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Ectoparasites, uropygial glands and hatching success in birds

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Abstract The uropygial gland of birds secretes wax that is applied to the plumage, where the secretions are hypothesized to eliminate fungi and bacteria, thereby potentially providing important benefits in terms of plumage maintenance. We analyzed variation in size of the uropygial gland in 212 species of birds to determine the function and the ecological correlates of variation in gland size. Bird species with larger uropygial glands had more genera of chewing lice of the sub-order Amblycera, but not of the sub-order Ischnocera, and more feather mites. There was a fitness advantage associated with relatively large uropygial glands because such species had higher hatching success. These findings are consistent with the hypothesis that the uropygial gland functions to manage the community of microorganisms, and that certain taxa of chewing lice have diverged as a consequence of these defenses.

Keywords Chewing lice · Feather mites · Hatching success · Preen gland

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Introduction

The diversity and abundance of microorganisms are enormous. Therefore, all organisms live in a matrix of microorganisms, of which many have beneficial effects, others are neutral, and yet others are pathogenic. Coevolutionary interactions between hosts and microorganisms have produced mutualistic relationships such as those between gut microorganisms and hosts (e.g., Hackstein and van Alen 1996). Such symbionts generally are superior competitors compared to pathogenic microorganisms through the process of bacterial interference (Brook 1999) consisting of certain bacteria impeding the establishment of competing bacteria by producing antibiotic substances (Riley and Wertz 2002), or modification of the environment for symbiotic bacteria by hosts (e.g., Hackstein et al. 1996). Weakened, sick or immunologically naive hosts may be unable to efficiently provide the optimal environment for mutualistic bacteria, which are then replaced by opportunistic, pathogenic bacteria (McCracken and Lorenz 2001).

In humans and domestic animals microorganisms are a common cause of disease or death, and a battery of defense mechanisms have evolved to cope with such infection, including behavioral parasite avoidance (Moore 2002), physiological anti-parasite defenses (Wakelin 1996), fever (e.g., Banet 1986; Blatteis 1986; Hart 1990), and removal of iron from the gut (e.g., Hart 1990). The immune system mainly produces specific cells and molecules that vary in

specificity against pathogens. First, lysozyme is a general component of innate antibacterial immune defense, which digests peptoglycans of bacterial cell walls (e.g., Sato and Watanabe 1976; Kudo 2000). Second, natural antibodies and complement are components of the constitutive innate immune system that recognize and initiate the complement enzyme cascade that eventually ends in cell lysis (Carroll and Prodeus 1998; Ochsenbein et al. 1999; Reid et al. 1997). Third, the uropygial gland of birds produces secretions that are hypothesized to constitute a defense against bacterial and fungal infections of feathers and skin (Jacob and Ziswiler 1982; Shawkey et al. 2003).

Recent comparative studies have demonstrated anti-bacterial defense ability, related to the ecological context, of lysozyme (Saino et al. 2007), natural antibodies (Matson et al. 2005; Soler et al. 2007; Lee et al. 2008), and secretions from the uropygial gland (Galván et al. 2008). Consequently, antimicrobial properties of uropygial secretions should affect not only the probability of parasitic infections of birds, but also indirectly the expression of other costly immunological defenses involved in such defenses. Using this scenario, we present a comprehensive study of ecological factors associated with interspecific variation in antibacterial defenses for the first time by relating the relative size of the uropygial gland to the abundance of different taxa of parasites, by investigating covariation between the relative size of the uropygial gland and other defense mechanisms, and by analyzing the role of the uropygial gland in hatching success, an important fitness component naturally affected by pathogenic bacteria (e.g., Baggott and Graeme-Cook 2002).

Previous studies have shown that large uropygial glands produce more waxes than small glands (Elder 1954; Sandilands et al. 2004; Oka and Okuyama 2000). Gland size is related to habitat (Kennedy 1971; Jacob and Ziswiler 1982; Johnston 1988; Montalti et al. 2005; Montalti and Salibián 2000), and aquatic bird species have larger glands than terrestrial species (Johnston 1988; Galván et al. 2008). Accordingly, the uropygial gland was originally suggested to function in waterproofing plumage (Jacob and Ziswiler 1982). However, the relationship with aquatic habitat could be explained by water affecting bacterial and fungal growth (e.g., Burtt and Ichida 2004; Shawkey et al. 2003). In accordance with this interpretation, Jacob and Ziswiler (1982) reviewed the old literature including experiments that removed the uropygial gland, which showed increased levels of fungi and bacteria on feathers, and higher levels of feather degradation, although not in all species.

The objectives of this comparative study were to investigate the ecological factors associated with greater relative size of the uropygial gland. First, we predicted that parasites that consume feathers and substances on feathers such as chewing lice should come into contact with wax from the uropygial gland, and diversifying selection on glands from interactions with microorganisms should also increase diversity of such parasites. We predicted that this effect should be more pronounced in chewing lice of the sub-order Amblycera than of the sub-order Ischnocera because the former taxon is already known to be involved in coevolutionary interactions with the immune system of the host (Møller and Rózsa 2005), and because parasites that are involved in coevolution with multiple defense systems of hosts should show stronger effects of antagonistic selection than parasites that only have to cope with a single defense system. Such antagonistic host defenses are common (e.g., Kraaijeveld and van Alphen 1995; Fineblum and Rausher 1995; Soler et al. 1999). The relationship between speciation by parasites/symbionts and host defenses depends on the extent to which parasites inflict fitness costs on their hosts (e.g., Møller et al. 2005a, b; Møller and Rózsa 2005; Janz et al. 2006; Krüger et al. 2009), with symbionts with no fitness costs for hosts showing little effect of host defenses on symbiont diversity (e.g., Moran 2006). Second, we predicted that the relative size of the uropygial gland should show positive covariation with other bacterial defenses like the relative size of the bursa of Fabricius, reflecting production of antibodies and differentiation and magnitude of the repertoire of B cells in young birds (Glick 1983, 1994; Toivanen and Toivanen 1987), and the level of natural antibodies (Abbas et al. 1994; Matson et al. 2005; Ochsenbein et al. 1999; Reid et al. 1997). Third, we tested whether the relative size of the uropygial gland predicted hatching success in birds, as expected if pathogenic bacteria that could be eliminated by wax from the uropygial gland are a common cause of hatching failure. Studies by Cook et al. (2003, 2005) have shown that bacteria are an important cause of egg mortality in birds under natural conditions, and extensive data from poultry demonstrate similar effects (review in Baggott and Graeme-Cook 2002). Given that pathogenic bacteria decrease hatching success, and that the uropygial gland has an antibacterial role, we predicted that species with larger glands should have higher hatching success. We tested these predictions using an extensive data set on the size of the uropygial gland in 212 species of birds.

Materials and methods

Uropygial glands

We extracted information on the mass of the uropygial gland from Jacob and Ziswiler (1982), and our own information recorded by J. E. for all birds brought to him as a taxidermist. More than 95% of all specimens were from Denmark and more than 50% from southern Jutland. Danish

taxidermists are by law enforced to register detailed information on specimens, including species, origin, date and cause of death. All this information was available for all specimens. The gland was carefully dissected out and weighed on a precision balance to the nearest 0.001 g. Estimates of the mass of the uropygial gland were highly repeatable when comparing ten common species from our data set and that of Jacob and Ziswiler (1982) [$F = 10.95$, $df = 9,10$, $r^2 = 0.91$, $P = 0.0004$, repeatability R (SE) = 0.83 (0.14)]. This provides evidence of consistency in estimates across sources. In total, we had information on the size of the uropygial gland of 212 species.

Ecological variables

We classified species as terrestrial (0; not commonly encountering water), partly aquatic (1; spending at least part of the time in water), or completely aquatic (2; spending most or all of the time in water) based on habitat descriptions in Cramp and Perrins (1977–1994) and del Hoyo et al. (1992–2008). While this habitat description hides intraspecific information, a more detailed description of the number of different breeding habitats (Belliure et al. 2000) did not provide different conclusions (results not shown).

Migration distance

We tested whether the relative size of the uropygial gland was related to migration distance because Galván et al. (2008) have shown previously that these two variables are correlated. We recorded migration status as: resident, when there was complete overlap between breeding and winter range (0); partial migrant when part of the population wintered in the same range as it breeds (1); and migratory (2) when breeding and winter range differed, relying on information from Cramp and Perrins (1977–1994) and del Hoyo et al. (1992–2008).

Taxonomic richness of chewing lice

We recorded richness of genera of the two sub-orders of chewing lice, Amblycera and Ischnocera, using data for all European host species reported in Price et al. (2003), combined with information from Vas et al. (2008) and Rékási and Kiss (1977). We restricted this data set to European host species because they are much more intensely and homogeneously studied than species from other continents. We obtained information on study intensity of different host species by recording the number of publications in the ISI Web of Knowledge (v. 4.3) by using the following search procedure: Topic = (epidemiol* OR pathogen* OR endoparasit* OR ectoparasit* OR helminth* OR Phthiraptera* Or Acari) AND Topic = ("Apus apus") AND Year Published = (1988–1997) Plus Year Published = (1998–2007). Because there was a maximum of 10 years in a search, we added the results for the two decades. In the case of *Troglodytes troglodytes*, we added the keyword "wren" to exclude hits on the chimpanzee (*Pan troglodytes troglodytes*). We extracted information on abundance of feather mites from Galván et al. (2008). We used information on mass of the bursa of Fabricius from Møller et al. (2005a, b) and Garamszegi et al. (2007).

We used previously published information on levels of natural antibodies in nestling and adult birds. Blood was collected from adult birds captured during the breeding seasons 2005–2007 in our Danish study sites in northern Jutland by puncturing the brachial vein and collecting two heparinized capillaries of 75 µl blood that were stored in a cooling box at a temperature just above freezing. Within a period of 2 h we centrifuged the capillaries for 10 min at 4,000 r.p.m. in the lab. Plasma and cells were separated and stored at −20°C until analysis at the lab.

To estimate the levels of circulating natural antibodies and complement we used the procedure developed by Matson et al. (2005) and adjusted by Møller and Haussy (2007). The agglutination part of the assay estimates the interaction between natural antibodies and antigens in rabbit blood, producing blood clumping. Quantification of agglutination is achieved by serial dilution in polystyrene 96-well assay plates, with the dilution step at which the agglutination reaction is stopped. We used fresh rabbit blood with Alsever's anticoagulant, 96 round well assay plates and an EPSON 4490 photo scanner that was set at professional mode, with document type color film, 48 bit color and 300 dpi. Whole rabbit blood was stored at 4°C. After determination of the level of hematocrit, the sample was diluted to obtain a solution of 1% of erythrocytes.

The protocol for hemagglutination was as follows. The plasma samples were thawed and homogenized using a vortex. Subsequently 25 ml of plasma was pipetted into each column, followed by addition of 25 ml of the solution to all wells. Wells subsequently contained a solution diluted by a factor 2 from a solution of 1:2 in the first well to a solution of 1:2,048 in the 11th well. Well number 12 only contained the dilution of erythrocytes, thus serving as a negative control. Subsequently 25 ml of the 1% solution of rabbit blood was added to all wells. The assay plate was then covered and shaken for 10 s, followed by incubation for 90 min in a bath at 37°C. The assay plate was then removed from the bath and left at 45° at ambient temperature for 20 min. The assay plate was then read and scanned. Scoring was based on negative wells having a small round agglutinate at the bottom thus forming a well-defined red round point, and positive wells having a diffuse film at the bottom. See Møller and Haussy (2007) for further details of procedures.

Hatching success

We used hatching success of eggs that had been incubated for the normal incubation period, thus excluded depredated or deserted eggs, as reported in Spottiswoode and Møller (2004) combined with data from Cramp and Perrins (1977–1994). Genetic similarity among conspecifics, as expressed by band-sharing coefficients, has previously been shown to predict hatching success (Spottiswoode and Møller 2004). We used published information on mean band-sharing coefficients among unrelated adults in local populations as reported by Møller et al. (2008). We recorded information on body mass of adult birds during the breeding season from our own data and Cramp and Perrins (1977–1994). The entire data set is reported in Appendix 1 (ESM).

Statistical analyses

All analyses were performed with the statistics software JMP (2000). Genera richness of Amblycera and Ischnocera, level of natural antibodies, mass of bursa of Fabricius, mass of the uropygial gland and body mass were $\log_{10}$-transformed before analysis, while hatching success was square-root arcsine transformed.

Relative mass of the uropygial gland was expressed as residuals of a linear regression where the slope was obtained from a log–log phylogenetically corrected regression of uropygial mass on body mass of 212 bird species. Residuals were simply observed mass of the uropygial gland minus the predicted mass from the phylogenetically corrected regression (see "Results"). The same procedure was adopted for contrast analyses. In this case, we first calculated contrasts of log(uropygial mass) on log(body mass), and then calculated residuals of these contrasts from the above-mentioned phylogenetically corrected regression equation.

Comparative analyses

We controlled for similarity in mass of the uropygial gland among species due to common ancestry by calculating standardized independent linear contrasts (Felsenstein 1985), using the computer program comparative analysis by independent contrasts (CAIC; Purvis and Rambaut 1995). Standardization of contrast values was checked by examination of absolute values of standardized contrasts versus their SDs (Garland et al. 1992). Plotting the resulting contrasts against the variances of the corresponding nodes revealed that these transformations made the variables suitable for regression analyses. In cases where extreme residuals were recorded, we tested for the robustness of the conclusions by excluding contrasts with Studentized residuals greater than 3.00 (Jones and Purvis 1997). In no case did we find qualitatively different conclusions. The phylogeny was based on Sibley and Ahlquist (1990), combined with more recent information from Bridge et al. (2005); Donne-Goussé et al. (2002); Griffiths et al. (2007); Johnson and Clayton (2000); Jønsson and Fjeldså (2006) and Thomas et al. (2004) [Appendix 2 (ESM)]. Two additional sets of analyses based on the phylogeny of Hackett et al. (2008) and the taxonomy of Sibley and Monroe (1990) provided qualitatively similar conclusions, and, therefore, we only report the first set of analyses for brevity.

Results

The size of the uropygial gland increased with body mass [Fig. 1; analysis of contrasts: $F = 375.44$, $df = 1,177$, $P < 0.0001$, slope (SE) = −0.42 (0.02)]. A model that excluded contrasts with extreme residuals (>3.00) had a slope that was slightly less steep [analysis of contrasts: $F = 747.17$, $df = 1,166$, $P < 0.0001$, slope (SE) = −0.41 (0.02)]. We subsequently used this slope to estimate relative mass of uropygial glands for all subsequent analyses, as described in "Materials and methods".

Bird species using aquatic habitats had relatively larger uropygial glands than terrestrial species [species-specific data, $F = 147.24$, $df = 1,210$, $r^2 = 0.41$, $P < 0.0001$, slope (SE) = 0.40 (0.03); contrasts, $F = 10.82$, $df = 1,177$, $r^2 = 0.06$, $P = 0.0012$, slope (SE) = 0.21 (0.06)]. There was no significant relationship between migration distance and relative size of the uropygial gland (species-specific data, $F = 1.45$, $df = 1,210$, $r^2 = 0.007$, $P = 0.23$; contrasts, $F = 2.72$, $df = 1,177$, $r^2 = 0.02$, $P = 0.10$), contrary to what was suggested by Galván et al. (2008) for a much smaller sample of species.

Bird species with relatively larger uropygial glands had more genera of Amblycera [Fig. 2a; analysis controlling for habitat, species-specific data, $F = 8.01$, $df = 1,39$, $r^2 = 0.17$, $P = 0.007$, slope (SE) = 0.21 (0.08); contrasts, $F = 6.17$, $df = 1,39$, $r^2 = 0.14$, $P = 0.017$, slope (SE) = 0.20 (0.08)],

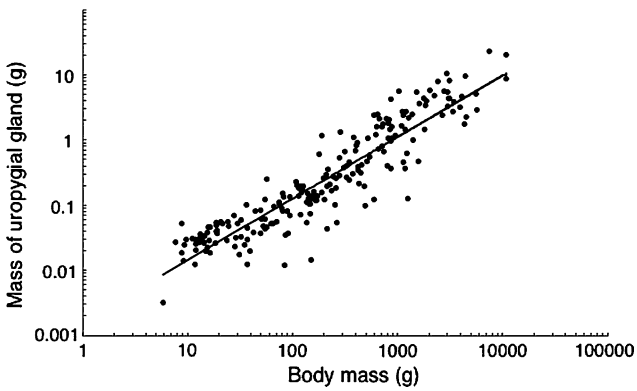

Fig. 1 Mass of the uropygial gland (*g*) in relation to body mass (*g*) in 212 species of birds. The linear regression line is shown

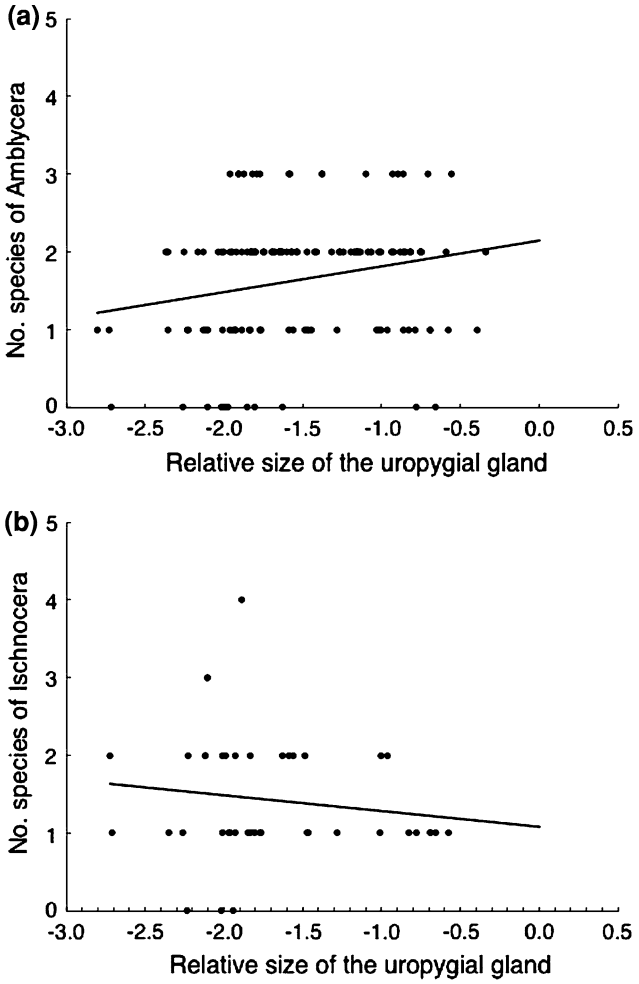

Fig. 2 Number of genera of **a** Amblycera and **b** Ischnocera chewing lice in relation to relative size of the uropygial gland in different species of birds. The size of the uropygial gland was adjusted for effects of body mass, as described in "Materials and methods". Linear regression lines are shown

but not more genera of Ischnocera (Fig. 2b; Table 1; analysis controlling for habitat, species-specific data, $F = 0.08$, $df = 1,38$, $r^2 = 0.00$, $P = 0.77$, slope (SE) = 0.06 (0.20); contrasts, $F = 1.09$, $df = 1,39$, $r^2 = 0.03$, $P = 0.30$). The relationship between genera richness of Amblycera and residual size of the uropygial gland was not significantly dependent on the effect of cell-mediated immunity in nestlings that has previously been shown to predict genera richness of Amblycera (Møller and Rózsa 2005; Table 2). Bird species with a larger abundance of feather mites tended to have relatively larger uropygial glands [species-specific data, $F = 5.98$, $df = 1,19$, $r^2 = 0.24$, $P = 0.024$, slope (SE) = 2.44 (1.00); contrasts, $F = 1.76$, $df = 1,19$, $r^2 = 0.08$, $P = 0.20$, slope (SE) = 0.06 (0.05)]. Abundance of feather mites was not significantly related to genera richness of chewing lice (Amblycera, species-specific data, $F = 0.38$, $df = 1,18$, $r^2 = 0.02$, $P = 0.55$; contrasts, $F = 0.19$, $df = 1,13$, $r^2 = 0.01$, $P = 0.67$; Ischnocera, species-specific data, $F = 0.20$, $df = 1,18$, $r^2 = 0.01$, $P = 0.66$; contrasts, $F = 0.30$, $df = 1,13$, $r^2 = 0.02$, $P = 0.59$).

Bird species with relatively large uropygial glands had relatively large bursa of Fabricius [Fig. 3; species-specific data, $F = 94.40$, $df = 1,79$, $r^2 = 0.54$, $P < 0.0001$, slope (SE) = 0.51 (0.05); contrasts, $F = 52.14$, $df = 1,73$, $r^2 = 0.42$, $P < 0.0001$, slope (SE) = 0.54 (0.07)].

Bird species with relatively large uropygial glands had low levels of agglutination in nestlings [species-specific data, $F = 6.14$, $df = 1,27$, $r^2 = 0.19$, $P = 0.020$, slope (SE) = −0.30 (0.12); contrasts, $F = 3.45$, $df = 1,24$, $r^2 = 0.13$, $P = 0.078$, slope (SE) = −0.18 (0.10)], but not in adults (species-specific data, $F = 1.52$, $df = 1,19$, $r^2 = 0.07$, $P = 0.23$; contrasts, $F = 0.87$, $df = 1,18$, $r^2 = 0.05$, $P = 0.36$).

There was a fitness advantage associated with relatively large uropygial glands because such species had higher

Table 1 Relative mass of the uropygial gland in relation to genera richness of Amblycera and Ischnocera, water habitat, research effort (number of publications) and body mass in birds

Variable	Sum of squares	*df*	*F*	*P*	Slope (SE)
Species					
Genera richness of Amblycera	0.678	1	4.37	0.039	0.505 (0.242)
Genera richness of Ischnocera	0.131	1	0.84	0.36	0.250 (0.272)
Water habitat	14.904	1	95.92	< 0.0001	0.382 (0.039)
No. publications	0.335	1	2.15	0.14	0.111 (0.075)
Error	19.422	125			
Contrasts					
Genera richness of Amblycera	0.112	1	4.01	0.048	0.310 (0.152)
Genera richness of Ischnocera	0.006	1	0.20	0.65	−0.127 (0.279)
Water habitat	0.261	1	9.23	0.0029	0.213 (0.070)
No. publications	0.111	1	3.93	0.050	0.133 (0.067)
Error	3.531	125			

The models had the statistics $F = 27.85$, $df = 4,125$, $r^2 = 0.47$, $P < 0.0001$ and $F = 4.54$, $df = 4,125$, $r^2 = 0.04$, $P = 0.0018$

Table 2 Number of genera of Amblycera in relation to relative mass of the uropygial gland, water habitat, cell-mediated immunity in nestling birds and research effort (number of publications)

Variable	Sum of squares	*df*	*F*	*P*	Slope (SE)
Species					
Uropygial mass	0.057	1	6.04	0.019	0.140 (0.057)
Water habitat	0.079	1	8.35	0.0064	−0.130 (0.045)
Cell-mediated immunity of nestlings	0.050	1	5.28	0.027	0.171 (0.075)
No. publications	0.043	1	4.51	0.040	0.072 (0.034)
Error	0.349	37			
Contrasts					
Uropygial mass	0.016	1	7.38	0.010	0.172 (0.063)
Water habitat	0.009	1	3.97	0.054	−0.140 (0.070)
Cell-mediated immunity of nestlings	0.002	1	0.97	0.33	0.079 (0.080)
No. publications	0.004	1	2.07	0.16	0.046 (0.032)
Error	0.080	37			

The models had the statistics $F = 5.36$, $df = 4,37$, $r^2 = 0.37$, $P = 0.0016$ and $F = 2.72$, $df = 4,37$, $r^2 = 0.07$, $P = 0.044$

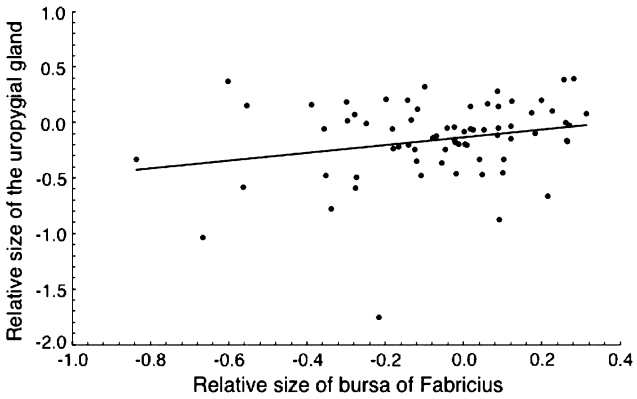

Fig. 3 Relative size of the uropygial gland in relation to relative size of the bursa of Fabricius in different species of birds. Both variables were adjusted for effects of body mass, as described in "Materials and methods". Linear regression line is shown

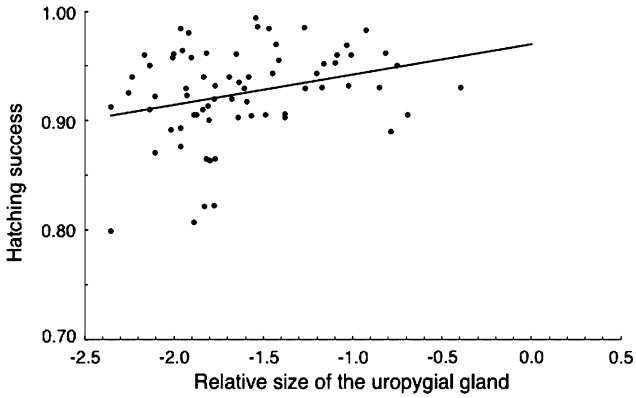

Fig. 4 Hatching success in relation to relative size of the uropygial gland in different species of birds. The size of the uropygial gland was adjusted for effects of body mass, as described in "Materials and methods". Linear regression line is shown

hatching success [Fig. 4; species-specific data, $F = 5.16$, $df = 1,67$, $r^2 = 0.07$, $P = 0.026$, slope (SE) = 0.049 (0.022); contrasts, $F = 3.45$, $df = 1,24$, $r^2 = 0.13$, $P = 0.078$, slope (SE) = −0.18 (0.10)]. This effect was independent of the effect of band sharing, body mass and water habitat (Table 3).

Discussion

The main results of this study were that the relative size of the uropygial gland increased with the abundance of feather mites and richness of genera of chewing lice of the sub-order Amblycera, but not of the sub-order Ischnocera. Bird species with relatively large uropygial glands also had relatively large bursa of Fabricius, but low innate immunity as reflected by natural antibodies in nestlings. Bird species with a relatively large uropygial gland enjoyed elevated hatching success.

Terrestrial species had relatively smaller uropygial glands than aquatic species, with partially aquatic species being intermediate. This confirms previous studies suggesting that uropygial glands are larger in aquatic birds (Kennedy 1971; Jacob and Ziswiler 1982; Johnston 1988; Montalti and Salibián 2000; Shawkey et al. 2003; Galván et al. 2008). We could not confirm a previous report that the size of the uropygial gland was related to migratory status (Galván et al. 2008), although our sample size was larger, providing us with considerably more statistical power.

Because the plumage of birds is regularly covered with wax from the uropygial gland during preening, any organism living on the plumage and coming into contact with wax from the uropygial gland will have to cope with and adjust to the biochemicals involved. The genera richness of

Table 3 Hatching success in relation to relative mass of the uropygial gland and band-sharing coefficient in birds

Variable	Sum of squares	*df*	*F*	*P*	Slope (SE)
Species					
Uropygial mass	0.034	1	5.73	0.026	0.089 (0.037)
Band-sharing coefficient	0.050	1	8.51	0.0080	−0.524 (0.180)
Error	0.129	22			
Contrasts					
Uropygial mass	0.003	1	8.54	0.0079	0.106 (0.036)
Band-sharing coefficient	0.006	1	16.75	0.0005	−0.533 (0.130)
Error	0.008	22			

The models had the statistics $F = 5.73$, $df = 2,22$, $r^2 = 0.34$, $P = 0.0099$ and $F = 11.42$, $df = 2,22$, $r^2 = 0.35$, $P = 0.004$

amblyceran chewing lice, but not ischnoceran lice, was positively associated with the relative size of the uropygial gland. While Amblycera come into contact with skin and tissue of the host from feeding on living tissue and feather barbs, Ischnocera rely on grazing feather barbs (Møller and Rózsa 2005). Gland wax kills microorganisms (Shawkey et al. 2003) that are subsequently eaten by feather mites (Proctor 2003), which are depredated by chewing lice, at least to a certain extent. The fact that we only found a positive relationship between relative size of the uropygial gland and genera richness of Amblycera, but not of Ischnocera suggests that the effect of biochemicals on diversification only acted in the sub-order that had to cope with two defense systems: immunity and wax from the uropygial gland. We confirmed the observation by Galván et al. (2008) that abundance of feather mites increased with the relative size of the uropygial gland, potentially through the effects of secretions on abundance of dead microorganisms that are subsequently consumed by feather mites (Proctor 2003).

The immune system constitutes an integrated system of physiological defense (Abbas et al. 1994) that together with other defenses [avoidance of infection, fever, nutrition and many others (see "Introduction")] help hosts keep parasites at bay. Thus, several mechanisms are simultaneously involved in regulating populations of microorganisms and fighting pathogenic strains. Therefore, we should expect different defenses to constitute integrated mechanisms of defense. Indeed, bird species with relatively large uropygial glands also had relatively large bursa of Fabricius. Because the latter is involved in the production of antibodies and differentiation and magnitude of the repertoire of B cells in young birds (Glick 1983, 1994; Toivanen and Toivanen 1987), secretions from the uropygial gland and products of the bursa would constitute alternative defenses against microorganisms. Furthermore, the level of natural antibodies in nestlings, but not in adults, was negatively correlated with the relative size of the uropygial gland in adults. Natural antibodies constitute the first line of defense against microorganisms (Carroll and Prodeus 1998; Ochsenbein et al. 1999; Reid et al. 1997), and the negative correlation between relative size of the uropygial gland and levels of natural antibodies may indicate that these are alternative modes of defense. The reason for the significant effect in nestlings but not in adults may be due to nestlings being more susceptible to parasite attacks than adults that already have encountered a large diversity of antigens during their life.

Interspecific variation in hatching success was positively associated with relative size of the uropygial gland. We can exclude the possibility that hatching failure was caused by the two taxa of ectoparasites that were positively correlated with relative size of the uropygial gland (feather mites; amblyceran chewing lice), because these parasites do not typically interact with birds' eggs (Galván et al. 2008; Møller and Rózsa 2005). There is extensive evidence suggesting that hatching failure in birds is caused by microorganisms including bacteria (e.g., Baggott and Graeme-Cook 2002; Cook et al. 2003, 2005), and that differences in anti-bacterial defense explain intraspecific variation in hatching success (Melek 1977; Krykanov 1982; Prusinowska and Jankowski 1996; Saino et al. 2002). Hatching success in birds is typically around 90%, with large variation among species (Spottiswoode and Møller 2004). Therefore, the association between hatching success and size of uropygial glands is consistent with the hypothesis that the gland plays an important role in defense against microorganisms.

In conclusion, investment into the anti-bacterial and anti-fungal defense of the plumage, as reflected by the relative size of the uropygial gland, is related to a terrestrial versus an aquatic way of life, abundance and taxonomic diversity of ectoparasites, size of the bursa of Fabricius, and levels of natural antibodies in nestlings. Species having larger uropygial glands are predicted to have less contaminated plumage, and thus their hatching success should suffer less from microbial contamination of eggs. Accordingly, we have shown that gland size covaries positively with hatching success.

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