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Sources of variation in uropygial gland size in European birds

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Defence mechanisms against parasites and pathogens are some of the most elaborate biological systems in animals. The oily secretion of the avian uropygial gland has been suggested to serve as a chemical defence against feather and eggshell bacteria. Yet, the traits associated with uropygial gland oil production are not well understood. We conducted a phylogenetic analysis comprising 132 European bird species aiming to test: (1) whether life-history and ecological traits drive gland size evolution by potentially promoting microbial infestation and (2) how these traits affects change in the gland size throughout the annual cycle. We show that the size of the uropygial gland is dynamic (i.e. increasing from the nonbreeding to the breeding season, independent of sex). Furthermore, we found that the year-round size of the gland was similar between sexes and was correlated with different ecological and life-history traits promoting microbial infection throughout the annual cycle. During the breeding season, the total eggshell surface area in a clutch correlated significantly and positively with the gland size, suggesting the importance of oil in protecting eggs from microbes. Social species exhibited a larger gland size increase during the breeding season compared to nonsocials; a change that was also predicted by the total eggshell surface area. Aquatic, riparian and non-migratory species had larger glands than terrestrials and migrants, respectively. The findings of the present study suggest that aquatic environments may promote the production of gland oil, through either the need of waterproofing the plumage and/or defending it against the intensified feather degradation in these moist conditions. Finally, we found a negative effect of the incubation period on uropygial gland size, which may suggest an energetic constraint imposed by other development-connected costly activities. Our results show that the role of the uropygial gland dynamically varies during the annual cycle, potentially in response to seasonal variation in parasitic infection risk. *Biological Journal of the Linnean Society*, 2013, **110**, 543–563.

ADDITIONAL KEYWORDS: habitat – life-history – microbial infection – seasonal change.

INTRODUCTION

Parasitic relationships between animal hosts and microorganisms are common in nature, yet the factors controlling infection are mostly unknown (Schmid-Hempel, 2011). Free-living animals and humans carry a wide variety of bacterial pathogens (Bush *et al*., 2001; Schmid-Hempel, 2011) against which a diverse defence repertoire has evolved (Bush *et al*., 2001; Moore, 2002). For example, the plumage of birds is known to provide habitat for diverse parasitic communities, some of which are detrimental

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to feathers because they decompose keratin (keratinolytic fungi and bacteria; Burtt & Ichida, 1999; Shawkey, Pillai & Hill, 2003; Ruiz-Rodríguez *et al*., 2009; Ruiz-de-Castañeda *et al*., 2012).

Empirical results suggest that uropygial gland oil plays a role in antimicrobial protection against feather-degrading bacteria (Shawkey *et al*., 2003; Reneerkens *et al*., 2008; Møller, Czirják & Heeb, 2009; Soler *et al*., 2012). For example, under laboratory conditions, the gland oil is shown to be an effective growth inhibitor of several feather degrading microorganisms (Shawkey *et al*., 2003; Reneerkens *et al*., 2008). Also, gland size was found to be negatively correlated with feather bacteria load (Møller *et al*., 2009). However, two recent *in vivo* studies found no significant negative effect of gland blocking or removing on the abundance of feather-degrading bacteria (Czirják *et al*., 2013; Giraudeau *et al*., 2013). These findings suggest that gland secretion might regulate harmful surface microbiota; however, its effect may differ between species with different ecological and life-history traits, and may vary during the avian annual cycle. Therefore, further studies are needed to obtain better insight into the diversity of the avian–microbial interactions potentially mediated by uropygial gland oil.

Exterior parasitic microbial communities not only inhabit the feathers and skin of birds, but also have been found to dwell on the surface of eggs, consequently reducing egg viability (Cook *et al*., 2003, 2005; Soler *et al*., 2012; but see also Peralta-Sanchez *et al*., 2010; Wang, Firestone & Beissinger, 2011). Shortly after laying, avian eggshells are colonized by microbes that proliferate rapidly under suitable ambient conditions, penetrate through shell pores, and infect egg contents, ultimately causing embryo mortality (Cook *et al*., 2003, 2005; Shawkey *et al*., 2009; Ruiz-de-Castañeda *et al*., 2011). Because uropygial gland oil can be directed to the bacteria community living in the nest during incubation, it has been suggested that gland secretions may serve as a complementary way of defence against bacteria-induced embryo mortality (Martín-Vivaldi *et al*., 2009; Shawkey *et al*., 2009; Møller, Erritzøe & Rózsa, 2010a). Furthermore, the increase in gland size and change in oil composition during incubation has been suggested to be an evolutionary response for egg protection by the incubating sex (Reneerkens *et al*., 2007; Martín-Vivaldi *et al*., 2009; Pap *et al*., 2010). Because the presence of parasitic microbial communities varies seasonally as a result of the breeding chronology of the host, we predicted that the selective pressures inflicted by the microorganisms on the avian host would subsequently vary during the annual cycle.

The importance of the secreted waxes originating from the uropygial gland is well known because birds spend a considerable part of their daily time budget preening, during which the oily secretions are spread throughout the plumage (Cotgreave & Clayton, 1994). Furthermore, there are only very few extant bird species that do not possess the uropygial gland, and most of the exceptions are either flightless species or the ones that produce powder down for plumage maintenance (Jacob & Ziswiler, 1982; Delhey, Peters & Kempenaers, 2007). Unexpectedly, however, associations between the size of the gland, which is a good proxy for the amount of waxes secreted (Møller *et al*., 2009; Pap *et al*., 2010), and life-history or ecological characteristics, which mirror pathogen infestation risks, are still poorly understood (Reneerkens *et al*., 2007; Møller *et al*., 2010a) despite the recent upsurge of investigations about the function of the uropygial gland secretions (Moyer, Rock & Clayton, 2003; Shawkey *et al*., 2003; Galván *et al*., 2008; Reneerkens *et al*., 2008; Martín-Vivaldi *et al*., 2009; Møller *et al*., 2009; Giraudeau *et al*., 2010a; Møller *et al*., 2010a; Møller, Erritzøe & Nielsen, 2010b; Amat *et al*., 2011; Mardon, Saunders & Bonadonna, 2011; Pérez-Rodríguez, Mougeot & Bortolotti, 2011; Whittaker *et al*., 2011; Leclaire *et al*., 2012). If uropygial gland secretion is an adaptive countermeasure of hosts against bacterial infestation, we would predict that the huge variation in gland size among birds parallels the pathogen selection regime that hosts might experience (Møller *et al*., 2010a, b; Soler *et al*., 2012). Epidemiological studies show that moisture, migration, sociality, and breeding in cavities expose host species to higher risks of infection (Møller & Erritzøe, 1996; Figuerola & Green, 2000; Tella, 2002; Cook *et al*., 2005). Because the incubation period may mediate the growth of microbes on the egg surface (Cook *et al*., 2003; Shawkey *et al*., 2009; Ruiz-de-Castañeda *et al*., 2011; Peralta-Sanchez *et al*., 2012) and the fledging period may constrain the development of elaborate and functional defence traits (Starck & Ricklefs, 1998), we included these traits in a multivariate analyses on gland size.

We conducted a phylogenetic comparative study of 2706 individuals from 132 European avian species. We collected data on gland size from both males and females during the nonbreeding and breeding season. With the premise that gland secretion may serve as 'antibiotics' on both the plumage and eggs, we tested predictions based on three concepts. First, we tested for potential factors explaining seasonal variation in uropygial gland size, such as the allocation of oily secretions to eggs during the breeding or the allocation to the feathers and skin throughout the year. We predicted that the gland size outside of breeding season would mainly be influenced by factors promoting microbial infection on plumage and/or skin (e.g. migratory behaviour, habitat), whereas the gland size

during breeding is associated with the microbial infection of the eggs (e.g. eggshell surface, sociality). Second, we tested whether the sexual dimorphism of gland size measured during the breeding season is predicted by the incubation share of each sex. We expected that the sex with greater incubation share would have a larger gland. Third, we predicted that there would be an increase in uropygial gland size from the nonbreeding to breeding season (Martín-Vivaldi *et al*., 2009; Pap *et al*., 2010), and that this morphological change would be related to ecological and life-history traits that might enhance the host's abilities to fight microbial infections during breeding.

MATERIAL AND METHODS

Field data collection

We collected year-round data on the size of the uropygial gland of adult birds between 2003 and 2012 at several sites across Romania and Norway. All captured birds were marked with an individually numbered aluminium ring, sexed (when possible), and the maximum length, width, and height of the uropygial gland was measured with a digital calliper (Microprecision Calibration Inc.; precision of 0.01 mm). Uropygial gland size was expressed as the product of these three measures *sensu* Galván & Sanz (2006). Data on the glaucous gull (*Larus hyperboreus*), black-legged kittiwake (*Rissa tridactyla*), and common eider (*Somateria mollissima*) were collected in Ny-Ålesund, on the island of Svalbard, from May to June 2010 by P.L.P. and Kjetil Sagerup, whereas birds from Romania were measured by O.V., P.L.P., C.I.V., and I.K. These data originating from captured birds were supplemented by data acquired from corpses (e.g. road kills) during 2010–2012. To minimize potential changes in gland size as a result of fatality, uropygial measurements were only taken from corpses found shortly after the time of death. In total, we had information available from Romania and Svalbard on the size of the uropygial gland of 2706 individuals from 132 species. To estimate the reliability of our measures, we used information on individual birds for which the uropygial gland size was measured by two different observers. The between observer repeatability was high ($R_{15} > 0.75$, $P < 0.001$). Additionally, within observer repeatability was also satisfactory ($R_{15} > 0.85$, $P < 0.0001$) based on repeated gland size measures of the same individuals. Finally, because we were restricted to measuring the size of only the external portion of the uropygial gland, we tested the reliability of our size estimates by comparing our data with total gland mass (i.e. dissected) reported by Møller *et al*. (2010a). The gland volume estimate was significantly and positively correlated with gland mass [phylogenetic least squares model: β (SE) = 0.83 (0.07), $t = 11.85$, $N = 59$, $P < 0.0001$, $\lambda = 0.92$], providing strong support for the reliability of our measurements.

Life-history and ecological variables

We obtained body mass data from Dunning (2008). In cases of species for which the data of several subspecies or populations were reported, we only used populations and subspecies within Europe. We extracted female and male mean body masses separately, although we also calculated an overall mean body mass irrespective of the sexes. Life-history variables, such as egg weight, clutch size, length of incubation and fledging periods, and sex-specific incubation share, were extracted from Cramp & Perrins (1977–1994). We calculated eggshell surface area for each species based on the mean egg weight of the species *sensu* Paganelli, Olszowka & Ar (1974). Total eggshell surface area of each species was calculated as the product of the surface of a single egg and the mean clutch size of the species. Species were classified on the basis of ecological characters (Cramp & Perrins, 1977–1994): (1) type of nest: breeding in open or hole nests; (2) habitat preferences: terrestrial (rarely encountering water), riparian (living in moist habitats, e.g. marshes and sedges) or aquatic (species with direct contact to water); (3) migration strategy: residents (species that have completely overlapping breeding and nonbreeding ranges), short-distance migrants (with breeding and nonbreeding ranges partially overlapping or with wintering ranges north to the Sahara) or long-distance migrants (species wintering in sub-Saharan Africa); (4) social behaviour during breeding: social (colonial breeders) or solitary (territorial species); (5) social behaviour outside the breeding season: social (gregarious during winters) or solitary (which do not exhibit flocking behaviour); (6) incubation share: egalitarian (approximately 50 : 50 share of males and females) or only-female (clutches mostly or fully incubated by the female). Information on uropygial gland size, life-history, and ecology are shown in the Appendices (Tables A1 and A2).

Statistical analysis

Our statistical analyses were performed on several levels. First, we investigated the overall mean gland size of species, including the calculation of the means of both sexes and all data collected at any time during the year (2706 individuals, 132 species). Second,

because uropygial gland may change significantly in size seasonally (Martín-Vivaldi *et al.*, 2009; Pap *et al.*, 2010), we analyzed the gland sizes separately for the breeding and nonbreeding periods. The two periods were arbitrarily defined as: reproductive season (between 1 April and 31 July, when most of the birds breed; based on our field observation) and nonreproductive season (between 1 August and 31 March). Third, we tested whether there is significant change in gland size between the reproductive and nonreproductive season across species using phylogenetic paired *t*-tests (Lindenfors, Revell & Nunn, 2010). The latter analyses were performed for males and females separately. Because there was a significant seasonal change in both sexes but no sex differences in either season (see below), we also computed the mean change in gland size per species, irrespective of sex. This was expressed as the difference between log-transformed uropygial gland size during the reproductive season minus nonreproductive season and was later used as a response variable in a phylogenetic least squares (PGLS) model. Fourth, we performed phylogenetic paired *t*-tests (Lindenfors *et al.*, 2010) to test whether sexes differ regarding their gland size during the breeding and nonbreeding season, respectively. Then, we calculated a difference between females and males, expressed as the difference in the log-transformed uropygial gland size of the sexes for each species during the breeding season. The latter difference was then included in a PGLS model as a response variable with body mass difference and incubation share as response variables. Sample size for the latter two analyses was somewhat reduced because sex determination for several species was not possible and/or because we did not capture both sexes of certain species. Body mass, total eggshell surface area, and the uropygial gland size were log-transformed in all models. Because the sociality of species may differ between the breeding and the nonbreeding season, we used two sets of categorization in the analyses of gland size measured during the two periods: (1) in the analysis of the overall gland size and the seasonal change in gland size, we used the nonbreeding social categorization, which largely corresponds with the sociality over the whole annual cycle, and (2) in the analysis of the gland size during the breeding season, we used the social categorization for this period.

To investigate the relationship between gland size, ecological, and life-history traits, we used PGLS models (Pagel, 1997, 1999). We conducted all analyses setting the degree of phylogenetic dependence (λ) to the most appropriate degree evaluated for each model (Freckleton, Harvey & Pagel, 2002). To represent phylogenetic relationships among taxa, we used the dated phylogeny reported by Thuiller *et al.* (2011). We report full and minimal models, with the latter being obtained by eliminating nonsignificant predictors, except body mass to control for allometry, from the full model in a stepwise backward manner using $\alpha = 0.05$. We are aware of possible collinearity problems caused by the body mass dependence of several explanatory variables used. To detect such problems, we repeated the multivariate models using residual uropygial gland volume, extracted from a log-log linear regression between gland volume and body mass. Our conclusions did not change using these models, nor did the models using raw gland volume show signs of multicollinearity. However, because working with residuals in PGLS models is not recommended (Freckleton, 2009), we report the result from models using raw uropygial gland volumes.

All statistical analyses were conducted in the R statistical environment (R Development Core Team, 2011) with 'nlme' and 'ape' add-on packages and the 'gls' function (Paradis, Claude & Strimmer, 2004; Pinheiro *et al.*, 2011). Our sample sizes differed among species. Such differences in sampling effort are known to be sources of bias because different estimates are not estimated with similar precision (Garamszegi & Møller, 2010, 2011). However, if within species variance is particularly small compared to between species variance, then ignoring this measurement error has no effect on type I error of phylogenetic analyses (Harmon & Losos, 2005). Conspecifics gland size was highly similar in species for which at least two individuals were available ($R_{105} = 0.94$, $P < 0.0001$); therefore, we present the unweighed models. Furthermore, unweighed PGLS models were more competitive (had the lowest Akaike information criterion values) than models weighed by sample size (data not presented), which further strengthens the minor effect of the within-species variance and the variation in the within-species sample size on the results. However, we repeated the analysis weighing the models by log-sample size. As expected, the results (Tables 1–4) were qualitatively similar to the models not weighed by sample size (see Appendix, Tables A3–A6).

To test seasonal change in uropygial gland size and sex differences during the breeding season, we used phylogenetic paired *t*-test, using the 'phyl.pairedttest' function of the 'phytools' package in R (Lindenfors *et al.*, 2010). We report the mean ± SE values and two-tailed statistical tests with $\alpha = 0.05$. Because the phylogenetic methods applied here do not allow the graphical presentation of phylogenetically corrected data, all reported values are based on raw species data.

Table 1. Full and minimal phylogenetic generalized least squares models explaining overall uropygial gland size

	Full model			Minimal model		
	β (SE)	*t*	*P*	β (SE)	*t*	*P*
Intercept	2.14 (0.63)	3.40	**0.0009**	2.55 (0.40)	6.33	< **0.0001**
Body mass	0.90 (0.08)	11.91	< **0.0001**	0.93 (0.05)	17.30	< **0.0001**
Incubation	–0.03 (0.02)	–2.00	**0.0482**	–0.04 (0.01)	–2.41	**0.0174**
Fledging	–0.01 (0.01)	–1.31	0.1921			
Total eggshell surface	0.14 (0.15)	0.98	0.3312			
Habitat: riparian*	0.19 (0.14)	1.38	0.1701			
Aquatic	0.41 (0.22)	1.89	0.0618			
Migration: short†	–0.05 (0.09)	–0.57	0.5664			
Long	–0.17 (0.10)	–1.70	0.0917			
Sociality	0.01 (0.08)	0.10	0.9240			
Nest type: open	0.01 (0.11)	0.07	0.9422			

The minimal models were obtained by eliminating nonsignificant predictors from the full models in a backward stepwise manner based on the largest *P*-value. Model intercepts implement the first level of each factor (i.e. terrestrial species in the case of habitat and resident species in the case of migratory behaviour). All other levels of the candidate factor are compared to the level implemented in the intercept. Significant *P*-values are shown in bold.
*No significant difference between groups riparian and aquatic: β (SE) = 0.23 (0.25), $t = 0.91$, $P = 0.3625$.
†No significant difference between groups short- and long-distance migrant: β (SE) = –0.11 (0.08), $t = -1.37$, $P = 0.1717$.

Table 2. Full and minimal phylogenetic generalized least squares models explaining breeding season uropygial gland size

	Full model			Minimal model		
	β (SE)	*t*	*P*	β (SE)	*t*	*P*
Intercept	1.37 (0.74)	1.85	0.0665	1.20 (0.69)	1.74	**0.0851**
Body mass	0.78 (0.09)	8.25	< **0.0001**	0.76 (0.09)	8.80	< **0.0001**
Incubation	–0.05 (0.02)	–2.27	**0.0250**	–0.05 (0.02)	–2.68	**0.0084**
Total eggshell surface	0.41 (0.19)	2.14	**0.0351**	0.45 (0.18)	2.44	**0.0161**
Habitat: riparian*	0.19 (0.14)	1.33	0.1863			
Aquatic	0.28 (0.24)	1.16	0.2502			
Migration: short†	–0.06 (0.10)	–0.54	0.5902			
Long	–0.15 (0.11)	–1.37	0.1727			
Fledging	0.00 (0.01)	–0.32	0.7489			
Sociality: social	0.03 (0.14)	0.23	0.8184			
Nest type: open	0.06 (0.12)	0.52	0.6012			

The minimal models were obtained by eliminating nonsignificant predictors from the full models in a backward stepwise manner based on the largest *P*-value. Model intercepts implement the first level of each factor (i.e. terrestrial species in the case of habitat and resident species in the case of migratory behaviour). All other levels of the candidate factor are compared to the level implemented in the intercept. Significant *P*-values are shown in bold.
*No significant difference between groups riparian and aquatic: β (SE) = 0.09 (0.27), $t = 0.32$, $P = 0.7487$.
†No significant difference between groups short- and long-distance migrant: β (SE) = –0.10 (0.09), $t = -1.05$, $P = 0.2965$.

RESULTS

Overall uropygial gland size

Life-history variables were important in explaining variation in uropygial gland size of European birds. Besides the effect of the body mass, the incubation period significantly explained the gland size in both full and minimal multivariate models (Fig. 1, Table 1). Species with long incubation periods had significantly smaller glands compared to those with

Table 3. Full and minimal phylogenetic generalized least squares models explaining nonbreeding season uropygial gland size

	Full model			Minimal model		
	β (SE)	*t*	*P*	β (SE)	*t*	*P*
Intercept	3.45 (0.71)	4.87	< **0.0001**	3.03 (0.45)	6.77	< **0.0001**
Body mass	0.96 (0.08)	11.38	< **0.0001**	0.91 (0.06)	14.30	< **0.0001**
Incubation	–0.04 (0.02)	–2.07	**0.0424**	–0.05 (0.02)	–2.77	**0.0071**
Habitat: riparian*	0.09 (0.13)	0.69	0.4904	0.06 (0.13)	0.46	0.6458
Aquatic	0.66 (0.23)	2.84	**0.0059**	0.60 (0.23)	2.58	**0.0118**
Migration: short†	–0.19 (0.11)	–1.67	0.0984	–0.10 (0.11)	–0.93	0.3545
Long	–0.32 (0.12)	–2.73	**0.0079**	–0.25 (0.11)	–2.27	**0.0262**
Sociality: social	–0.18 (0.11)	–1.72	0.0889			
Nest type: open	0.00 (0.11)	0.01	0.9893			
Fledging	–0.01 (0.01)	–1.36	0.1789			
Total eggshell surface	–0.09 (0.16)	–0.54	0.5900			

The minimal models were obtained by eliminating nonsignificant predictors from the full models in a backward stepwise manner based on the largest *P*-value. Model intercepts implement the first level of each factor (i.e. terrestrial species in the case of habitat and resident species in the case of migratory behaviour). All other levels of the candidate factor are compared to the level implemented in the intercept. Significant *P*-values are shown in bold.
*No significant difference between groups riparian and aquatic: β (SE) = –0.54 (0.26), $t = -1.71$, $P = 0.0916$.
†Significant difference between groups short- and long-distance migrant: β (SE) = 0.54 (0.26), $t = 2.06$, ***P* = 0.0427.**

Table 4. Full and minimal phylogenetic generalized least squares models explaining seasonal change in uropygial gland size (difference between the values during the reproductive season minus the value obtained during the nonreproductive season)

	Full model			Minimal model		
	β (SE)	*t*	*P*	β (SE)	*t*	*P*
Intercept	–1.18 (0.64)	–1.84	0.0716	–0.83 (0.52)	–1.61	0.1116
Body mass	–0.08 (0.08)	–1.04	0.3022	–0.10 (0.07)	–1.38	0.1728
Total eggshell surface	0.45 (0.18)	2.47	**0.0169**	0.34 (0.16)	2.19	**0.0324**
Sociality: social	0.36 (0.11)	3.29	**0.0018**	0.30 (0.08)	3.59	< **0.0001**
Incubation	–0.02 (0.02)	–1.21	0.2315			
Fledging	0.00 (0.01)	0.36	0.7216			
Habitat: riparian*	0.07 (0.10)	0.65	0.5197			
Aquatic	0.02 (0.17)	0.10	0.9215			
Migration: short†	0.05 (0.11)	0.40	0.6881			
Long	0.13 (0.11)	1.19	0.2409			
Nest type: open	–0.06 (0.08)	–0.71	0.4794			

The minimal models were obtained by eliminating nonsignificant predictors from the full models in a backward stepwise manner based on the largest *P*-value. Model intercepts implement the first level of each factor (i.e. terrestrial species in the case of habitat and resident species in the case of migratory behaviour). All other levels of the candidate factor are compared to the level implemented in the intercept. Significant *P*-values are shown in bold.
*No significant difference between groups riparian and aquatic: β (SE) = –0.06 (0.20), $t = -0.28$, $P = 0.7776$.
†No significant difference between groups short- and long-distance migrant: β (SE) = –0.10 (0.08), $t = -0.31$, $P = 0.2303$.

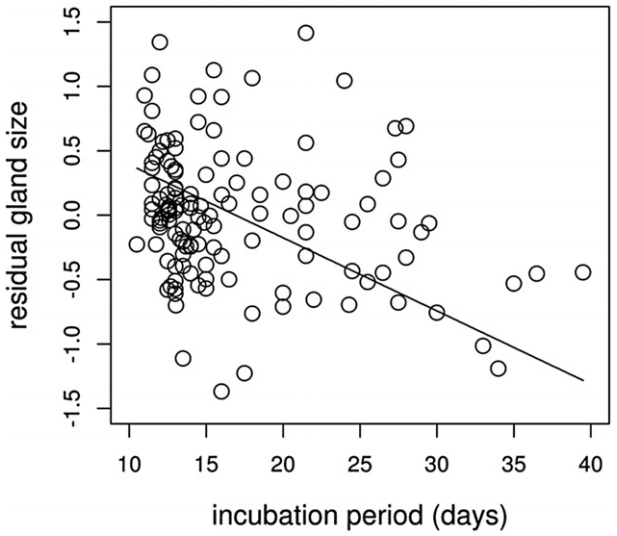

Figure 1. The relationship between the residual overall uropygial gland size (measured during the whole annual cycle and corrected for the body mass) and incubation period of 132 European bird species. Slope obtained from standard linear regressions are shown.

short incubation periods. The overall gland size was marginally explained by the migratory behaviour and habitat (Table 1), which was strengthened by the significant effect of these traits in the PGLS weighed models (see Appendix, Table A3).

Uropygial gland size during and outside the reproductive season

The varying influence of the breeding and nonbreeding season versus ecological and life-history traits on uropygial gland size indicates that this organ is differentially affected by a variety of factors (Tables 2 and 3). During the breeding season, incubation period significantly and negatively explained the gland size, whereas total eggshell surface had a significant positive effect (Fig. 2A, Table 2). In the nonbreeding season, the negative effect of incubation period still holds, whereas the effect of the total eggshell surface lost support (Fig. 2B, Table 2). Additionally, the gland size during the nonbreeding season was explained by migratory behaviour and habitat (Fig. 3A, B, Table 3), with gland size gradually decreasing with increasing migratory distance. Aquatic species had significantly larger gland sizes compared to terrestrial birds, whereas riparian species living in moist habitats were intermediate between the two. Nest type and fledging period had no effect on gland size during the breeding and nonbreeding periods.

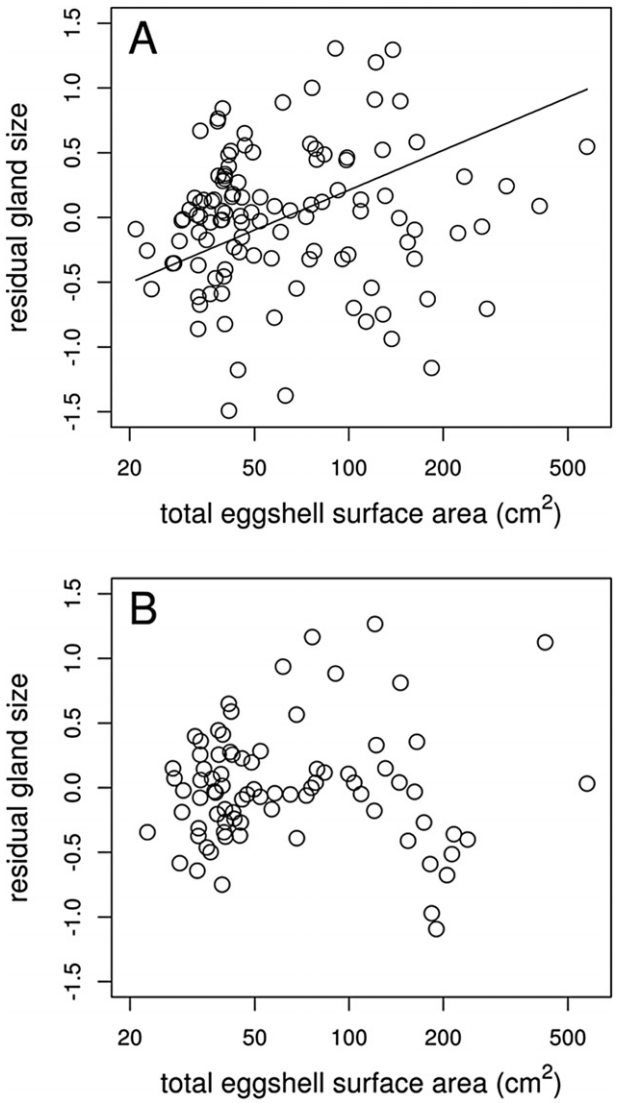

Figure 2. The relationship between the relative uropygial gland size (corrected for the body mass) measured during the breeding (A) and nonbreeding (B) season and the total eggshell surface area. The slope obtained from standard linear regression is shown.

Sexual dimorphism in uropygial gland size

There was no difference in the uropygial gland size measured during the breeding season between the sexes across species (phylogenetic paired t-test, $t = 0.10$, $N = 73$, $P = 0.9193$, $\lambda = 0.54$). Similarly, the difference between sexes in gland size measured during the nonbreeding season was nonsignificant ($t = 0.08$, $N = 13$, $P = 0.99392$, $\lambda = 0.00$), although sample size was low. Sex differences in uropygial gland size during the reproductive season were positively correlated with body size dimorphism [PGLS, β (SE) = 1.12 (0.36), $t = 3.11$, $P = 0.0027$, $\lambda = 0.06$] and

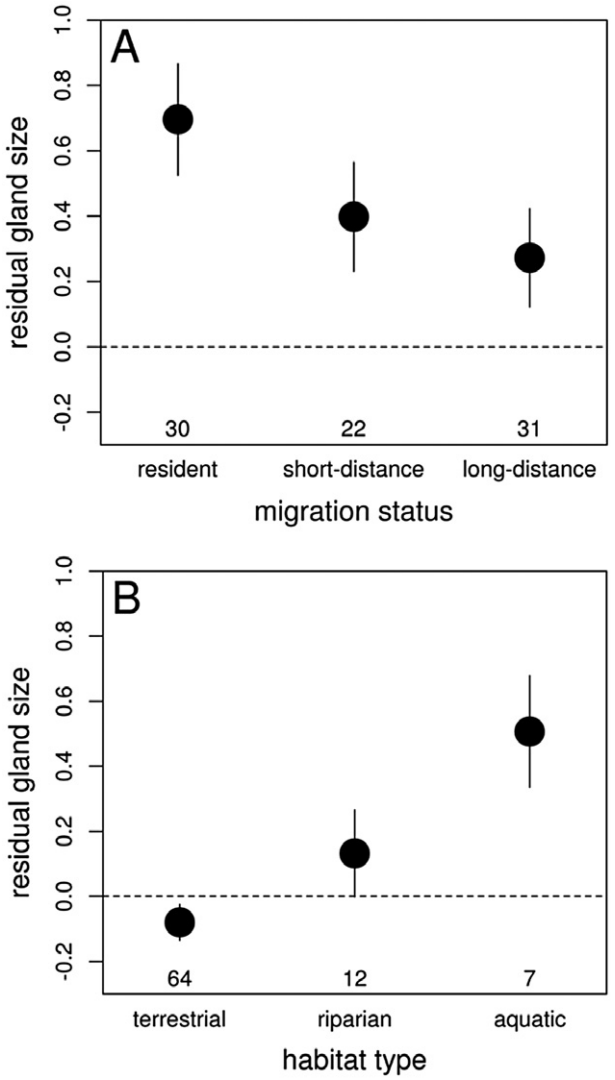

Figure 3. The relationship between the relative uropygial gland size (corrected for the body mass) measured during the nonreproductive season and the migratory behaviour (A) and habitat use (B). Error bars represent the SEs of the means. Numbers denote corresponding sample sizes.

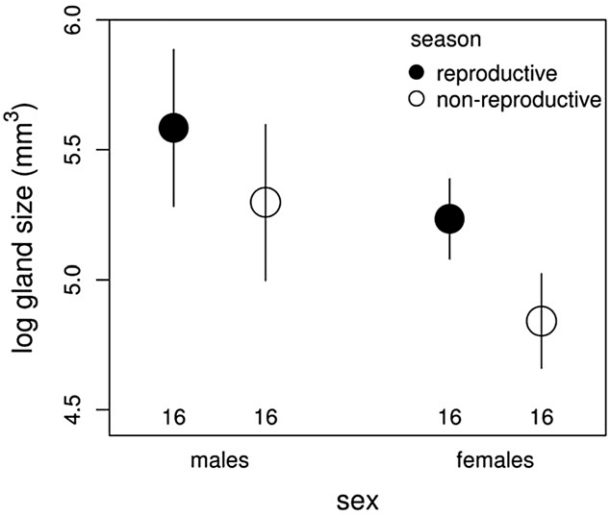

Figure 4. The difference in the uropygial gland sizes of males and females measured during the nonbreeding and breeding seasons. Numbers denote corresponding sample sizes.

were not explained significantly by the incubation share of the sexes [β (SE) = −0.01 (0.07), $t = 3.11$, $P = 0.9175$].

Seasonal change in uropygial gland size

Uropygial gland size increased significantly during the breeding compared to the nonbreeding season across species, in both males (phylogenetic paired t-test, $t = 2.21$, $N = 16$, $P = 0.0459$, $\lambda = 0.83$) and females ($t = 2.52$, $N = 16$, $P = 0.0254$, $\lambda = 0.95$; Fig. 4). Social species exhibit a larger increase in uropygial gland size during the reproductive season compared to the nonreproductive period than do nonsocial species (Fig. 5A, Table 4). Additionally, the increase in gland size during breeding was positively correlated with total eggshell surface (Fig. 5B, Table 4). Body mass did not predict the change in uropygial gland size, although we retained in the model to control for potential allometric effect of the size.

DISCUSSION

The function of the gland during breeding

Owing to the possible antimicrobial properties of avian uropygial gland secretions, our results are consistent with the hypothesis that life-history and ecological traits promoting infestation play an important role in host–microorganism interactions. We found that the total eggshell surface area is significantly and positively correlated with the size of the uropygial gland measured during the reproductive season, but not with measures outside the breeding season. This finding is consistent with our prediction that the variation in gland size between species is influenced by the amount of gland oil needed to coat the surface of eggs in a clutch (Cook *et al*., 2003, 2005; Shawkey *et al*., 2009; Ruiz-de-Castañeda *et al*., 2011; Soler *et al*., 2012). Interestingly, none of the life-history and ecological traits that may promote microbial infection (Møller & Erritzøe, 1996; Figuerola & Green, 2000; Tella, 2002; Cook *et al*., 2005), and some of which proved to significantly influence the variation of the gland size during the nonbreeding period, had an effect during breeding (Tables 2, 3). This suggests that the gland secretion may be directed against different microbial communities during the

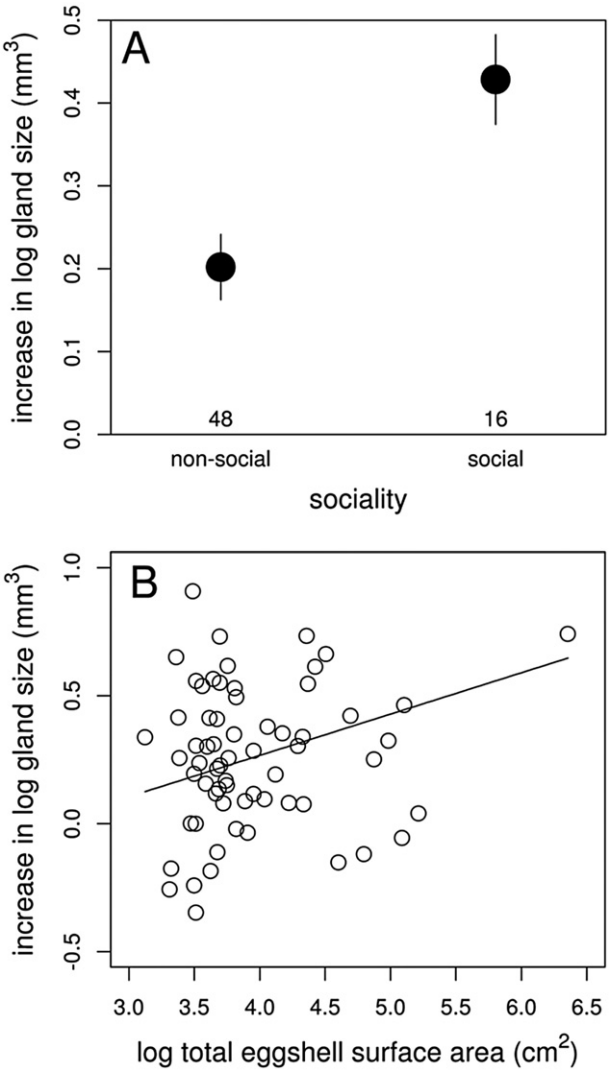

Figure 5. The increase of the uropygial gland size between the nonbreeding and breeding season in relation to sociality (A) and total eggshell surface area (B). Error bars represent the SEs of the means. Numbers denote corresponding sample sizes. The slope obtained from standard linear regression is shown.

nonbreeding and breeding periods. We also found that gland size is significantly larger during breeding compared to the nonbreeding period, and the magnitude of size increment is positively correlated with the eggshell surface area and sociality. This association strengthens our previous result on the need to protect the eggs with gland oil. However, owing to the cost of oil production (Piault *et al*., 2008; Pap *et al*., 2013), only those species whose life-history and ecological traits promote the proliferation of microbes during breeding may invest much in antimicrobial defence. Our results show that the amount of investment in reproduction and sociality is a factor that may influence microbial infection and hence antimicrobial defence. The positive association between eggshell surface and gland size is in concert with several studies. First, uropygial gland size is positively associated with hatching success in birds; thus, there is an apparent direct fitness consequence of the produced oil amounts (Møller *et al*., 2010a). Second, uropygial secretion reduces bacterial loads of eggshells and hatching failures of European birds (Soler *et al*., 2012). Third, it is consistent with findings that microorganisms have a negative effect on egg viability as a result of trans-shell infections during incubation (Cook *et al*., 2003, 2005).

By contrast to that found in two avian species (Martín-Vivaldi *et al*., 2009; Pap *et al*., 2010), we found no sexual size dimorphism in gland size during the breeding season across species. Furthermore, sexual dimorphism was not explained by the incubation share of the sexes. The results of the present study show that both sexes are equally exposed to selection by microbes during the breeding period (but see also Reneerkens *et al*., 2007). Our results show that the gland size increase during breeding is a general phenomenon and applies to a wide range of avian species. Our results strengthen the previous findings about the change in the quantity and composition of the gland oil during the annual cycle in birds, which may be regulated by the seasonal variation of its function (Reneerkens, Piersma & Sinninghe Damsté, 2005; Martín-Vivaldi *et al*., 2009; Pap *et al*., 2010). Alternatively, there may be other reasons for such a seasonal effect not only including lower temperatures and hence lower microbial growth, but also lower activity and hence less dirt being deposited on the plumage when wintering. These hypotheses, however, remain to be tested.

The function of the gland outside the breeding season

We found that aquatic species have larger glands than terrestrial birds during the nonbreeding season, a finding that is consistent with the originally suggested waterproofing function of the gland (Jacob & Ziswiler, 1982; Giraudeau *et al*., 2010a). The relationship between gland size and the use of aquatic habitat could, however, be additionally explained by the indirect effect of moisture facilitating microbial activity and growth (Burtt & Ichida, 1999; Cook *et al*., 2005). Under this scenario, microorganisms may have greater effect on the host's plumage in aquatic and riparian habitats than in drier environments. Our findings demonstrated that gland size increased from terrestrial to aquatic species, with riparian species having intermediate sizes. Thus, our study suggests that an aquatic environment may directly or

indirectly affect the production of gland oil, through the need of waterproofing the plumage and/or defending it against potentially intensified feather parasitism in moist conditions. Our findings propose that increased gland activity in moist environments may provide an additive defence against bacteria other than melanin-based plumage pigmentation ('Gloger's rule'; Burtt & Ichida, 2004). Birds living in moist environments are known to have an increased risk of parasitism by eggshell microbes (Cook *et al*., 2005; Ruiz-de-Castañeda *et al*., 2011); however, it is unknown whether avian species living in these environments have an increased risk of parasitism by keratinolytic microorganisms and/or richer feather degrading bacteria communities than those living in dry habitats.

Among the ecological traits that we tested, the migratory behaviour for many different host groups is known to influence risk of infection (Figuerola & Green, 2000). Most avian pathogens are mesophilic (Madigan *et al*., 2012), which suggests that tropical conditions promote greater parasite diversity and abundance. Therefore, we expected a larger investment in gland size in migratory species compared to resident birds. In addition, migration might increase infection risk not only through greater parasite abundance in tropics, but also as a result of increased coloniality and connections with other species. However, by contrast to our prediction, we found that long-distance migrants had the smallest (and residents the largest) gland sizes, at least during the migratory period when all migrants were measured. Burtt & Ichida (1999) found that feather-degrading bacteria pressure was the highest during winter in temperate resident birds, and Bisson *et al*. (2009) found that resident birds had higher plumage microbial diversity than migrants in the Nearctic. These results are in line with our findings on larger gland size of residents compared to migrants. Alternatively, an allocation conflict with a competing costly trait, such as migratory behaviour, may over-ride the benefits of producing large quantities of waxes provided that gland activity is also expensive (Piault *et al*., 2008; Moreno-Rueda, 2010; Pap *et al*., 2013). It is important to note that, in the present study, the gland size of long-distance migratory species was measured between spring and fall and therefore we have no information on the size of their glands during the winter. Birds are known to seasonally change their uropygial oil production (Martín-Vivaldi *et al*., 2009; Pap *et al*., 2010), with only one study actually following the change in gland size throughout the entire annual cycle, showing a dramatic increase during breeding (Pap *et al*., 2010). However, except for this study on a sedentary bird species, we do not know how the gland size changes seasonally in migratory bird species. Further studies are required that measure gland size and microbiota community in migratory and resident birds throughout the year with the aim of understanding of the seasonal adaptive change in oil production.

Developmental period and gland size

Following the hypothesis that there is selection pressure for larger gland oil production in response to long exposure time of the eggs to microbes, we expected a positive association between the gland size measured during the breeding season and length of the incubation period. However, by contrast to this hypothesis, we found a year-round significant negative correlation between the incubation period and gland size, a finding that suggests a long-term carry-over effect of the incubation period on gland size. We speculate that species with slower growth rate (i.e. long incubation period) regularly live slow and die old. Species with a slower 'pace-of-life' invest more in immunocompetence and antioxidant system to ensure a longer lifespan (Ricklefs, 1992; Lee *et al*., 2008). We argue that this might constrain gland activity for three reasons. First, gland activity and immune response are conflicting commodities (Piault *et al*., 2008). Second, components of the constitutive immune system responsible for antimicrobial protection (e.g. lysozyme) might be partially complementary with the defence provided by the gland oils (Giraudeau *et al*., 2010b; Soler *et al*., 2011). Giraudeau *et al*. (2010b) experimentally demonstrated increased lysozyme concentrations of female birds with no access to their preen glands compared to control birds. Also, a comparative study by Soler *et al*. (2011) showed a negative relationship between innate immunity (natural antibodies and complement) and eggshell bacterial load. Third, gland activity and other survival-enhancing functions (e.g. immune and antioxidant system) are genetically linked by pleiotropic genes (Ducrest, Keller & Roulin, 2008). In conclusion, these studies are consistent with our results and suggest that slow-living species with long development and long lifespan prioritize physiological defence systems over an effective defence through gland oils to increase survival expectancy. However, this hypothesis deserves future investigations.

In conclusion, the present study provides (strong) support for the important role played by uropygial gland secretions as a defence mechanism against feather and eggshell microorganisms in birds. We show that the amount of secretions produced dynamically varies along the year across species and the change is a complex response to ecological and life-history circumstances.

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APPENDICES

Table A1. Summary information on overall uropygial gland (UG) sizes (mm^3) and values for the nonbreeding and breeding season of males and females

Species	UG overall	Noverall	UG nonreproductive	*N* nonreproductive	UG reproductive	*N* reproductive	UG female reproductive	*N* female reproductive	UG female nonreproductive	*N* female nonreproductive	UG male reproductive	*N* male reproductive	UG male nonreproductive	*N* male nonreproductive
Accipiter gentilis	1468.2	1	1468.2	1					1468.2	1				
Accipiter nisus	244.7	2	244.7	2					203.0	1				
Acrocephalus arundinaceus	179.4	51	169.1	17	184.5	34	191.9	9			147.9	8		
Acrocephalus palustris	95.9	60	95.9	17	96.0	43	99.6	4	114.3	1	96.6	6		
Acrocephalus schoenobaenus	81.3	69	81.4	30	81.3	39	108.4	2			52.9	1		
Acrocephalus scirpaceus	65.9	52	79.0	14	61.1	38	80.5	2			62.0	11		
Actitis hypoleucos	206.3	5	225.3	2	193.6	3								
Aegithalos caudatus	67.3	33	46.6	10	76.4	23	71.8	7			60.1	3		
Alauda arvensis	517.4	1			517.4	1					517.4	1		
Alcedo atthis	244.0	19	179.9	11	332.1	8	380.3	3			379.2	2		
Anas platyrhynchos	12055.1	4	15883.0	3	571.6	1	571.6	1						
Anthus campestris	190.1	3			190.1	3	258.8	1						
Anthus spinoletta	283.4	2			283.4	2					283.4	2		
Anthus trivialis	154.8	18	131.4	6	166.5	12	273.8	3			139.9	6		
Aquila pomarina	1992.0	4			1992.0	4					2313.0	2		
Asio flammeus	655.9	1	655.9	1										
Asio otus	917.8	9			917.8	9	875.5	4						
Athene noctua	307.6	1			307.6	1								
Bombycilla garrulus	255.1	12			255.1	12	264.5	5			226.3	5		
Botaurus stellaris	1797.6	2			1797.6	2								
Bubo bubo	3062.5	1	3062.5	1										
Buteo buteo	726.0	5	714.4	3	743.3	2					1111.3	1		
Carduelis cannabina	133.8	2			133.8	2	137.9	1			129.6	1		
Carduelis carduelis	99.0	24	82.1	7	106.0	17	94.3	4	78.8	3	111.1	11	80.2	3
Carpodacus erythrinus	207.5	5			207.5	5	191.4	2			218.2	3		
Cecropis daurica	75.5	7			75.5	7	82.3	2			72.7	5		
Cinclus cinclus	715.3	16	631.9	6	765.3	10	753.1	5			775.5	3		
Circus cyaneus	565.2	1	565.2	1					565.2	1				
Coccothraustes coccothraustes	243.8	30	215.5	18	286.3	12	388.2	2	205.9	2	293.1	8	225.3	4
Columba livia	321.4	9			321.4	9	492.1	2			277.6	2		
Coracias garrulus	454.4	4			454.4	4	493.7	1			436.2	2		
Corvus cornix	1849.4	1			1849.4	1								
Corvus frugilegus	1705.1	2	1705.1	2										
Crex crex	902.9	8			902.9	8					993.0	6		
Cyanistes caeruleus	65.9	52	57.7	36	84.3	16	95.2	4	51.6	5	78.1	8	51.4	6
Delichon urbicum	57.0	22			57.0	22	61.6	7			57.5	14		
Dendrocopos major	1002.6	20	947.1	5	1021.1	15	1060.5	6			968.7	7		
Dendrocopos medius	420.9	1	420.9	1										
Dryocopus martius	3888.9	2			3888.9	2					3888.9	2		
Emberiza calandra	431.7	3			431.7	3					467.9	2		
Emberiza cia	140.2	1			140.2	1					140.2	1		
Emberiza citrinella	200.9	68	178.1	44	242.7	24	253.7	6	188.2	10	232.6	16	175.7	15

Table A1. *Continued*

Species	UG overall	Noverall	UG nonreproductive	*N* nonreproductive	UG reproductive	*N* reproductive	UG female reproductive	*N* female reproductive	UG female nonreproductive	*N* female nonreproductive	UG male reproductive	*N* male reproductive	UG male nonreproductive	*N* male nonreproductive
Emberiza hortulana	172.2	5			172.2	5					172.2	5		
Emberiza schoeniclus	190.0	15	180.1	5	195.0	10	274.2	1			176.5	5		
Erithacus rubecula	85.8	56	71.6	18	92.5	38	115.7	6			91.7	15		
Falco subbuteo	687.1	1	687.1	1										
Falco tinnunculus	1323.0	20	848.5	1	1347.9	19	1685.0	11			878.4	4		
Falco vespertinus	681.4	13			681.4	13	928.1	5			527.3	8		
Ficedula albicollis	56.9	5	56.9	5									86.4	1
Ficedula hypoleuca	57.7	10	53.1	4	60.8	6	56.2	4			70.0	2		
Ficedula parva	39.1	8	36.0	7	61.5	1	61.5	1						
Fringilla coelebs	138.6	53	128.9	21	145.0	32	173.8	13	133.7	2	121.2	15	123.1	13
Fringilla montifringilla	107.4	8	107.4	8					117.8	2			102.0	3
Galerida cristata	202.3	1			202.3	1								
Gallinula chloropus	1049.4	1			1049.5	1								
Garrulus glandarius	674.8	15	527.7	7	803.6	8	1246.9	3			835.2	1		
Hippolais icterina	64.3	9	66.5	8	47.0	1								
Hirundo rustica	110.2	158	48.5	22	120.2	136	125.2	69	33.5	1	115.8	66	56.6	5
Ixobrychus minutus	219.3	5			206.8	4	213.7	3			186.1	1		
Jynx torquilla	269.5	18	181.6	6	313.5	12	423.9	1			329.8	1		
Lanius collurio	185.3	62	176.9	38	198.6	24	230.4	7	80.6	1	185.5	17	165.1	4
Lanius excubitor	453.0	3	263.2	1	547.9	2	575.3	1			520.6	1		
Lanius minor	444.1	3			444.1	3	478.5	1			426.8	2		
Larus hyperboreus	4881.7	13			4881.7	13	4513.4	8			5470.9	5		
Larus ridibundus	877.8	1	877.8	1										
Locustella fluviatilis	111.1	7	111.1	7										
Locustella lusciniodes	174.1	30	107.4	5	187.4	25	207.6	4						
Luscinia luscinia	113.7	44	113.9	42	109.9	2	126.8	1						
Luscinia megarhynchos	156.8	20			156.8	20	174.7	4			155.9	12		
Merops apiaster	146.2	24			146.2	24	138.5	11			170.5	8		
Motacilla alba	129.1	16	131.0	5	128.3	11	133.4	6			120.4	4		
Motacilla cinerea	112.5	17	91.9	1	113.8	16	122.5	7			107.0	9		
Motacilla flava	118.1	4	76.8	2	159.4	2					157.0	1		
Muscicapa striata	54.7	27	57.5	21	45.1	6								
Oenanthe oenanthe	72.2	3			72.2	3					72.2	3		
Oenanthe pleschanka	38.5	5			38.6	5	38.4	1			38.6	4		
Oriolus oriolus	209.9	13	204.6	9	221.8	4			272.9	1	218.6	3	197.2	1
Otus scops	251.8	11			251.8	11	367.1	3			213.4	7		
Panurus biarmicus	142.0	18			142.0	18	132.5	5			145.6	13		
Parus major	100.1	206	88.5	142	125.9	64	128.8	22	88.1	24	124.5	42	88.0	47
Passer domesticus	254.3	104	235.0	57	277.8	47	306.6	22	229.8	30	252.5	25	240.8	27
Passer hispaniolensis	260.7	17			260.7	17					260.7	17		
Passer montanus	220.9	99	161.5	51	284.0	48	334.3	19	222.4	4	250.0	28	158.8	1
Pastor roseus	446.3	11			446.3	11	485.0	4			424.2	7		
Perdix perdix	3451.5	1	3451.5	1					3451.5	1				
Periparus ater	44.9	21	36.9	3	46.2	18	63.8	3			53.2	5	36.9	2
Phasianus colchicus	2923.7	42	2646.9	38	5552.5	4	3765.9	1			6148.0	3	2745.6	27
Philomachus pugnax	2160.0	1			2160.0	1								
Phoenicurus ochruros	58.6	8	40.7	1	61.2	7	59.9	4			63.0	3		

Table A1. *Continued*

Species	UG overall	Noverall	UG nonreproductive	*N* nonreproductive	UG reproductive	*N* reproductive	UG female reproductive	*N* female reproductive	UG female nonreproductive	*N* female nonreproductive	UG male reproductive	*N* male reproductive	UG male nonreproductive	*N* male nonreproductive
Phylloscopus collybita	48.1	67	53.3	27	44.7	40	36.7	2			39.1	11		
Phylloscopus sibilatrix	37.2	11	32.7	2	38.2	9					41.8	2		
Phylloscopus trochilus	38.0	45	37.6	43	45.7	2								
Pica pica	865.8	6	757.8	3	973.9	3	973.9	3						
Picoides minor	267.5	2			267.5	2	270.6	1			264.3	1		
Picus canus	1326.5	3	1057.1	1	1461.3	2	1340.3	1			1582.2	1	1057.1	1
Picus viridis	1853.7	5	2036.4	1	1808.0	4	1981.0	2			2472.5	1		
Poecile palustris	71.0	22	47.1	6	80.0	16	84.5	7			78.0	3		
Porzana parva	125.5	1			125.5	1	125.5	1						
Porzana porzana	377.2	1			377.2	1					377.2	1		
Prunella modularis	142.5	20	130.9	9	152.1	11	163.5	2			156.8	6		
Rallus aquaticus	447.5	13			447.5	13	259.2	3			490.0	9		
Regulus ignicapillus	41.4	2			41.4	2	41.4	2						
Regulus regulus	32.6	4	27.6	3	47.8	1					47.8	1		
Remiz pendulinus	52.2	9	38.5	1	53.9	8	69.8	2			48.6	6		
Riparia riparia	67.7	31	38.9	6	74.6	25	74.4	11			74.8	14		
Rissa tridactyla	2436.7	70			2436.7	70	1969.6	10			2552.3	36		
Saxicola rubetra	72.9	17	59.7	8	84.6	9	72.0	3			98.6	5		
Saxicola rubicola	65.5	12	77.5	1	64.4	11	88.1	2	77.5	1	58.9	8		
Serinus serinus	73.8	6			73.8	6	77.5	3			70.1	3		
Sitta europaea	102.6	17	93.8	1	103.2	16	126.8	5			94.2	10		
Somateria mollissima	5668.7	1			5668.7	1	5668.7	1						
Sterna hirundo	835.0	5			835.1	5								
Streptopelia decaocto	143.3	5			143.3	5					147.5	2		
Strix aluco	1201.8	10	1235.6	5	1168.0	5	1502.9	2			944.7	3	1167.6	1
Strix uralensis	1454.7	1	1454.7	1										
Sturnus vulgaris	652.5	19			652.5	19	718.9	11			600.9	6		
Sylvia atricapilla	110.3	88	84.4	35	127.5	53	139.8	22	111.8	4	119.0	29		
Sylvia borin	108.7	43	99.8	32	134.8	11	150.0	8			70.0	1		
Sylvia communis	101.2	58	85.1	27	115.2	31	123.3	10	82.6	3	111.9	17		
Sylvia curruca	73.2	83	51.8	16	78.4	67	80.9	30			61.3	8		
Sylvia nisoria	137.7	5	91.1	2	168.7	3	130.7	1			234.0	1		
Tachymarptis melba	209.7	2			209.7	2								
Tringa nebularia	334.3	2	334.3	2										
Tringa ochropus	232.9	1	232.9	1										
Troglodytes troglodytes	79.9	13	84.0	7	75.2	6	69.0	1			108.2	1		
Turdus iliacus	163.2	1	163.2	1										
Turdus merula	486.2	94	379.3	19	513.3	75	568.1	25	460.2	1	484.8	47	411.7	1
Turdus philomelos	331.6	25	266.9	10	374.7	15	457.6	5	453.3	1	405.6	5		
Turdus pilaris	547.4	15			547.4	15	745.4	4			483.7	9		
Turdus torquatus	382.8	1			382.8	1	382.8	1						
Turdus viscivorus	381.5	1			381.5	1	381.5	1						
Upupa epops	877.6	4	597.2	2	1158.0	2								

N, sample size for each category. For sources, see Material and methods.

Table A2. Summary information on species specific body mass (g) and the values of males and females, incubation period (days), fledging period (days), egg weight (g), clutch size (number of eggs), total eggshell surface area (cm^2), incubation share (F, mainly or fully by the female; E, equal shares), nest type (O, open nest; H, hole breeding), habitat (T, terrestrial; R, riparian; A, aquatic), migration (R, resident; S, partial/short-distance migrant; L, long-distance migrant), and sociality (S, territorial/solitary; C, flocking/colonial breeder)

Species	Body mass	Female body mass	Male body mass	Incubation period	Fledging period	Egg weight	Clutch size	Total eggshell surface area	Incubation share	Nest type	Habitat	Migration	Sociality during breeding	Sociality outside the breeding season
Accipiter gentiles	934.8	1137.0	912.0	36.5	38.5	53.5	3.6	239.8	F	O	T	R	S	S
Accipiter nisus	237.5	246.0	70.0	34.0	27.0	23.0	5.0	190.6	F	O	T	S	S	S
Acrocephalus arundinaceus	30.0	30.0	30.0	14.0	13.0	3.2	4.7	48.9	F	O	R	L	S	S
Acrocephalus palustris	11.5	11.5	11.5	13.0	10.5	1.9	4.5	32.1	E	O	R	L	S	S
Acrocephalus schoenobaenus	11.2	11.2	11.2	14.0	13.5	1.7	5.0	33.4	F	O	R	L	S	S
Acrocephalus scirpaceus	12.3	12.3	12.3	10.5	11.0	1.8	3.9	27.4	E	O	R	L	S	S
Actitis hypoleucos	48.0	48.0	48.0	21.5	27.0	12.0	4.0	99.5	E	O	A	L	S	S
Aegithalos caudatus	8.6	8.6	8.6	14.7	16.0	0.9	10.1	45.6	F	O	T	R	S	C
Alauda arvensis	40.0	37.2	42.7	11.0	19.0	3.4	3.7	39.6	F	O	T	S	S	S
Alcedo atthis	35.8	35.8	35.8	20.0	25.0	4.2	6.7	83.9	E	H	A	S	S	S
Anas platyrhynchos	1141.0	1095.0	1246.0	27.5	55.0	51.0	10.1	658.5	F	O	A	S	S	C
Anthus campestris	23.0	23.0	23.0	13.0	13.5	2.7	4.2	39.6	F	O	T	S	S	S
Anthus spinoletta	23.9	23.9	23.9	14.5	14.5	2.7	5.0	46.5	F	O	T	S	S	S
Anthus trivialis	23.4	25.1	21.7	13.0	13.0	2.4	4.0	34.5	F	O	T	L	S	S
Aquila pomarina	1370.0	1540.0	1200.0	39.5	58.0	83.0	2.0	179.5	F	O	T	L	S	S
Asio flammeus	325.0	378.0	315.0	26.5	25.5	21.0	6.0	217.0	F	O	T	S	S	S
Asio otus	299.0	337.0	261.0	27.5	30.0	22.0	4.2	154.5	F	O	T	R	S	S
Athene noctua	164.0	164.0	164.0	27.5	32.5	15.1	3.9	113.3	F	H	T	R	S	S
Bombycilla garrulus	54.5	52.5	52.5	14.5	14.5	3.8	5.2	60.9	F	O	T	S	S	C
Botaurus stellaris	1324.5	1440.0	1209.0	25.5	52.5	40.0	5.0	275.9	F	O	A	S	S	S
Bubo bubo	2686.0	2992.0	2380.0	35.0	55.0	73.0	2.6	212.7	F	H	T	R	S	S
Buteo buteo	776.0	969.0	781.0	33.0	52.5	53.0	2.8	184.9	E	O	T	R	S	S
Carduelis cannabina	19.6	18.9	20.2	12.0	13.5	1.7	4.6	31.2	F	O	T	R	S	C
Carduelis carduelis	16.0	16.0	16.0	12.1	14.7	1.5	4.8	29.7	F	O	T	R	S	C
Carpodacus erythrinus	24.0	23.0	25.0	11.5	11.5	2.3	4.9	40.4	F	O	T	L	S	S
Cecropis daurica	22.2	22.2	22.2	14.5	24.0	2.0	4.3	33.1	F	H	T	L	C	C
Cinclus cinclus	61.7	55.4	64.2	16.0	22.0	4.6	4.6	61.6	F	H	A	R	S	S
Circus cyaneus	401.0	430.0	300.0	30.0	37.0	31.0	4.4	206.4	F	O	T	S	S	S
Coccothraustes coccothraustes	56.7	55.3	58.0	12.0	12.5	3.8	4.5	51.9	F	O	T	R	S	C
Columba livia	354.5	340.0	369.0	17.5	36.0	18.0	1.9	62.8	E	O	T	R	C	C
Coracias garrulus	146.0	146.0	146.0	18.0	26.5	12.2	3.8	95.6	F	H	T	L	S	S
Corvus cornix	570.0	570.0	570.0	18.5	32.2	18.9	4.3	145.5	F	O	T	R	S	S
Corvus frugilegus	453.5	418.0	489.0	17.0	33.0	16.0	4.1	122.7	F	O	T	R	C	C
Crex crex	155.5	142.0	169.0	17.5	36.0	13.0	8.9	232.8	F	O	R	L	S	S
Cyanistes caeruleus	10.6	10.6	10.6	14.2	19.0	1.1	11.0	58.0	F	H	T	R	S	C
Delichon urbicum	14.5	14.5	14.5	15.0	26.7	1.7	3.5	23.6	F	H	T	L	C	C
Dendrocopos major	76.8	72.7	76.0	11.5	22.0	5.0	5.5	76.7	E	H	T	R	S	S
Dendrocopos medius	59.0	59.0	59.0	12.5	22.5	4.0	5.6	68.0	E	H	T	R	S	S
Dryocopus martius	321.0	321.0	321.0	12.0	26.0	12.4	4.8	122.7	E	H	T	R	S	S
Emberiza calandra	48.8	43.9	53.6	13.0	11.0	3.8	4.3	49.4	F	O	T	S	S	C

Table A2. *Continued*

Species	Body mass	Female body mass	Male body mass	Incubation period	Fledging period	Egg weight	Clutch size	Total eggshell surface area	Incubation share	Nest type	Habitat	Migration	Sociality during breeding	Sociality outside the breeding season
Emberiza cia	23.5	22.7	24.2	13.0	11.5	2.7	3.9	36.2	F	O	T	R	S	S
Emberiza citrinella	29.7	29.7	29.7	13.0	12.0	2.9	3.9	38.5	F	O	T	R	S	C
Emberiza hortulana	19.9	19.9	19.9	11.5	12.5	2.5	4.6	40.0	F	O	T	L	S	S
Emberiza schoeniclus	18.5	17.2	19.7	13.0	11.0	2.3	4.9	41.3	F	O	R	S	S	S
Erithacus rubecula	17.7	17.7	17.7	13.7	13.4	2.4	5.0	42.9	F	O	T	S	S	S
Falco subbuteo	209.5	233.0	186.0	29.5	31.0	25.0	2.6	103.5	F	O	T	L	S	S
Falco tinnunculus	184.0	201.0	167.0	28.0	29.5	20.0	4.7	164.0	F	O	T	R	S	S
Falco vespertinus	152.5	170.0	135.0	22.5	28.5	17.0	3.5	108.9	E	O	T	L	C	C
Ficedula albicollis	12.7	12.5	12.9	13.0	16.5	1.6	5.8	38.1	F	H	T	L	S	S
Ficedula hypoleuca	13.9	15.6	12.2	14.0	15.5	1.7	5.8	40.0	F	H	T	L	S	S
Ficedula parva	9.9	9.9	9.9	12.5	12.5	1.5	5.7	35.2	F	H	T	L	S	S
Fringilla coelebs	24.0	27.0	28.3	12.6	13.9	2.5	4.5	38.9	F	O	T	S	S	C
Fringilla montifringilla	23.2	22.6	22.6	11.8	13.5	2.1	5.9	47.5	F	O	T	S	S	C
Galerida cristata	42.8	41.9	39.0	12.0	16.5	3.3	4.3	45.6	F	O	T	R	S	S
Gallinula chloropus	305.0	271.0	339.0	21.5	45.0	25.0	6.6	265.1	E	O	A	S	S	S
Garrulus glandarius	168.0	164.0	172.0	16.5	21.5	8.7	5.4	108.9	F	O	T	R	S	S
Hippolais icterina	13.2	13.2	13.2	13.5	13.5	1.7	4.8	33.4	F	O	R	L	S	S
Hirundo rustica	18.0	18.2	18.2	15.3	19.5	1.9	4.4	32.8	F	H	T	L	C	C
Ixobrychus minutus	118.0	118.0	118.0	18.0	27.5	12.0	5.5	137.0	E	O	A	L	S	S
Jynx torquilla	35.0	36.5	36.5	12.8	20.0	2.6	8.7	79.0	F	H	T	L	S	S
Lanius collurio	28.5	29.0	27.9	14.0	14.5	3.2	5.1	51.9	F	O	T	L	S	S
Lanius excubitor	63.4	63.4	63.4	16.0	16.5	5.2	5.5	78.3	F	O	T	R	S	S
Lanius minor	46.5	47.3	45.7	15.5	17.0	4.3	6.0	75.2	F	O	T	L	S	S
Larus hyperboreus	1412.5	1249.0	1576.0	27.5	47.5	108.0	3.0	317.3	E	O	A	S	C	C
Larus ridibundus	284.0	284.0	284.0	24.5	35.0	38.0	2.7	145.5	E	O	A	S	C	C
Locustella fluviatilis	16.1	16.1	16.1	11.5	15.0	2.4	4.9	41.7	F	O	R	L	S	S
Locustella luscinioides	13.9	13.8	13.8	11.0	13.0	1.9	4.6	33.4	F	O	R	L	S	S
Luscinia luscinia	23.8	23.8	23.8	13.3	9.6	3.2	4.8	49.9	F	O	T	L	S	S
Luscinia megarhynchos	18.3	18.3	18.3	13.0	11.0	2.7	4.8	44.3	F	O	T	L	S	S
Merops apiaster	56.6	56.6	56.6	20.0	22.5	6.9	6.0	103.5	E	H	T	L	C	C
Motacilla alba	21.0	21.0	21.0	12.6	13.7	2.3	5.4	45.6	F	H	T	S	S	S
Motacilla cinerea	17.6	17.2	18.0	12.5	13.5	2.0	5.2	39.6	E	H	R	S	S	S
Motacilla flava	17.6	17.6	17.6	12.4	16.0	1.9	5.5	40.4	F	O	R	L	S	C
Muscicapa striata	15.9	15.9	15.9	13.0	14.0	1.9	4.5	33.1	F	O	T	L	S	S
Oenanthe oenanthe	25.6	22.3	24.0	13.1	15.0	2.9	6.0	58.0	F	H	T	L	S	S
Oenanthe pleschanka	19.4	20.6	18.2	13.5	13.5	2.3	5.3	44.3	F	H	T	L	S	S
Oriolus oriolus	79.0	79.0	79.0	16.5	16.5	7.3	3.8	68.0	E	O	T	L	S	S
Otus scops	92.0	92.0	92.0	24.5	25.0	13.0	4.5	117.9	F	H	T	L	S	S
Panurus biarmicus	13.9	15.0	14.0	11.8	12.5	1.8	5.9	41.3	E	O	R	R	C	C
Parus major	18.3	17.6	18.9	13.5	16.2	1.7	9.6	64.7	F	H	T	R	S	C
Passer domesticus	27.7	27.4	28.0	12.0	14.0	2.7	4.5	42.1	F	H	T	R	C	C
Passer hispaniolensis	24.2	24.2	24.2	11.3	13.3	2.7	5.0	46.5	F	H	T	S	C	C
Passer montanus	20.8	20.8	20.8	12.5	17.5	2.1	4.8	38.5	F	H	T	R	S	C
Pastor roseus	73.3	66.9	79.6	15.0	24.0	6.6	5.5	92.8	E	H	T	L	C	C
Perdix perdix	405.5	393.0	418.0	24.0	15.0	14.5	15.0	424.1	F	O	T	R	S	C
Periparus ater	9.2	9.2	9.2	15.0	19.0	1.0	8.4	40.4	F	H	T	R	S	C
Phasianus colchicus	1135.0	953.0	1317.0	25.5	75.0	33.0	11.9	578.2	F	O	T	R	S	S

Table A2. *Continued*

Species	Body mass	Female body mass	Male body mass	Incubation period	Fledging period	Egg weight	Clutch size	Total eggshell surface area	Incubation share	Nest type	Habitat	Migration	Sociality during breeding	Sociality outside the breeding season
Philomachus pugnax	136.0	102.0	170.0	21.5	26.5	22.0	3.7	138.4	F	O	A	L	S	C
Phoenicurus ochruros	16.5	16.5	16.5	15.0	15.5	2.2	4.9	39.3	F	H	T	S	S	S
Phylloscopus collybita	8.3	8.3	8.3	14.0	15.0	1.2	5.1	27.7	F	O	T	S	S	S
Phylloscopus sibilatrix	9.2	9.2	9.2	13.0	12.0	1.5	5.8	36.2	F	O	T	L	S	S
Phylloscopus trochilus	8.7	8.7	8.7	13.0	13.0	1.2	6.1	33.1	F	O	T	L	S	S
Pica pica	206.0	191.0	221.0	21.5	27.0	9.8	6.0	130.3	F	O	T	R	S	S
Picoides minor	19.8	19.8	19.8	11.5	19.0	2.0	5.0	38.1	E	H	T	R	S	S
Picus canus	137.0	137.0	137.0	14.5	26.0	7.5	8.0	146.9	E	H	T	R	S	S
Picus viridis	176.0	176.0	176.0	18.0	25.0	8.5	6.1	121.5	E	H	T	R	S	S
Poecile palustris	10.9	10.9	10.9	14.9	18.5	1.2	8.2	45.2	F	H	T	R	S	C
Porzana parva	49.7	49.7	49.7	22.0	47.5	8.0	6.8	129.0	E	O	A	L	S	S
Porzana porzana	87.1	87.1	87.1	18.5	25.0	6.0	10.3	162.4	E	O	A	L	S	S
Prunella modularis	20.3	19.7	19.7	12.5	11.5	2.3	5.1	42.5	F	O	T	S	S	S
Rallus aquaticus	111.5	98.0	125.0	20.5	25.0	13.0	8.5	223.6	F	O	A	S	S	S
Regulus ignicapillus	5.6	5.6	5.6	15.5	23.0	0.7	8.8	33.4	F	O	T	R	S	C
Regulus regulus	5.6	5.5	5.6	16.0	19.0	0.8	9.8	40.4	F	O	T	R	S	C
Remiz pendulinus	9.3	9.3	9.3	14.0	22.2	1.0	4.6	22.6	E	O	R	S	S	C
Riparia riparia	12.7	13.9	13.0	14.5	22.3	1.4	4.8	28.8	E	H	R	L	C	C
Rissa tridactyla	416.3	394.0	421.0	27.3	42.7	48.0	2.1	127.7	E	O	A	S	C	C
Saxicola rubetra	16.6	16.6	16.6	12.5	12.5	2.1	5.8	44.7	F	O	T	L	S	S
Saxicola rubicola	15.2	15.3	15.3	13.5	13.5	1.9	5.1	37.7	F	O	T	S	S	S
Serinus serinus	11.2	11.2	11.2	12.6	15.2	1.2	3.8	20.9	F	O	T	S	S	C
Sitta europaea	22.6	22.6	22.6	15.5	23.5	2.0	7.4	56.8	F	H	T	R	S	S
Somateria mollissima	2066.5	1915.0	2218.0	26.5	70.0	110.0	3.8	407.5	F	O	A	S	C	C
Sterna hirundo	120.0	120.0	120.0	21.5	25.0	21.0	2.7	98.5	F	O	A	L	C	C
Streptopelia decaocto	149.0	146.0	152.0	16.0	17.0	9.6	1.9	41.7	E	O	T	R	S	S
Strix aluco	475.0	524.0	426.0	29.0	34.5	40.0	2.9	162.4	F	H	T	R	S	S
Strix uralensis	784.5	863.0	706.0	28.0	40.0	46.0	2.9	174.2	F	H	T	R	S	S
Sturnus vulgaris	86.0	84.4	87.6	12.2	21.0	8.7	4.9	98.5	F	H	T	S	S	C
Sylvia atricapilla	16.7	16.7	16.7	13.0	10.0	2.2	4.6	37.3	E	O	T	S	S	S
Sylvia borin	18.2	18.2	18.2	11.5	10.0	2.4	4.3	36.6	E	O	T	L	S	S
Sylvia communis	15.1	15.1	15.1	11.5	11.0	1.8	4.8	33.4	E	O	T	L	S	S
Sylvia curruca	11.1	11.1	11.1	12.0	11.5	1.4	4.9	29.4	F	O	T	L	S	S
Sylvia nisoria	22.5	22.5	22.5	12.5	11.0	2.6	4.7	42.9	F	O	T	L	S	S
Tachymarptis melba	104.0	104.0	104.0	20.0	50.0	6.2	2.5	40.4	E	H	T	L	C	C
Tringa nebularia	187.0	187.0	187.0	24.3	28.0	31.0	3.9	181.3	E	O	A	L	S	C
Tringa ochropus	71.4	71.4	71.4	21.5	28.0	16.0	4.0	120.3	F	O	A	L	S	S
Troglodytes troglodytes	9.6	9.3	9.3	16.0	17.3	1.6	6.1	39.6	F	H	T	S	S	S
Turdus iliacus	61.2	61.2	61.2	12.7	10.0	15.2	5.3	154.5	F	O	T	S	S	S
Turdus merula	113.0	113.0	113.0	12.6	13.6	7.4	4.0	73.0	F	O	T	S	S	S
Turdus philomelos	67.8	66.6	68.9	13.4	13.2	6.2	4.7	75.9	F	O	T	S	S	S
Turdus pilaris	106.0	106.0	106.0	11.5	13.5	6.0	5.2	82.3	F	O	T	S	S	C
Turdus torquatus	109.0	109.0	109.0	13.0	15.0	7.8	4.2	77.5	F	O	T	S	S	S
Turdus viscivorus	117.5	123.0	112.0	13.5	13.5	7.8	4.0	75.2	F	O	T	S	S	C
Upupa epops	61.4	61.4	61.4	15.5	27.5	4.5	7.0	90.9	F	H	T	L	S	S

For sources, see Material and methods.

Table A3. Full and minimal phylogenetic generalized least squares weighted models explaining relative overall uropygial gland size

	Full model			Minimal model		
	β (SE)	*t*	*P*	β (SE)	*t*	*P*
Intercept	2.09 (0.42)	4.93	< **0.0001**	2.66 (0.24)	11.13	< **0.0001**
Body mass	0.85 (0.07)	11.91	< **0.0001**	0.89 (0.05)	18.70	< **0.0001**
Incubation	–0.02 (0.02)	–1.51	0.1336	–0.03 (0.01)	–2.04	**0.0433**
Habitat: riparian*	0.23 (0.11)	2.11	**0.0373**	0.22 (0.11)**	1.99	**0.0486**
Aquatic	0.46 (0.18)	2.52	**0.0131**	0.48 (0.18)	2.64	**0.0093**
Migration: short†	–0.06 (0.08)	–0.70	0.4866	–0.06 (0.08)††	–0.74	0.4592
Long	–0.18 (0.09)	–2.10	**0.0382**	–0.19 (0.08)	–2.25	**0.0261**
Fledging	–0.01 (0.01)	–1.04	0.3027			
Total eggshell surface	0.20 (0.13)	1.53	0.1295			
Sociality	–0.01 (0.08)	–0.07	0.9498			
Nest type: open	–0.03 (0.09)	–0.31	0.7545			

The minimal models were obtained by eliminating nonsignificant predictors from the full models in a backward stepwise manner based on the largest *P*-value. Model intercepts implement the first level of each factor (i.e. terrestrial species in the case of habitat and resident species in the case of migratory behaviour). All other levels of the candidate factor are compared to the level implemented in the intercept. Significant *P*-values are shown in bold.

*No significant difference between groups riparian and aquatic: β (SE) = 0.19 (0.21), $t = 0.91$, $P = 0.3623$.

†No significant difference between groups short- and long-distance migrant: β (SE) = –0.12 (0.07), $t = -1.74$, $P = 0.0841$.

**No significant difference between groups riparian and aquatic: β (SE) = 0.26 (0.21), $t = 1.28$, $P = 0.2035$.

††No significant difference between groups short- and long-distance migrant: β (SE) = –0.13 (0.07), $t = -1.85$, $P = 0.0673$.

Table A4. Full and minimal phylogenetic generalized least squares weighted models explaining relative breeding season uropygial gland size

	Full model			Minimal model		
	β (SE)	*t*	*P*	β (SE)	*t*	*P*
Intercept	1.66 (0.49)	3.40	**0.0009**	1.49 (0.48)	3.12	**0.0023**
Body mass	0.77 (0.09)	8.65	< **0.0001**	0.68 (0.08)	8.77	< **0.0001**
Total eggshell surface	0.37 (0.17)	2.23	**0.0276**	0.36 (0.15)	2.31	**0.0228**
Incubation	–0.03 (0.02)	–1.42	0.1592			
Habitat: riparian*	0.21 (0.12)	1.83	0.0697			
Aquatic	0.36 (0.21)	1.70	0.0921			
Migration: short†	–0.14 (0.09)	–1.62	0.1087			
Long	–0.18 (0.10)	–1.82	0.0721			
Fledging	–0.01 (0.01)	–0.63	0.5322			
Sociality: social	0.09 (0.11)	0.79	0.4327			
Nest type: open	0.04 (0.10)	0.38	0.7065			

The minimal models were obtained by eliminating nonsignificant predictors from the full models in a backward stepwise manner based on the largest *P*-value. Model intercepts implement the first level of each factor (i.e. terrestrial species in the case of habitat and resident species in the case of migratory behaviour). All other levels of the candidate factor are compared to the level implemented in the intercept. Significant *P*-values are shown in bold.

*No significant difference between groups riparian and aquatic: β (SE) = 0.15 (0.23), $t = 0.62$, $P = 0.5361$.

†No significant difference between groups short- and long-distance migrant: β (SE) = –0.03 (0.08), $t = -0.42$, $P = 0.6739$.

Table A5. Full and minimal phylogenetic generalized least squares weighted models explaining relative nonbreeding season uropygial gland size

	Full model			Minimal model		
	β (SE)	*t*	*P*	β (SE)	*t*	*P*
Intercept	2.89 (0.47)	6.19	< **0.0001**	3.01 (0.25)	12.15	< **0.0001**
Body mass	0.87 (0.07)	12.00	< **0.0001**	0.85 (0.05)	16.37	< **0.0001**
Incubation	–0.04 (0.02)	–2.33	**0.0228**	–0.05 (0.01)	–3.16	**0.0023**
Habitat: riparian*	0.29 (0.13)	2.20	**0.0307**	0.26 (0.13)**	2.02	**0.0473**
Aquatic	0.64 (0.21)	3.07	**0.0030**	0.67 (0.20)	3.28	**0.0016**
Migration: short†	–0.17 (0.11)	–1.58	0.1195	–0.17 (0.10)††	–1.69	0.0947
Long	–0.36 (0.11)	–3.25	**0.0018**	–0.37 (0.10)	–3.57	**0.0006**
Sociality: social	–0.18 (0.11)	–1.72	0.0889	0.22 (0.10)	2.24	**0.0280**
Nest type: open	0.00 (0.11)	0.01	0.9893			
Fledging	–0.01 (0.01)	–0.96	0.3380			
Total eggshell surface	0.03 (0.15)	0.21	0.8340			

The minimal models were obtained by eliminating nonsignificant predictors from the full models in a backward stepwise manner based on the largest *P*-value. Model intercepts implement the first level of each factor (i.e. terrestrial species in the case of habitat and resident species in the case of migratory behaviour). All other levels of the candidate factor are compared to the level implemented in the intercept. Significant *P*-values are shown in bold.
*No significant difference between groups riparian and aquatic: β (SE) = 0.35 (0.25), $t = 1.40$, $P = 0.1650$.
†Significant difference between groups short- and long-distance migrant: β (SE) = –0.19 (0.08), $t = -2.47$, $P = 0.0160$.
**No significant difference between groups riparian and aquatic: β (SE) = 0.41 (0.24), $t = 1.70$, $P = 0.0924$.
††Significant difference between groups short- and long-distance migrant: β (SE) = –0.20 (0.08), $t = -2.62$, $P = 0.0105$.

Table A6. Full and minimal phylogenetic generalized least squares weighted models explaining seasonal change in uropygial gland size (difference between the values during the reproductive season minus the value obtained during the nonreproductive season)

	Full model			Minimal model		
	β (SE)	*t*	*P*	β (SE)	*t*	*P*
Intercept	–1.08 (0.55)	–1.95	0.0560	–0.82 (0.43)	–1.91	0.0612
Body mass	–0.04 (0.07)	–0.58	0.5626	–0.07 (0.07)	–0.90	0.3693
Total eggshell surface	0.34 (0.17)	2.00	0.0512	0.29 (0.14)	2.07	**0.0428**
Sociality: social	0.36 (0.10)	3.57	**0.0008**	0.30 (0.08)	3.79	**0.0004**
Incubation	–0.01 (0.02)	–0.86	0.3930			
Fledging	0.01 (0.01)	0.74	0.4599			
Habitat: riparian*	0.02 (0.10)	0.16	0.8739			
Aquatic	0.07 (0.18)	0.39	0.6965			
Migration: short†	0.11 (0.11)	1.04	0.3035			
Long	0.20 (0.11)	1.82	0.0748			
Nest type: open	–0.07 (0.08)	–0.88	0.3826			

The minimal models were obtained by eliminating nonsignificant predictors from the full models in a backward stepwise manner based on the largest *P*-value. Model intercepts implement the first level of each factor (i.e. terrestrial species in the case of habitat and resident species in the case of migratory behaviour). All other levels of the candidate factor are compared to the level implemented in the intercept. Significant *P*-values are shown in bold.
*No significant difference between groups riparian and aquatic: β (SE) = –0.05 (0.21), $t = -0.27$, $P = 0.7911$.
†No significant difference between groups short- and long-distance migrant: β (SE) = –0.08 (0.07), $t = -1.14$, $P = 0.2607$.