, Allander K, Dufva R (1993) Parasites and the evolution of host social behavior. *Adv Study Behav* 22:65–102
31. Møller AP, Czirják GÁ, Heeb P (2009) Feather micro-organisms and uropygial anti-microbial defenses in a colonial passerine bird. *Funct Ecol* 23:1097–1102
32. Møller AP, Hobson KA, Mousseau TA, Peklo AM (2006) Chernobyl as a population sink for barn swallows: tracking dispersal using stable-isotope profiles. *Ecol Appl* 16:1696–1705
33. Møller AP, Mousseau TA (2001) Albinism and phenotype of barn swallows *Hirundo rustica* from Chernobyl. *Evolution* 55:2097–2104
34. Møller AP, Mousseau TA (2006) Biological consequences of Chernobyl: 20 years on. *Trends Ecol Evol* 21:200–207
35. Møller AP, Mousseau TA (2007) Species richness and abundance of forest birds in relation to radiation at Chernobyl. *Biol Lett* 3:483–486
36. Møller AP, Mousseau TA (2009) Reduced abundance of raptors in radioactively contaminated areas near Chernobyl. *J Ornithol* 150:239–246
37. Møller AP, Mousseau TA (2009) Reduced abundance of insects and spiders linked to the radiation at Chernobyl 20 years after the accident. *Biol Lett* 5:356–359
38. Møller AP, Mousseau TA, Milinevski G, Peklo A, Pysanets E, Szép T (2005) Condition, reproduction and survival of barn swallows from Chernobyl. *J Anim Ecol* 74:1102–1111
39. Møller AP, Szép T (2002) Survival rate of adult barn swallows *Hirundo rustica* in relation to sexual selection and reproduction. *Ecology* 83:2220–2228
40. Moysich KB, Menezes RJ, Michalek AM (2002) Chernobyl-related ionizing exposure and cancer risk; an epidemiological review. *Lancet Oncol* 3:269–279
41. Petridou E, Trichopoulos D, Dessypris N, Flytzani V, Haidas S, Kalmanti M, Kolioukas D, Kosmidis H, Piperopoulou F, Tzortzatou F (1996) Infant leukaemia after in utero exposure to radiation from Chernobyl. *Nature* 382:352–353
42. Pugh GJF (1972) The contamination of birds' feathers by fungi. *Ibis* 114:172–177
43. Revis HC, Waller DA (2004) Bactericidal and fungicidal activity of ant chemicals on feather parasites: an evaluation of anting behavior as a method of self-medication in songbirds. *Auk* 121:1262–1268
44. Romanovskaya VA, Rokitko PV, Malashenko YuR, Krishtab TP, Chernaya NA (1999) Sensitivity of soil bacteria isolated from the alienated zone around the Chernobyl Nuclear Power Plant to various stress factors. *Microbiol* 68:465–469
45. Romanovskaya VA, Rokitko PV, Mikheev AN, Gushcha NI, Malashenko YuR, Chernaya NA (2002) The effect of γ -radiation and desiccation on the viability of the soil bacteria isolated from the alienated zone around the Chernobyl Nuclear Power Plant. *Microbiol* 71:608–613
46. Romanovskaya VA, Sokolov IG, Rokitko PV, Chernaya NA (1998) Effect of radioactive contamination on soil bacteria in the 10-km zone around the Chernobyl Nuclear Power Plant. *Microbiol* 67:274–280
47. Romanovskaya VA, Stolyar SM, Malashenko YuR, Shatokima EF (1996) The effect of prolonged radiation on the diversity of heterotrophic bacteria in the soils of the 10-km zone around Chernobyl NPP. *Mikrobio Zh Kiev* 58:3–11
48. Sangali S, Brandelli A (2000) Feather keratin hydrolysis by a *Vibrio* sp. strain kr2. *J Appl Microbiol* 89:735–743
49. Shawkey MD, Mills KL, Dale C, Hill GE (2005) Microbial diversity of wild bird feathers revealed through culture-based and culture-independent techniques. *Microb Ecol* 50:40–47
50. Shawkey MD, Pillai SR, Hill GE (2003) Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. *J Avian Biol* 34:345–349
51. Shawkey MD, Pillai SR, Hill GE (2009) Do feather-degrading bacteria affect sexually selected plumage color? *Naturwissenschaften* 96:123–128
52. Shestopalov VM (1996) Atlas of Chernobyl exclusion zone. Ukrainian Academy of Science, Kiev
53. Smit E, Leeftang P, Gommans S, van den Broek J, van Mil S, Wernars K (2001) Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl Environ Microbiol* 67:2284–2291
54. Vinogradov AE, Chubinshvili AT (1999) Genome reduction in a hemiclinal frog *Rana esculanta* from radioactively contaminated area. *Genetics* 151:1123–1125
55. Williams CM, Richter CS, MACKenzie JM Jr, Shih JCH (1990) Isolation, identification, and characterization of a feather-degrading bacterium. *Appl Environ Microbiol* 56:1509–1515
56. Yablokov AV, Nesterenko VB, Nesterenko AV (2009) Chernobyl: consequences of the catastrophe for people and nature. New York Academy of Sciences, New York
57. Zavigelsky GB, Abilev SK, Sukhodolets VV, Ahmad SI (1998) Isolation and analysis of UV and radio-resistant bacteria from Chernobyl. *J Photochem Photobiol B* 43:152–157
58. Zhdanova NN, Zakharchenko VA, Vember VV, Nakonechnaya LT (2000) Fungi from Chernobyl: mycobiota of the inner regions of the containment structures of the damaged nuclear reactor. *Mycol Res* 104:1421–1426