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Feather mites and birds: an interaction mediated by uropygial gland size?

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Abstract

Feather mites (Arachnida: Acari: Astigmata) feed mainly on secretions of the uropygial gland of birds. Here, we use analyses corrected for phylogeny and body size to show that there is a positive correlation between the size of this gland and mite abundance in passerine birds at an interspecific level during the breeding season, suggesting that the gland mediates interactions between mites and birds. As predicted on the basis of hypothesized waterproofing and antibiotic functions of uropygial gland secretions, riparian/marsh bird species had larger glands and higher mite loads than birds living in less mesic terrestrial environments. An unexpected pattern was a steeper relationship between mite load and gland size in migratory birds than in residents. If moderate mite loads are beneficial to a host but high loads detrimental, this could create complex selection regimes in which gland size influences mite load and vice versa. Mites may exert selective pressures on gland size of their hosts that has resulted in smaller glands among migratory bird species, suggesting that smaller glands may have evolved in these birds to attenuate a possible detrimental effect of feather mites when present in large numbers.

Introduction

Feather mites (Astigmata: Analgoidea, Freyanoidea, Pterolichoidea) are among the most important ectosymbionts living on birds (Proctor & Owens, 2000). Despite their abundance and prevalence in almost all groups of birds (Proctor, 2003), evolutionary and ecological relationships between feather mites and birds are poorly known. For example, the basic biology of most plumicolous feather mites is not understood and, although some authors report detrimental effects for their hosts suggesting a parasitic nature of these mites, others suggest neutral or even mutualistic relationships between mites and birds (Galván & Sanz, 2006 and references therein). Results from an increasing number of studies are consistent with the idea that many plumicolous feather mites provide benefits to their hosts (Brown *et al.*, 2006; Galván & Sanz, 2006).

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The mechanisms by which feather mites could benefit their bird hosts are unknown, and only one process has been proposed to explain the possible mutualism. Most vane-dwelling feather mites feed on the lipidic secretions produced by the host's uropygial gland that are smeared onto the plumage during preening (Proctor, 2003). Enzymatic alterations of waxes that may change their properties can occur over time (Jacob, 1976) and the high stability of these substances can allow them to remain on feathers for several decades (Sweeney *et al.*, 2004). It seems likely that mites benefit birds by removing old waxes and associated micro-organisms that accumulate on feathers (Blanco *et al.*, 1997, 2001; Jovani & Blanco, 2000; Reneerkens, 2007). Indeed, it has been reported that an excess of waxes makes the plumage lose its capacity for heat retention (Sandilands *et al.*, 2004). Thus, feather mites could benefit from the food source

provided by uropygial gland secretions and birds benefit from mite feeding that helps to maintain plumage in proper condition. In support of this idea, a recent study showed that the abundance of feather mites increased with the size of the uropygial gland in great tits *Parus major*, and that birds bearing more mites exhibited more intensely coloured plumage (Galván & Sanz, 2006).

Thus, the uropygial gland seems to play an important role in the interactions between feather mites and birds and hence may mediate coevolutionary processes between these organisms (Proctor & Owens, 2000). Furthermore, it has been suggested that the selective pressures exerted by feather mites on their hosts are partly responsible for the high variability in the chemical composition of uropygial gland waxes among bird species (Haribal *et al.*, 2005).

The first aim of the present study was to test for a relationship between the abundance of vane-dwelling feather mites and the size of the uropygial gland of their hosts. This relationship has previously been found within a single species of host (Galván & Sanz, 2006), but in order to demonstrate that this holocrine gland could have had a role in the evolutionary interactions between feather mites and their bird hosts, it is necessary to find a general trend among bird species. Thus, our goal was to move from the individual to the specific level to test the prediction that those bird species with relatively larger uropygial glands would harbour more mites in their feathers. This prediction is based on the assumption that larger uropygial glands secrete larger amounts of waxes (Elder, 1954). This seems likely for several reasons: (1) the secretary capsules (which produce the lipidic substances) occupy the greatest proportion of the gland (Sandilands *et al.*, 2004); (2) the size of the gland is positively correlated with the diameter of its artery (Özcan *et al.*, 2004); and (3) uropygial gland weight is positively correlated with bird body weight within species (Kennedy, 1971; Johnston, 1979), which in turn is correlated with the weight of gland secretions (Oka & Okuyama, 2000).

Secondly, we tested for a link between host habitat and feather mite abundance. It has long been observed that birds living in mesic environments seem to have both greater densities of feather mites and larger uropygial glands than birds living in drier habitats (Dubinin, 1951; see also Johnston, 1988 for older references). This is possibly because of aquatic species having greater need to waterproof their plumage, which is one of the several functional aspects proposed for uropygial gland secretions (although the waterproofing function is not supported by all authors; Reneerkens, 2007). In addition, bacteria and fungi are better able to colonize feathers in humid environments (Burtt & Ichida, 2004), so birds may need to produce more antibiotic uropygial secretions to protect their feathers (Shawkey *et al.*, 2003) in these habitats. As feather mites consume fungal spores and bacteria trapped in uropygial oil (Proctor, 2003), mite abundance could increase because of this additional source of nutrition.

Various authors have provided observational evidence for the link between habitat type and uropygial gland size by measuring the weight of dissected glands from many species of birds (Kennedy, 1971; Jacob & Ziswiler, 1982; Johnston, 1988; Montalti *et al.*, 2005; but see Montalti & Salibián, 2000). However, enzymatic alterations can occur on the secretions of the uropygial gland if not collected immediately after gland excision (Jacob, 1976), which may affect the gland weight. Furthermore, the secretory activity of the gland is affected by levels of certain hormones (e.g. Ghosh & Bhattacharyya, 1996), and so gland size might vary seasonally. As the authors cited above do not provide information about the dates at which uropygial glands were investigated, it is not known whether their data on species living in different habitats are comparable. Indeed, seasonal variation found by Bhattacharyya & Chowdhury (1995) in the weight of the gland in the red-vented bulbul *Pycnonotus cafer* indicate the need to control for seasonality in analyses concerning the size or weight of this organ. Another caveat is that the above studies did not control for the effect of common ancestry among bird species, which is necessary to make interspecific inferences about correlated characters (Montalti *et al.*, 2005; and see Garland *et al.*, 2005 for a recent review). Therefore, we controlled for season and phylogeny when testing whether birds living in riparian or marshy environments present higher abundances of feather mites than terrestrial birds, and whether there is an interaction effect of uropygial gland size. We predicted that mite load would be higher during the breeding season than during the nonbreeding period because certain hormones produced during that period (e.g. androgenic steroids, glucocorticoids and secretions from the thyroid gland) seem to stimulate the growth of the uropygial gland and the production of glandular lipids (Blanco & Frías, 2001 and references therein).

Finally, we explored the relationship between migratory behaviour and feather mite load. As migratory bird species move to areas with lower densities of conspecifics than in resident species' populations (Møller & Erritzøe, 1998) and feather mites need physical contact between birds to colonize new hosts (Proctor, 2003), migrant birds may present lower numbers of mites than residents.

Material and methods

Field data

We captured birds from January to October 2006 at five different localities in Eastern Spain. In this way, we considered an entire annual cycle: wintering period, breeding season and, in the case of migrants, spring and most of the autumn migration periods. Localities were selected to include different habitats, two of them

Table 1 Location and dominant habitat type of the study areas.

Locality	Coordinates	Habitat type
Sagunto, Valencia	39°42′N, 0°15′W	Extensive plantations of orange trees
Gaibiel, Castellón	39°55′N, 0°30′W	Traditional olive *Olea europaea* and almond *Prunus dulcis* tree plantations
Cocentaina, Alicante	38°45′N, 0°25′W	Mixed riverine forest with marsh vegetation (cattail *Typha* sp., reedbeds *Phragmites australis*, giant reed *Arundo donax*, poplar *Populus alba*
Pego, Alicante (Pego-Oliva Natural Park)	38°51′N, 0°03′W	Reedbeds *Phragmites australis*, with isolated groups of rush *Juncus* sp. together with small open water areas and water channels
Alcoi, Alicante (La Font Roja Natural Park)	38°41′N, 0°31′W	Pine trees *Pinus halepensis*, evergreen holm oaks *Quercus ilex* and nearby olive and almond trees

man-made (orange, and olive and almond plantations) and three natural (evergreen holm oak forest, marshland and riverine forest; Table 1), with the aim of maximizing the number of bird species captured. At all sites, birds were trapped with mist nets that were operated weekly within a Constant Effort Site (CES) ringing program. Total length of the nets varied among sites, but all had 14 mm mesh size. In all cases, nets were placed just before sunrise and were operated for 4 h (e.g. Belda *et al.*, 2007).

Each bird captured was ringed with an individually numbered metal ring and its tarsus length measured with digital callipers to the nearest 0.1 mm. Feather mites were counted by exposing the extended wings of the birds to daylight (e.g. Jovani & Serrano, 2001). Only the right wing was examined because the number of feather mites on both wings of the same bird are highly correlated (Jovani & Serrano, 2004). The total number of feather mites was determined on flight feathers only, where mite abundance tends to be high (Jovani & Blanco, 2000).

In the winter–spring of 2007, we collected 1–2 wing feathers from one or two individuals of most species of birds involved in this study in order to determine what species of mites were present. We did not collect feathers from four of the species (Appendix S1). Feather samples were sent to HP's laboratory at the University of Alberta for identification of mites. Feathers were first soaked in 70% EtOH for several days to rehydrate the mites. Feathers and ethanol were examined at 20–40× magnification using a dissecting microscope and all mites were removed. We did not find mites on the sample feathers of three of the bird species (Appendix S1). Several exemplars of each mite morphotaxon from each sample were cleared overnight in lactic acid and were then mounted in PVA mounting medium (BioQuip Products; Rancho Dominguez, CA, USA) on glass slides. The slides were cured at 45 °C on a warming tray for 4 days. Slide-mounted mites were examined at 400× using differential interference contrast (DIC) lighting on a Leica DMLB compound microscope (Leica Microsystems Inc., Richmond Hill, ON, Canada). We identified mites to genus and species when possible using a range of taxonomic literature, the most important being Atyeo & Braasch (1966), Santana (1976) and Gaud & Atyeo (1996). Exemplars of each feather mite species have been deposited in the E.H. Strickland Entomological Museum, Department of Biological Sciences, University of Alberta, Canada.

The uropygial gland was measured following the method of Galván & Sanz (2006), taking three linear measurements with a digital calliper (maximum length, width and 'height') and then multiplying them to obtain an estimate of the volume of the gland (mm^3). Although this is a rough approximation to real size, this measure should provide an estimate of the secretory capacity of the gland (see Introduction) adequate for comparative purposes.

Measurements were taken by seven of the authors (IG, EB, RP, JLC, VC, JSM and FA). In order to estimate repeatabilities, we used information on 24 and 43 individual birds in which the number of feather mites and the size of the uropygial gland size, respectively, was measured by two different observers (seven observers involved). The repeatability of measurements was high for both variables (number of feather mites: $r = 0.94$, $F_{23,24} = 30.25$, $P < 0.0001$; uropygial gland size: $r = 0.93$, $F_{42,43} = 27.30$, $P < 0.0001$). Additionally, we used information on 45 birds for which mite abundance and uropygial gland size had been determined twice by the same observer (six observers involved) to calculate intra-observer repeatability, that was also high (number of feather mites: $r = 0.94$, $F_{44,45} = 33.71$, $P < 0.0001$; uropygial gland size: $r = 0.97$, $F_{44,45} = 71.51$, $P < 0.0001$). Although, for a given bird, measurements of mite abundance and uropygial gland size were taken by the same observer, we consider that the possibility of unconscious bias in estimates is unlikely because gland volume was calculated from three linear measurements (see above) and hence it would be difficult to bias towards an unconsciously 'desired' volume on the basis of these individual values.

The migratory behaviour of a bird species can change along its distribution range, so we considered the behaviour exhibited at each of the study areas. We grouped the species either as resident (those present all year round at the study area) or migrant (leaving the area for breeding or wintering or only passing through on migration).

We considered as breeding season in each of the study areas as the period of the year during which a species exhibited signs of reproductive activity (e.g. presence of brood patches or cloacal protuberances and captures of fledglings; roughly from March to August), and as nonbreeding season the rest of the year (roughly from September to February).

We considered data collected during the period of spring migration as belonging to the breeding season because during this period there is an increase in certain hormones that predispose birds to migrate and initiate breeding activity. On the contrary, data collected during the autumn migration period were considered to belong to the nonbreeding season because an increase in hormonal levels and breeding activity does not occur at this time (e.g. Campbell *et al.*, 1981; Blanco & Frías, 2001). From all the birds captured, we included in analyses only those species for which we had measured three or more individuals in at least one of the seasons (i.e. breeding or nonbreeding), and we used mean values per species (see Statistical analyses). In total, 621 passerine birds belonging to 38 species and 10 families are considered here (Fig. 1 and Appendix S1).

Finally, we categorized bird species into either 'riparian/marsh' or 'terrestrial' in order to consider effect of habitat in the analyses. Given that this categorization is a simplification of a continuous gradient in habitat use, we considered as riparian/marsh those species that are strongly associated with aquatic environments (rivers and marshes in our case), and as terrestrial the rest of the species. Those species that occur in mesic habitats only at some times of the year or in some locations (e.g. the willow warbler *Phylloscopus trochilus*) were categorized as terrestrial.

Statistical analyses

In all the statistical analyses, we used the mean values obtained per species. In order to explore the possible relationship between feather mite abundance and uropygial gland size, we performed general linear models (GLM) in which $\log_{10}$ number of feather mites + 1 was the dependent variable and uropygial gland size a covariate. Habitat (marsh/riparian vs. terrestrial) and migratory behaviour (migrant or resident) were introduced as fixed factors. Tarsus length was also introduced as a covariate in order to control for body size. The reason for controlling for body size is that a positive correlation between mite abundance and gland size could arise in

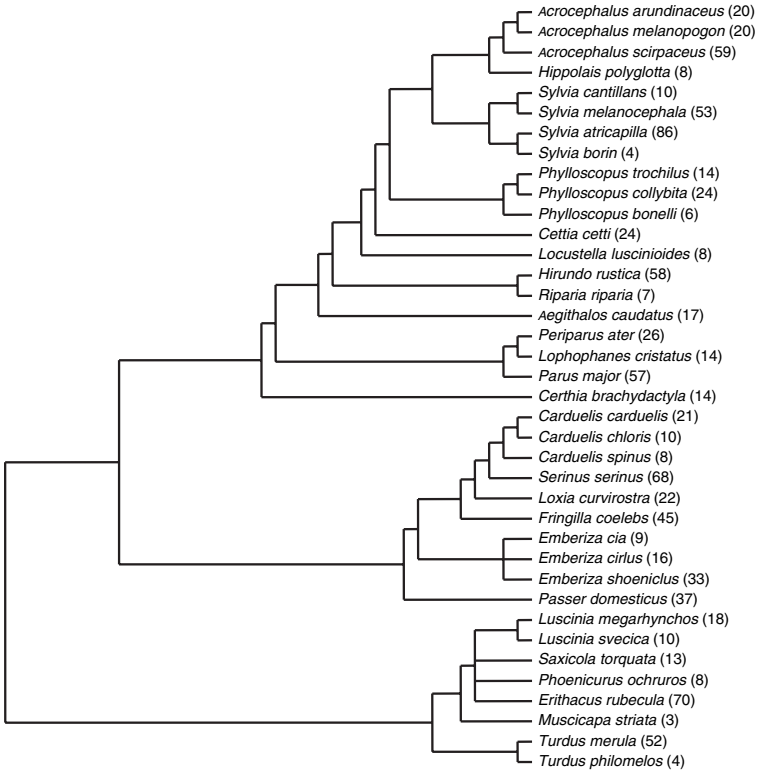

Fig. 1 Phylogenetic hypothesis for the passerine bird species treated in this study. Sample sizes (individual birds) are indicated in parentheses.

part because larger host species have greater surface area of flight feathers and thus can harbour more mites. We started with the saturated model with all third-order interactions (except interactions with body size) and nonsignificant terms were removed setting a probability of 0.1 to abandon the model. Nonsignificant terms were maintained if interactions including any of these terms were significant. Body size was always maintained in the models. We performed a model for data from the breeding season (29 species) and another for data corresponding to the nonbreeding season (26 species). The distribution of residuals generated from the models was explored to determine if normality assumptions were fulfilled.

In those bird species for which we had captured individuals from both breeding and nonbreeding seasons, we were interested in investigating possible seasonal effects on both feather mite abundance and uropygial gland size. As these variables were in fact correlated (see Results), we introduced the residuals obtained after regressing each of them against the values of tarsus length corresponding to each season as dependent variables in a repeated measures MANOVA in which season (breeding vs. nonbreeding) was the within-subjects factor for both variables. Habitat and migratory behaviour were also introduced as fixed factors, and thus we could explore the effect of the interaction between seasonal variation and each of these factors.

Comparative analyses

As characteristics of the different species of birds cannot be considered independent data because of the effect of common ancestry (e.g. Garland *et al.*, 2005), we tested our predictions taking into account phylogenetic relationships among hosts. We used the phylogeny of passerine birds of the Western Palaearctic compiled by Figuerola & Jovani (2001) that is based on different DNA phylogenies (Sibley & Ahlquist, 1990; Blondel *et al.*, 1996; Leisler *et al.*, 1997; Price *et al.*, 1997) with additional information from Gill *et al.* (2005) for Paridae. The phylogenetic relationships between the species used in the present study are shown in Fig. 1.

In order to control for these relationships, we performed the same models as previously described for the traditional statistical analyses but with data simulated on the basis of the phylogeny of bird species and following a particular model of evolution. For this purpose, we introduced the phylogenetic relationships and data on the continuous variables (feather mite abundance, uropygial gland size and tarsus length) in the module PDTREE of the software PDAP, and then used this information to generate 100 Monte Carlo computer simulations with PDSIMUL (Garland *et al.*, 1993). As the phylogeny of birds was inferred through different methodologies, branch lengths could not be estimated and were set equal to unity, and the simulations were made according to a speciational Brownian motion model of evolution (Garland *et al.*, 1993). We set the correlation between the traits to simulate as zero in order to obtain data of independently evolved traits and then compare them with real data.

Although variances of the simulated data are, on average, the same as those of the observed data, realistic limits can be exceeded during the course of simulations (Garland *et al.*, 1993). Thus, we simulated the data adding a lower bound to the evolution of uropygial gland size (see Lovegrove, 2001 for an example of bounded simulations). Because we have no information on uropygial gland size in ancestral species (such soft structures rarely fossilize), we set a lower bound of zero as morphological traits obviously cannot acquire negative values, and the same was applied to tarsus length. We also added a lower bound of zero to the number of feather mites because this variable was +1 log-transformed, but upper bounds were ignored because there is no information about the maximum number of feather mites that can be carried by birds. To keep data above the lower bound while running simulations (see for example Lovegrove, 2001), we used the 'replace' algorithm implemented in PDSIMUL. This algorithm checks at each step if the next added change will land the trait out of bounds, and if so, a different change is used, then checking and replacing if necessary the new step. We did not consider bounds in the simulations carried out to perform the phylogenetically correct MANOVA because this analysis was performed with residual values.

We employed a conventional statistical model (see previous section) for each of these simulations, thereby obtaining phylogenetically correct distributions of *F*-ratios. The significance of the conventional statistical tests performed without controlling for phylogeny was then tested against this distribution using the 95th percentile as the critical value for $\alpha = 0.05$. Exact *P*-values were calculated by dividing the number of *F*-ratios of simulated data exceeding the corresponding empirical *F*-ratio by the number of simulations performed. The different factors considered in the analyses are not confounded with phylogeny (Fig. 1 and Appendix S1), which increases the statistical power of our tests to detect differences (Garland *et al.*, 1993).

Results

In the field, we observed feather mites on the flight feathers of all species of birds with the exceptions of the rock bunting *Emberiza cia* and the coal tit *Periparus ater* (Appendix S1). For seven of the species that did have mites in the field, no mites were observed on the one or two feathers that were plucked and examined in the laboratory (Appendix S1); this is not a surprising result as feather mites are not evenly distributed on all flight feathers of the host (e.g. Jovani & Serrano, 2001), and the few mites present may have been knocked off in the

process of collecting. Members of the genus *Proctophyllodes* (Proctophyllodidae) were the most common mites on the examined feathers, occurring in samples from 23 (85%) of the 27 bird species whose mites were identified (Appendix S1). Some *Proctophyllodes* 'species' are likely to be arrays of several closely related species that are poorly morphologically differentiated (e.g. *P. pinnatus*) (Atyeo & Braasch, 1966), and are therefore recorded as members of a species group rather than a particular species. The next most common genus was *Trouessartia* (Trouessartiidae), found in the samples from four (15%) species of birds (*Hirundo rustica* and three *Acrocephalus* sp.). Other genera occurred only in a single sample from one host species: *Scutulanyssus* (Pteronyssidae) from *H. rustica*, *Mesalgoides* (Psoroptoididae) from *Loxia curvirostra*, *Monojoubertia* (Proctophyllodidae) from *Fringilla coelebs* and *Dolichodectes* (Proctophyllodidae) from *Acrocephalus arundinaceus*. Although most feather samples included only a single mite species, a second species sometimes occurred in low numbers. Most of the observed mite taxa have been previously reported from these hosts (Appendix S1). Mites that occur on more than one species of host tend to be on closely related bird species rather than species that happen to share the same habitat or migratory behaviour, consistent with other studies showing strong cophylogenetic patterns in feather mites and their hosts (e.g. Mironov & Dabert, 1999). Many of the slide-mounted mites were observed to have fungal spores in their guts.

The final model obtained for data collected during the breeding season accounted for 49% of variance in feather mite abundance (Table 2). As expected, the number of mites harboured by birds was positively correlated with uropygial gland size ($\beta = 1.37$, $P = 0.001$), and the effect was maintained when the significance was tested against the phylogenetically correct distribution of *F*-ratios. This indicates that mites were more abundant in species with larger glands.

Conventional statistical test showed that, as predicted, the abundance of feather mites was significantly higher on riparian/marsh birds than on terrestrial birds although the effect disappeared when phylogenetic relationships were taken into account (Table 2). The results obtained from conventional and phylogenetically corrected statistics were consistent, however, in the case of migratory behaviour, with the number of feather mites carried by migrant species being larger than that observed on residents (Fig. 2). The interaction term between migratory behaviour and uropygial gland size was also significant (Table 2) because of the more pronounced positive relationship between feather mite abundance and gland size in migrants ($\beta = 1.24$,

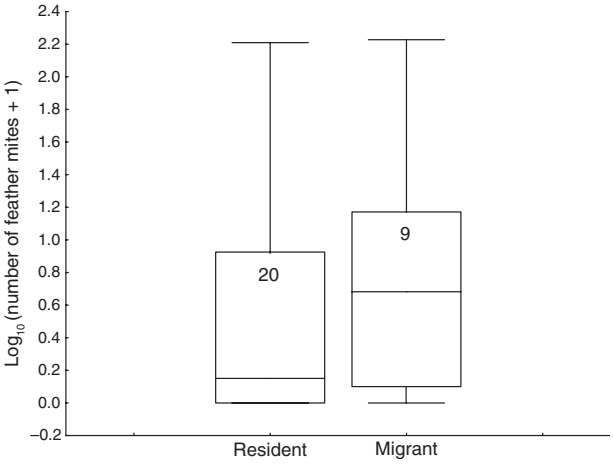

Fig. 2 Box-plot for results obtained from the model of Table 2, showing the difference in the number of feather mites harboured by migratory and resident bird species during the breeding season. Median, inter-quartile range and nonoutlier range are shown. Numbers are sample sizes (species).

Table 2 Results of the general linear model (GLM) exploring the effects of habitat, migratory behaviour and uropygial gland size on the number of feather mites harboured by passerine bird species during the breeding season.

				Conventional tables		Phylogenetically correct distributions	
Source of variation	d.f.	MS	*F*	Critical value	*P*	Critical value	*P*
Habitat	1	1.11	4.80	4.30	0.039	7.03	0.10
Migratory behaviour	1	1.28	5.54	4.30	0.028	3.64	< 0.01
Body size	1	0.81	3.51	4.30	0.074	7.46	0.18
Uropygial gland size	1	3.03	13.10	4.30	0.001	8.16	0.02
Habitat × migratory behaviour	1	1.91	8.27	4.30	0.009	5.02	0.01
Migratory behaviour × uropygial gland size	1	1.52	6.57	4.30	0.018	4.50	0.02
Error	22	0.23					

Only the factors that remained in the final model are shown. Critical values and corresponding *P*-values calculated from both conventional statistical tables and phylogenetically correct computer simulations (see text for details) are shown.
Model: adjusted $R^2 = 0.49$, $F_{6,22} = 5.47$, $P = 0.001$.

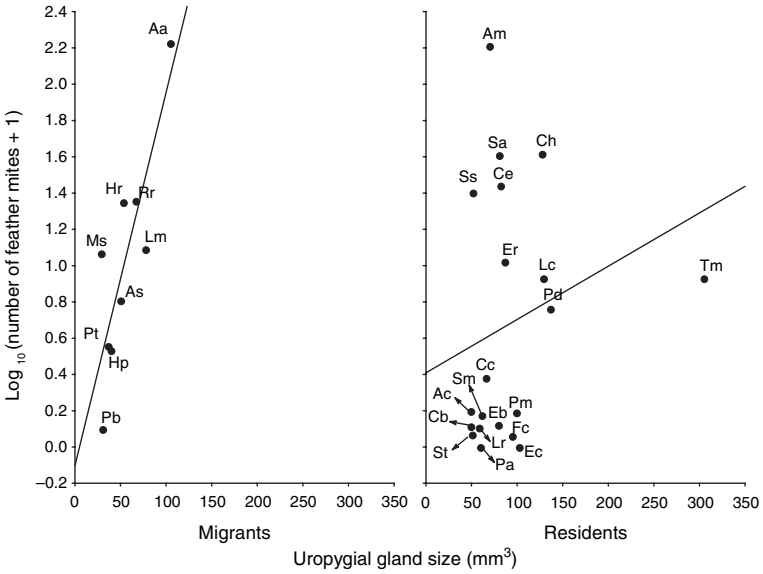

Fig. 3 Relationship between feather mite abundance and uropygial gland size in migratory and resident bird species. Note that the regression line is much more pronounced in migratory birds than in residents. Species codes are indicated in Appendix S1.

$P = 0.004$) than in resident birds ($\beta = 0.55$, $P = 0.018$; Fig. 3).

The interaction between habitat and migratory behaviour was significant both with conventional tests and after controlling for phylogeny (Table 2), and showed that feather mite abundance was higher in riparian/marsh than in terrestrial species only in the case of resident birds (Scheffé's test: residents, $P = 0.015$; migrants, $P = 0.625$; Fig. 4).

When data corresponding to the nonbreeding period were analyzed, the above patterns found in the breeding season did not hold, and the model could explain only 12% of variance in feather mite abundance. Although the number of feather mites tended to be positively correlated with uropygial gland size ($\beta = 0.44$, $P = 0.088$) and higher in riparian/marsh than in terrestrial species (Scheffé's test: $P = 0.093$), these effects were not significant after applying phylogenetic corrections (Table 3).

Univariate results for the repeated measures analysis performed to explore seasonal effects showed that uropygial gland size did not change between the breeding season and the rest of the year, and neither the effects of habitat nor migratory behaviour masked seasonal effects on this variable (Table 4). However, the number of feather mites changed seasonally but only in interaction with the migratory behaviour of hosts (Table 3). *Post hoc* tests showed that the differences only occurred among resident species, which presented lower mite numbers

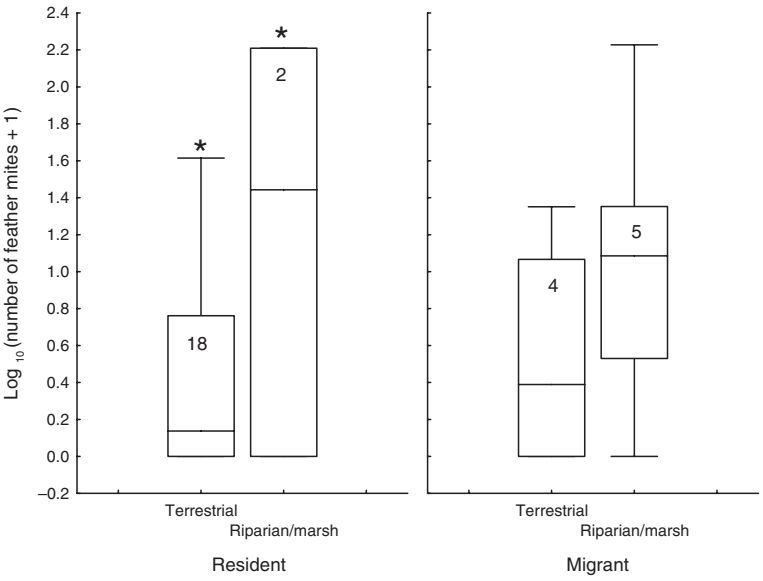

Fig. 4 Box-plot of results from the model of Table 2 showing the difference in the number of feather mites harboured by migratory and resident bird species during the breeding season in relation to habitat type. Median, inter-quartile range and nonoutlier range are shown. Asterisks show the groups where significant differences were observed. Numbers are sample sizes (species).

Table 3 Results of the general linear model (GLM) exploring the effects of habitat, migratory behaviour and uropygial gland size on the number of feather mites harboured by passerine bird species during the nonbreeding season.

				Conventional tables		Phylogenetically correct distributions	
Source of variation	d.f.	MS	*F*	Critical value	*P*	Critical value	*P*
Habitat	1	2.03	4.16	4.30	0.053	5.81	0.11
Body size	1	1.04	2.13	4.30	0.159	14.16	0.24
Uropygial gland size	1	1.55	3.19	4.30	0.088	14.81	0.26
Error	22	0.49					

Only the factors that remained in the final model are shown. Critical values and corresponding *P*-values calculated from both conventional statistical tables and phylogenetically correct computer simulations (see text for details) are shown.
Model: adjusted $R^2 = 0.12$, $F_{3,22} = 2.14$, $P = 0.124$.

Table 4 Results of the repeated measures multivariate analysis of variance (MANOVA) performed on the number of feather mites harboured by passerine birds and their uropygial gland size to explore differences between the breeding and nonbreeding season (within-subjects factor).

						Feather mite abundance					Uropygial gland size				
Source of variation	Wilks	*F*	*F* critical value	d.f.	*P*	MS	*F*	*F* critical value	d.f.	*P*	MS	*F*	*F* critical value	d.f.	*P*
Conventional tables															
Habitat	0.69	2.94		2	0.088	–	–		–	–	–	–		–	–
Migratory behaviour	0.93	0.47		2	0.637	–	–		–	–	–	–		–	–
Season	0.87	1.00		2	0.395	0.04	0.44		1	0.519	250.39	1.36		1	0.263
Season × habitat	0.89	0.76		2	0.487	0.10	0.95		1	0.345	74.20	0.40		1	0.536
Season × migratory behaviour	0.51	6.12		2	0.013	1.27	12.56		1	0.003	2.61	0.01		1	0.907
Error				13		0.101			14		184.52			14	
Phylogenetically correct distributions															
Habitat			7.71	2	0.32				–					–	
Migratory behaviour			3.53	2	0.72				–					–	
Season			47.20	2	0.91			55.70	1	0.90			58.95	1	0.77
Season × habitat			6.30	2	0.77			9.23	1	0.52			8.20	1	0.85
Season × migratory behaviour			3.27	2	0.02			4.93	1	< 0.01			4.70	1	0.91
Error				13					14					14	

Results from both conventional statistical tables and phylogenetically correct computer simulations (see text for details) are included. For clarity, critical values for *F*-ratios from conventional tables are not shown.

during the breeding season than during the rest of the year (Scheffé's test: $P = 0.013$; Fig. 5). Although the abundance of feather mites during the breeding season tended to be larger in migrant birds, the difference was not significant (Scheffé's test: $P = 0.130$; Fig. 5).

The multivariate analysis showed that the seasonal effect in interaction with the migratory behaviour of birds was the only factor contributing to explain seasonal differences in both feather mite abundance and uropygial gland size (Table 4). This means that the abundance of mites was higher in the nonbreeding season among resident birds even after controlling for the effects of uropygial gland size.

Discussion

The use that feather mites make of uropygial gland secretions of birds as a food source (Proctor, 2003) suggests that variations in mite load could depend on variations in the morphological trait reflecting the secretory capacity of the gland, uropygial gland size. This hypothesis was previously supported in an intraspecific comparison of one species of bird (the great tit; Galván & Sanz, 2006). Our comparative study goes further and shows that there is a positive correlation between abundance of feather mites and uropygial gland size of their host species, thus indicating that reproductive rate and/or carrying capacity of these ectosymbionts likely depends on the food source provided by birds. Although we have restricted our analysis to passerine birds, it is likely to be a general trend among bird species, as deduced from the observation of Dubinin (1951, in Proctor, 2003) that species from different bird orders with larger glands present higher mite loads.

Therefore, we hypothesize that both inter- and intraspecific variations in uropygial gland size determine the abundance of feather mites by limiting food provision to these organisms. It is also possible that, if feather mites

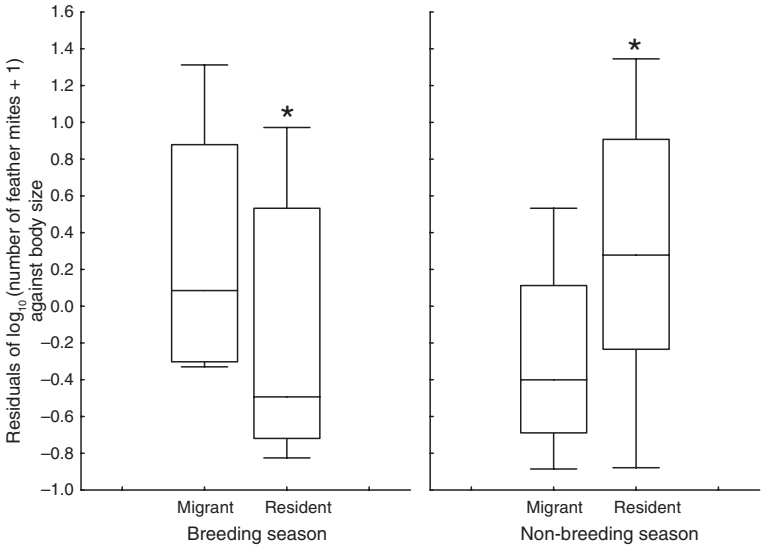

Fig. 5 Seasonal effects on the number of feather mites harboured by migrant (filled symbols and continuous line; $n = 6$) and resident (open symbols and dashed line; $n = 17$) bird species. Values are from the model of Table 4. Asterisks show the groups where significant differences were observed.

have an effect on host fitness, over evolutionary time the size of the uropygial gland may be influenced by selection imposed by mites on their hosts. We are currently unable to differentiate which of these two (non-exclusive) alternatives is the main evolutionary force determining the interactions between feather mites and their hosts. It is noteworthy to mention, however, that although many functions have been proposed for uropygial gland secretions (and demonstrated in the case of protection against feather-degrading bacteria and mammalian predation; Reneerkens, 2007), several studies involving excision of uropygial glands have found no significant changes in what is often considered to be the main function of these uropygial secretions, i.e. increasing waterproofing and plumage flexibility and appearance (Jacob & Ziswiler, 1982; Reneerkens, 2007 and references therein). Indeed, only a small amount of all time spent preening involves the application of uropygial gland waxes onto the plumage (Møller, 1991; Reneerkens, 2007). Together with the observation that feather mites often seem to provide some benefits to their bird hosts (Galván & Sanz, 2006 and references therein; Brown *et al.*, 2006), this makes us suggest that the uropygial gland size may have evolved in birds, at least in part, as a consequence of the positive influence of feather mites on their hosts. This agrees with the idea proposed by Haribal *et al.* (2005) that the great variability in the chemical composition of uropygial gland waxes is an evolutionary consequence mediated by the pressures of feather mites and other ectosymbionts.

As feather mite abundance in at least one species is positively correlated with the intensity of plumage colour (Galván & Sanz, 2006), a common sexually selected trait (see Hill & McGraw, 2006 for review) and with individual survival (Brown *et al.*, 2006), the fitness of individuals bearing large numbers of mites may be higher than those with few or no mites on their feathers. If so, any strategy promoting higher mite densities would be favoured, and increasing the size of the uropygial gland may be one of them. This may be the reason for the pattern found here at an interspecific level. Many morphological adaptations have evolved in feather mites as a consequence of their life on feathers (Proctor & Owens, 2000; Proctor, 2003), but this is the first time that a morphological trait of birds is proposed to be (partly) the result of their interactions with mites. However, only future experimental manipulation of mite abundance (and perhaps also of uropygial gland size or secretory output) will allow us to make conclusions about the directionality of the evolutionary relationships proposed here.

Although migratory birds are considered to be exposed to lower densities of conspecifics than residents (Møller & Erritzøe, 1998), migrant birds in this study harboured more mites on their feathers. As a possible explanation, it may be that the relatively constant environments to which migrants are exposed as compared with residents (Berthold, 2001) favour the thriving of feather mites even to the extent of compensating for lower densities of hosts. It could also be that horizontal transfer of feather mites among unrelated birds is relatively unimportant compared with vertical transfer between parents and offspring, in which case lower densities of migratory host species would have little negative effect on feather mite densities. In any case, the higher mite numbers observed in migrant birds appeared to be the result of a significant interaction between migratory behaviour and uropygial gland size, as the regression line between feather mite load and gland size was much more pronounced in migrants than in residents. However, the slope of the regression line was also positive and significant in the case of residents, which uncovers an interesting result: Although there is a correlation between mite abundance

and gland size, migrant species tended to have more mites per unit gland size than residents. This could suggest a strategy from the species with higher mite loads to attenuate the number of these organisms, which could be compared, for example, to the development of strong immune responses of migratory birds in response to the offence of parasites (Møller & Erritzøe, 1998). Although this result is contrary to the suggestions made above, there is a positive relationship between feather mite abundance and uropygial gland size for the bird species overall, and the possibility exists that mites are, on average, beneficial to their hosts but detrimental if present in very large numbers, as proposed by Haribal *et al.* (2005). Only experimental manipulative studies that demonstrate the existence of benefits or detrimental effects (or both) of feather mites to their hosts will be able to resolve this discrepancy.

On the contrary, we predicted that, as birds living in aquatic environments have more needs of waterproofing their plumage, they would have larger glands (although some authors have failed to support the waterproofing effect of this gland; Reneerkens, 2007) and hence higher mite numbers than terrestrial birds, as Dubinin (1951) had hypothesized more than half a century ago. The same result should be predicted from the fact that bacteria and fungi, which are eaten by feather mites (Proctor, 2003), are better able to colonize feathers in humid environments (Burtt & Ichida, 2004), and uropygial gland secretions are bactericidal (Shawkey *et al.*, 2003). The results were in the direction of the hypothesis, but only when the habitat effect interacted with the migratory behaviour of birds, indicating that the abundance of mites was higher in riparian/marsh than in terrestrial species only for resident birds. The overall correlation between feather mite abundance and uropygial gland size indicates that this habitat effect was due to riparian/marsh species having larger glands than terrestrial ones. Therefore, what is probably a functional adaptation of riparian/marsh birds to their environment allows them to support higher mite numbers, lending credence to the idea that variations in the size of the uropygial gland exert a selection on feather mites rather than the opposite. Although our observations may appear to contradict those of Montalti & Salibián (2000), who concluded that there were no differences in uropygial gland weight between riparian/marsh and terrestrial species of birds, we feel this is mostly because of differences in analysis. Apart from ignoring the effect of common ancestry and not making a detailed statistical analysis, Montalti & Salibián (2000) could have reached this conclusion because of overlooking differences in migratory behaviour of birds.

All the results discussed above pertain only to the breeding season of birds. In contrast, no significant factors explaining variations in the number of feather mites were found during the nonbreeding period. As analysis did not reveal seasonal differences in uropygial gland size, it is possible that the dependence of feather mites on uropygial gland output takes place only during the hosts' breeding season as a consequence of seasonal variations in the breeding biology of the mites themselves (Haribal *et al.*, 2005). Almost nothing is known about feather mite phenology, but Mironov (2000) and Mironov & Malyshev (2002) found that numbers of the feather mite *Monojoubertia microphylla* decreased in adult female chaffinches *Fringilla coelebs* during the brooding period because mites moved onto the nestlings, whereas the number of mites in males, which do not brood, increased during this period. Thus, the results of the present study may suggest that mites synchronize their breeding biology with that of their bird hosts so that motile stages are reached at the time when there are more possibilities of transmission to other birds. This is likely, as feather mite transmission occurs through physical contact between birds (Proctor & Owens, 2000). Thus, it could be speculated that the relationship between feather mite abundance and uropygial gland size is only detected during the breeding season of birds because it is only at that time when the mites are at their most metabolically active and hence are most constrained by food availability. The possibility that feather mites make a differential use of uropygial gland secretions of different chemical composition, which indeed change seasonally in some birds (Reneerkens, 2007), remains unexplored.

In any case, the fact that significant seasonal differences in mite abundance were observed only in resident bird species is interesting and not readily explained. Superficially it agrees with predictions of theory on local adaptation in host–parasite (and other symbiont) systems, in which hosts and symbionts living in sympatry have more opportunities to coevolve (e.g. Soler & Møller, 1990). In addition, it has been hypothesized that migration has evolved in birds to, among other functions, alter the parasite–host dynamics because the risk of parasite infection is usually density-dependent and migratory populations have lower abundances of parasites as compared with resident populations (Møller & Erritzøe, 1998; Møller *et al.*, 2004). However, this theory is not particularly relevant for the feather mite/bird system because, unlike many other avian symbionts (e.g. *Plasmodium*, most nematodes and flatworms), feather mites do not have a life history stage off of the host (Proctor, 2003). Therefore, birds cannot 'escape' their mites by migrating.

The fact that there were no seasonal effects on the uropygial gland size is somewhat surprising as other authors have reported that hormonal levels increase the secretory activity of the gland (Blanco & Frías, 2001 and references therein), and that this organ increases in size during the spermatogenic phase (Bhattacharyya & Chowdhury, 1995). However, the role of hormones as factors regulating the activity of the uropygial gland is poorly known (Ghosh & Bhattacharyya, 1996), and an

example of this is the fact that some authors have failed to find sexual differences in feather mite load even when it has been established that androgen levels increase the activity of the gland and thus feather mite numbers should be higher in males (Galván & Sanz, 2006 and references therein).

In conclusion, the abundance of feather mites carried by a species of bird can be partly explained by the size of its uropygial gland. In particular, the higher mite loads observed in riparian/marsh birds are likely a consequence of the relatively larger glands of these birds. Other observations are not readily explained, however, including the restriction of a strong relationship between gland size and mite load to the breeding season of the hosts, and the steeper relationship between mite load and gland size in migratory species. If moderate mite loads are beneficial to a host but high loads detrimental, this could create complex selection regimes in which gland size influences mite load and vice versa. But to test this or any other hypothesis about bird-mite coadaptation, more experimental field and laboratory studies are required to determine effects of mites on host fitness, and how quantity and quality of uropygial secretions affect fitness of feather mites.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1 List of the species of passerine birds used in this study, together with their habitat type, migratory behaviour, season during which captures occurred, identity of mites found on feathers, feather mite taxa previously recorded from hosts, mean and maximum number of feather mites and mean uropygial gland size.

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