



Chronic coccidian infestation compromises flight feather quality in house sparrows *Passer domesticus*

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Parasites usurp indispensable resources for birds during a moult, and this is particularly relevant for those parasites residing in host intestines. This might compromise the nutritionally demanding moult and, thus, feather functionality. Although lower feather quality has profound and multifaceted adverse effects on residual fitness, surprisingly, little is known about parasites' effect on feather traits, especially over the longer term. We conducted an aviary experiment by medicating half of a group of naturally infested house sparrows *Passer domesticus* against intestinal coccidians for 15 months, spanning two consecutive postnuptial moults, whereas the other half was kept infested (i.e. without medication). Coccidian infestation significantly and negatively affected the size of the uropygial gland during the second moulting period compared to the medicated group. Furthermore, wing length was significantly shorter after the second moulting in the non-medicated compared to the medicated female birds, which indicates that the negative effects of coccidians emerge only after a prolonged exposure to parasite infestation. Non-medicated birds grew poorer quality flight feathers detected in a large number of feather traits both after the first and second moults. In the case of non-medicated birds, the primaries were lighter and shorter, and had a smaller vane area, thinner rachis and decreased stiffness, although a higher barb and barbule density, which may have various consequences for fitness through reducing flight performance. Our findings demonstrate that, besides the well-known immediate consequences for host breeding success, parasites might also have serious, long-lasting effects through influencing feather quality and, ultimately, fitness of the host. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **108**, 414–428.

ADDITIONAL KEYWORDS: bending stiffness – feather structure – infection – moult – uropygial gland size.

INTRODUCTION

The pathogenic effects of parasites have long been recognized. Studies have shown a causal relationship between parasites and breeding success, survival, and the expression of the secondary sex characters of avian hosts (Møller, 1997; Møller *et al.*, 2009). However, our knowledge about the effect of parasites on moulting is scarce (Langston & Hillgarth, 1995; Pap *et al.*, 2011). Parasites can affect moulting and thus the quality of feathers produced in a number of ways: they usurp resources, increase

metabolism, trigger immune response, elevate corticosterone levels, and induce oxidative stress (Hörak *et al.*, 2004; Raouf *et al.*, 2006; Baeta *et al.*, 2008; Monaghan, Metcalfe & Torres, 2009; Mougeot *et al.*, 2009; Sepp *et al.*, 2012). Moulting depends on nutrients and on antioxidants such as glutathione (i.e. the main reservoir of cysteine) and is metabolically expensive (Murphy & King, 1990; Lindström, Visser & Daan, 1993; Pap *et al.*, 2008). Furthermore, moulting is traded off with immune investment and suppressed by corticosterone (Romero, Storchlic & Wingfield, 2005; Moreno-Rueda, 2010a). Hence, all these inter-relationships are expected to be reflected in the moult pattern and/or feather features known to influence fitness.

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The primary function of flight feathers is to generate thrust and lift, thereby enabling flight. Feather structure and composition are known to vary among species, populations, and individuals, which in turn determine the variation in avian flight (Aparicio, Bonal & Cordero, 2003; Weber *et al.*, 2005; de la Hera, Pérez-Tris & Tellería, 2009; de la Hera, Pérez-Tris & Tellería, 2010a). The amount of keratin invested and the physical texture of the shaft and the vane, which are responsible for the elasticity of the feather and ultimately its dynamic response to air pressure and cyclic load, are among the traits that may determine the functionality of flight feathers (Jenni & Winkler, 1994; Bonser, 1995; Dawson *et al.*, 2000; Weber *et al.*, 2005; Ruiz-de-Castañeda *et al.*, 2012). Owing to the complex structure of the flight feather and its relationship with flight, information about the way feather structure varies between individuals is important to better understand how differences in feather structure originate (Dawson *et al.*, 2000; Weber *et al.*, 2005; DesRochers *et al.*, 2009). Flight feathers are composed of a rachis with regularly spaced branches (barbs) on each side, which are in turn equally branched with barbules. The flexibility of the shaft, the vane area, the number of barbs and barbules, and the way they are attached to each other, all largely determine the deviance from their optimal response to air pressure, which may finally determine flight performance. To maintain the functionality of flight feathers, birds exhibit many behavioural and morphological adaptations, such as preening to relock the unlocked barbules, lower the number of feather-degrading parasites, and increase secretion of gland oil, which inhibits the growth of feather decomposing microbes and maintains feather rigidity and integrity (Jacob & Ziswiler, 1982; Cotgreave & Clayton, 1994; Shawkey, Pillai & Hill, 2003; Moreno-Rueda, 2010b, 2011; Soler *et al.*, 2012). Furthermore, birds that spend considerable time in flight and/or are exposed to environmental conditions that deteriorate the keratin structure invest more in mechanical properties of the feathers to ensure their strength and durability between subsequent moulting events (Jenni & Winkler, 1994; Weber *et al.*, 2005). However, the extent of this variation and its underlying proximate mechanisms remain largely unexplored. One of the most energy- and nutrition-demanding periods in a bird's life is the time of increased somatic cell growth during the regular replacement of body and flight feathers (i.e. moulting; Lindström *et al.*, 1993; Jenni & Winkler, 1994; Klaassen, 1995). Therefore, if an environmental perturbation alters the optimal allocation into the moult, we may expect that the quality of feathers decreases (Dawson *et al.*, 2000; Hall & Fransson,

2000; Serra, 2001; Pap *et al.*, 2008; de la Hera *et al.*, 2009, 2010a; DesRochers *et al.*, 2009; Vágási *et al.*, 2012).

Some of the most prevalent and abundant avian parasites are coccidians, which are microscopic, unicellular organisms that inhabit the epithelium of the host's intestine (Greiner, 2008). In wild birds, coccidian infestation can reduce the signalling value of ornaments (Hill & Brawnner, 1998; Hōrak *et al.*, 2004; Baeta *et al.*, 2008; Mougeot *et al.*, 2009; Dolnik & Hoi, 2010) and significantly decrease an individual's condition (Hōrak *et al.*, 2004; Aguilar *et al.*, 2008; Mougeot *et al.*, 2009; Pap *et al.*, 2009). During moult, stressors such as infestations by parasites have the potential to compromise an individual's fitness because the conditions prevailing during plumage replacement determine the quality of the feathers that are produced and, ultimately, the flight performance of individuals (Swaddle *et al.*, 1996). Coccidians reduce the general absorption and drainage of essential nutrients (e.g. amino acids), stimulate the immune system (Allen & Fetterer, 2002), and cause oxidative damage (Sepp *et al.*, 2012). Thus, coccidian infestation during moult can induce a range of costs, including a prolonged moult, reduced feather quality, and increased postmoult mortality resulting from reduced predator escape ability. Furthermore, because most passerines moult flight feathers only once per year and feather quality cannot be enhanced between moults, these effects can be long-lasting. Previous studies on the house sparrow *Passer domesticus* showed that, under aviary conditions, several measures of feather quality, such as the length and mass of the primary flight feathers, were reduced in the infested birds compared to medicated house sparrows (Pap *et al.*, 2009, 2011). Such studies on the moulting house sparrows and generally those addressing coccidians and their effect on avian hosts suffer from two caveats. First, the effects of coccidians are generally monitored during a short period of several days or weeks, when the negative effect of these parasites may not be detected. Coccidians commonly inhabit the alimentary tract of the birds for life-long duration and their effects emerge only during stressful periods. Ignoring the effect of parasites on complex features of flight feathers may prevent the accumulation of valuable knowledge on host–parasite interactions. Therefore, experimental studies where the number of coccidians is manipulated for a longer period, covering multiple annual stages during multi-annual periods, may be particularly insightful and reveal the potential hidden cost of parasitism on the host's fitness. To our knowledge, studies on the effects of parasites on moulting and feather quality of the same host individuals during multiannual periods are very rare. Second, in our previous studies on moulting

house sparrows, we have characterized the quality of flight feathers through three measures only (primary length and mass, rachis width), whereas, in the present study, we extended these measures with further structural traits (vane area, barb and barbule density, bending stiffness).

To study the short- and long-term effect of coccidian infestation on the condition and feather quality of the house sparrow, we introduced wild adult birds to large outdoor aviaries shortly before they started their regular postnuptial moult. We followed the condition and moulting, and measured the quality of the primary flight feathers during two subsequent annual moults, when the level of infestation by coccidians was suppressed in the medicated birds and kept untreated in control groups. Based on our previous knowledge on the effect of coccidians on moulting birds (see above), we hypothesized that parasite infestation decreases the quality of flight feathers. Furthermore, we predicted that the negative effect of coccidians will be more pronounced over the long term (i.e. after 1 year of medication treatment). However, because our information about nutritional and physiological costs, the developing mode, and the functionality of feather quality measures is very limited, and the results are often contradictory (DesRochers *et al.*, 2009), we have no a priori prediction about the individual response of these traits to short- and long-term medication treatment except for feather mass, feather length, and rachis width. Based on our previous results (Pap *et al.*, 2009, 2011), we expect that these traits are negatively affected in the non-medicated birds. The uropygial gland, which secretes the gland oil, generally plays an important role in the maintenance of feathers (Jacob & Ziswiler, 1982; Giraudeau *et al.*, 2010; Moreno-Rueda, 2010b, 2011); therefore, we may expect that the amount of gland oil produced may influence certain properties (e.g. flexibility) of the feathers. Thus, we also measured the size of the uropygial gland of the birds to test whether the coccidian infestation affects the production of the gland oil and to control for the possible confounding effects of the oil on feather quality measures. Finally, we intercorrelated all feather quality measures to test for possible trade-offs in allocation between different structural traits.

MATERIAL AND METHODS

GENERAL PROCEDURE

Thirty adult male and 30 adult female house sparrows were caught with mist nets (Ecotone, Poland) at a farm near Bălcăciu, central Transylvania, Romania (46°11'N, 24°03'E), just before the annual postnuptial moult on 30 July 2010. Birds were transported to the campus of the Babeş-Bolyai University in Cluj

Napoca and housed in four groups ($N = 15$ birds in each, seven males and eight females or, inversely, alternated among groups) in outdoor aviaries ($5 \times 2 \times 2.5$ m; length $\times$ width $\times$ height) placed near each other and the experimental groups were arranged alternately (two replicates for each treatment group; see below). Birds were fed *ad libitum* with a mixture of seeds containing ground corn, sunflower, wheat, and oat throughout the experiment. In addition, on every second day, birds were given a protein supplement of two grated boiled eggs and fresh tap water was provided daily. No birds had begun to moult at capture. Of the 60 captured house sparrows, four individuals died from unknown causes (three from medicated and one from infested groups). All other individuals were released in good health at the end of the experiment on 3 November 2011. During the 15-month confinement, birds were measured and sampled four times: pre- and postmoult of the first and second moult (30 July and 11 December 2010, and 2 August and 29 October 2011, respectively). Some house sparrows built nests, laid eggs, and even raised chicks during the breeding season, although the identity of birds with nests was not determined. This shows that birds followed their normal annual cycle (i.e. they moulted after the breeding season in 2010 and 2011; during August to November as is the case in wild-living house sparrows in the population of origin).

EXPERIMENTAL PROTOCOL

At capture, we measured wing length, body mass, uropygial gland size, and tarsus length, after which the birds were transported and introduced in aviaries where they were allowed to acclimatize for 2 weeks (Fig. 1). The maximum length, width, and height of the uropygial gland was measured with a digital calliper (0.01 mm precision) and, based on these measures, we calculated the gland size as length $\times$ width $\times$ height, which is a good surrogate of gland volume and the amount of waxes secreted (Pap *et al.*, 2010). Afterwards, on 15 August, we placed the birds in individual cages to quantify the pre-experimental level of coccidian infestation. To assess infestation, we measured the rate of oocyst-shedding over 2 days (Pap *et al.*, 2009). In a previous study on the house sparrow, we demonstrated that *Isospora lacazei* was the most common coccidian in the faecal samples (Pap *et al.*, 2011). Because coccidians from the genus *Isospora* shed oocysts predominantly during the late afternoon (Filipiak, Mathieu & Moreau, 2009), faeces were collected just before sunset. The number of oocysts was counted in McMaster chamber as described previously Pap *et al.* (2011) and the concentration was expressed as

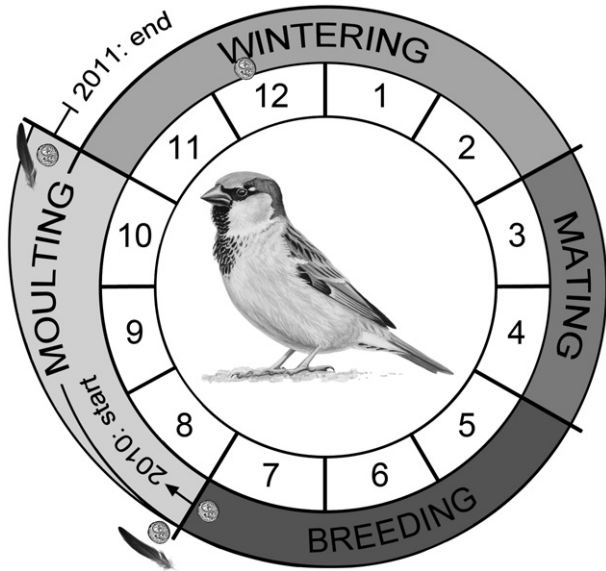


Figure 1. The annual cycle of a house sparrow and a schematic representation of the experimental protocol. The numbers within the circle show the months (i.e. 1 = January), the arrow indicates the start (30 July 2010), and the blunt-tip line marks the end of the experiment (3 November 2011). Wing length, body mass, uropygial gland size, and the infestation rate by coccidian oocysts were measured before and after the first (30 July 2010 and 11 December 2010, respectively) and second postnuptial moult (2 August 2011 and 29 October 2011, respectively). These events are indicated by the coccidian oocysts inserts. During the last two sessions, feather samples were also collected (feather inserts). Male house sparrow drawing courtesy of Zsoldos Márton.

number of oocysts per gram of faeces. The mean values of the oocyst numbers collected during the 2 days were used in the analyses. We treated the medicated birds against coccidians 2 days per week because, under aviary conditions, the reinfestation of birds from the environment is highly probable (P. L. Pap, C. I. Vágási, pers. observ.), making it necessary to continuously suppress the reproduction of these parasites. Our previous study shows that the drug used in the present experiment has no negative side-effect on the condition and physiology of house sparrows (Pap *et al.*, 2011). The next day, we randomly assigned birds to one of the four aviaries and each aviary to either: (1) medicated with an anticoccidial drug (Med; $N = 30$) or (2) naturally infested with coccidia (Cocc; $N = 30$) (i.e. we had two replicates for each treatment group). All Med birds were treated with the anticoccidial drug provided in their drinking water for 2 days per week through the experiment (1 mL of Baycox®, Bayer; 2.5% in 1 L of drinking water), whereas Cocc birds received tap water, which allowed the coccidians to persist in the alimentary tract (Fig. 2A).

At the beginning of moulting (i.e. premoult) of the first and second year, we assessed the progression of the moult of primaries of each individual according to the following scheme: we scored the dropped feathers as 1, a quarter-, half- or three-quarter regrown feathers as 2, 3 and 4, respectively, and the fully-regrown feathers as 5 (for details, see Pap *et al.*, 2008). Old feathers received a score of 0. On each occasion, we calculated the moult score for each individual, where moult score is the sum of the scores of individual primary feathers (total 9, the tenth is rudimentary) and ranges from 0 (before the beginning of the moult: all primaries old) to 45 (moult finished: all primaries completely replaced).

FEATHER QUALITY MEASURES

We plucked the seventh primaries (counted from the innermost) of both wings before and after the second moult to assess the effect of experimental manipulation on the feather quality during two subsequent moults. Note that feather samples collected before the second moult mirror the state prevailing during the first moult. For each feather, we determined: (1) length, (2) vane area, (3) mass, (4) rachis width, (5) barb density, (6) barbule density, and (7) vertical bending stiffness. We used the mean values of the two primaries to increase the robustness of data. Feather length was measured with a ruler to the nearest 0.5 mm and rachis width with a digital calliper to the nearest 0.01 mm at 1.5 cm from the base of the shaft. Feathers were weighed on an electronic balance to the nearest 0.1 mg. We took digital photographs of the feathers on a metric grid background or using a stage micrometer for measurements of the vane area, and barb and barbule density, which were imported into the IMAGEJ, version 1.37 (<http://rsb.info.nih.gov/ij/>). For vane area, we encircled the vane and expressed this surface in cm². Barb and barbule density were measured on the inner vane, at the middle part along the rachis, and in the case of barbules along the proximal side of the barbs. The density of barbs and barbules was expressed by counting ten consecutive barbs and barbules and measuring the distance occupied by these structures (i.e. higher distance values indicate a lower density). The stiffness of the primary feathers was measured using the method described by Dawson *et al.* (2000). Two grams were attached to the rachis at 22 mm from the distal end of the feather (approximately one-third along its length from the distal end), and the vertical (downward) deflection was measured. The calamus was fixed into a hole between two thick rubber bands so that it emerged at a point corresponding to the insertion point of the feather into the skin of the bird's wing (approximately 10 mm from the proximal end). The holder was

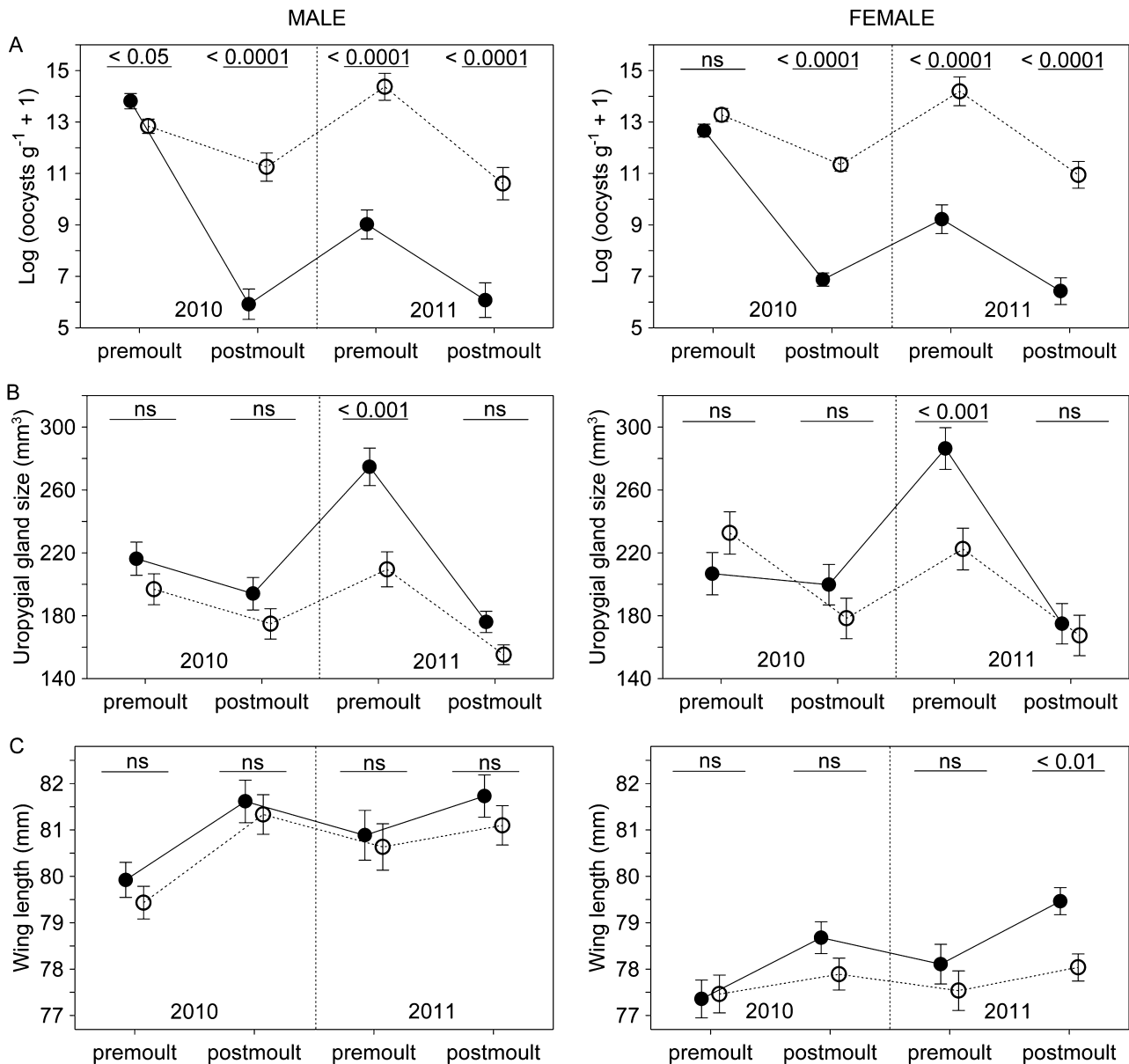


Figure 2. The effect of treatment on the coccidian infestation (A), uropygial gland size (B), and wing length (C) during the premoult and postmoult periods of the first (2010) and second study year (2011) of male and female house sparrows (Med group treated with coccidiostatic drug – black dots, continuous line; Cocc group with natural coccidian infestation – open dots, dashed line). Significance levels for the planned comparison tests from the general linear models (Table 1) are shown above the horizontal bars. ns, not significant. Values are the mean $\pm$ SE.

mounted on a tripod in front of a metric grid, with the dorsal surface of the feather upwards. The weight was attached to the rachis by means of a short piece of cotton. Vertical deflection of the feather was measured from digital pictures taken without and with a weight attached and was expressed as the distance between the two measures. Higher deflection values thus indicate a decreased stiffness. To test the validity of feather quality measures on feathers from the

aviary birds (see below), we compared these with the same primary feathers from 22 adult house sparrows (ten males and 12 females) from a natural population captured on 18 March 2012.

STATISTICAL ANALYSIS

The effects of experimental manipulation and sex on coccidian infestation, body mass, uropygial gland size,

wing length, and feather quality measures were analyzed using repeated-measures general linear models (GLMs), where the dependent variables were entered in separate models and the experimental groups and sex were set as factors. Within treatment groups, the effect of aviary was nonsignificant (i.e. no difference between replicates) on any morphological and parasitological measures ($P > 0.5$); therefore, the data of birds from different aviaries within groups were pooled. Because of the skewed distribution of coccidian oocysts, we normalized coccidian number by $\log_{10}$ -transformation and used transformed values in all subsequent analyses to follow the requisite of normal distribution of parametric statistics. Data were examined for normality and homogeneity of variance (Bartlett test) and most were found to satisfy these assumptions, necessary for parametric analysis, except some of the repeated measures of the coccidian infestation, uropygial gland size, and wing size (Table 1). Lindman (1974) had shown that the F -statistic is quite robust against violation of the assumption of the heterogeneity of variances. However, to be sure in the robustness of our results, we repeated these analyses using a variance function (varIdent of nlme library) in the R statistical environment (Pinheiro *et al.*, 2011; R Development Core Team, 2011) that permits different variances for each level of treatment. These results confirmed the validity of our conclusions based on repeated-measures GLMs. Repeated-measures analysis of variance containing factors with more than two levels (Table 1) should meet the sphericity assumption, which requires that the variances (pooled within-groups) and covariances (across subjects) of the repeated measures are homogeneous. We tested for this assumption with Mauchly's sphericity test. In none of the cases was the assumption violated. However, because the significance tests hardly reveal the degree of violation of the sphericity, we calculated the adjusted results with the Greenhouse–Geisser correction, which included epsilon factors used to multiply the degrees of freedom of the mean squares for the effect and the error (Quinn & Keough, 2002). In all cases, the adjusted F -statistics was similar with the uncorrected values. Data are reported as the mean $\pm$ SE unless otherwise stated. As a result of the significant associations among different feather quality measures (Broggi *et al.*, 2011), we tested the independent explanatory value of each traits. Accordingly, we intercorrelated quality measures of feathers collected after the first and second moult, where the effect of experimental group and sex was controlled. Finally, because the association and trade-offs between quality traits of feathers grown under aviary condition may loosen as a result of the surplus of food, we repeated the correlation analyses on feathers

Table 1. Results of repeated-measures general linear models on the effect of treatment on coccidian infestation and condition before and after the two subsequent moulting periods of the male and female house sparrows

Source	Coccidian infestation			Body mass			Uropygial gland size			Wing length		
	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>
Group	208.28	1	< 0.0001	0.06	1	0.815	5.78	1	0.020	2.24	1	0.140
Sex	0.29	1	0.593	4.72	1	0.035	0.80	1	0.376	58.44	1	< 0.0001
Group $\times$ Sex	0.03	1	0.866	0.00	1	0.998	0.53	1	0.471	0.13	1	0.725
Subject within groups	–	52	–	–	52	–	–	52	–	–	52	–
Repeated measures	90.16	3	< 0.0001	96.91	3	< 0.0001	105.94	3	< 0.0001	38.77	3	< 0.0001
Group $\times$ Repeated-measures	28.77	3	< 0.0001	1.69	3	0.171	18.32	3	< 0.0001	2.56	3	0.057
Sex $\times$ Repeated-measures	0.67	3	0.572	6.68	3	0.0003	0.43	3	0.729	3.16	3	0.026
Repeated-measures $\times$ Subjects within groups	–	156	–	–	156	–	–	156	–	–	156	–

Significant effects are shown in bold and all three-way interactions are nonsignificant.

collected from wild birds. $P < 0.05$ was considered statistically significant.

RESULTS

THE EFFECT OF TREATMENT ON COCCIDIANS, CONDITION, AND MOULT

The anticoccidial drug significantly reduced the number of oocysts shed in the faeces (Fig. 2A, Table 1) as revealed by the significant group and group $\times$ repeated-measures interaction in the repeated-measures GLM analysis, where the numbers of oocysts counted during the four sessions were entered as dependent variables, with group and sex as factors. The success of medication cure was similar between sexes. House sparrows in the Med group had a significantly larger uropygial gland than Cocc birds in both sexes (Fig. 2B, Table 1), although only during the second premoult period. The volume of the gland was larger during the premoult than after moult (Pap *et al.*, 2010). Wing length was marginally significantly and negatively affected by coccidians (Table 1), but only in females and over the long term (i.e. after the second moult) (Fig. 2C). Body mass was similar between experimental groups and the lack of an effect of coccidian infestation persisted during the whole period as revealed by the nonsignificant group $\times$ repeated-measures interaction (Table 1).

The moulting score at the beginning of the first moulting period on 15 August 2010 was similar between experimental groups ($F_{1,56} = 0.01$, $P = 0.937$), was significantly advanced in males related to females ($F_{1,56} = 11.58$, $P = 0.001$), and the difference between sexes was similar in Med and Cocc groups (group $\times$ sex interaction: $F_{1,56} = 0.90$, $P = 0.346$). Similarly, medication treatment had no effect on the moulting score measured during the beginning of the second moulting period on 2 August 2011 ($F_{1,53} = 2.07$, $P = 0.157$), and the moulting was similar between sexes (sex: $F_{1,53} = 0.00$, $P = 0.948$; group $\times$ sex: $F_{1,53} = 0.02$, $P = 0.883$).

THE EFFECT OF TREATMENT ON FEATHER QUALITY MEASURES

Coccidian infestation negatively affected the general quality of the primary flight feathers both after the first and second moult, as indicated by the significant group effect in the case of feather length, vane area, feather mass, rachis width, and barb and barbule density (Fig. 3A–G, Table 2). The coccidian treatment had similar effect on males and females (nonsignificant group $\times$ sex interactions). The difference between experimental groups was similar after the first and second moult (nonsignificant

group $\times$ repeated-measures interactions). The effect of experimental manipulation on the feather mass remained significant in the case of both the first and second moult, even after the positive effect of feather length and vane area on feather mass was controlled for (after first moult: $F_{1,51} = 5.97$, $P = 0.018$; after second moult: $F_{1,50} = 4.38$, $P = 0.041$; Table 3). This indicates that the relative feather mass (i.e. the amount of keratin invested per unit feather length or area) was reduced in birds from Cocc related to Med group. Because the bending stiffness is strongly positively related to feather length (both moults) and rachis width (second moult only; Table 3), we repeated the analysis by controlling for these inter-correlations. In this model, the coccidian infestation significantly reduced the stiffness of feathers grown after the first ($F_{1,52} = 5.14$, $P = 0.028$), but not in the case of feathers grown after the second moult ($F_{1,51} = 0.18$, $P = 0.675$).

Finally, we tested how feather quality measures of feathers grown at the same position change between the first and second moults (Fig. 3A–F). The exception is bending stiffness of which variation largely depends on feather length. The planned comparison analysis showed that feather length ($F_{1,52} = 20.84$, $P < 0.0001$), vane area ($F_{1,52} = 5.01$, $P = 0.030$), rachis width ($F_{1,52} = 58.68$, $P < 0.0001$), and barbule density ($F_{1,52} = 6.82$, $P = 0.012$) significantly increased, whereas feather mass ($F_{1,52} = 52.93$, $P < 0.0001$) and barb density ($F_{1,52} = 6.89$, $P = 0.011$) significantly decreased between the first and second moults (Fig. 3A, B, C, D, E, F). The changes in feather quality measures between the first and second moults were independent of the coccidian treatment (in all cases nonsignificant group $\times$ repeated-measures; Table 2).

THE RELATIONSHIP AMONG FEATHER QUALITY MEASURES

The relationship among quality measures of feathers grown after the first and second moults was similar, except bending stiffness, where the negative effect of the rachis width on deflection was significant only after the second moult (Table 3). As expected, feather length, vane area, feather mass, and rachis width are significantly inter-relating measures, except for the nonsignificant correlation between rachis width, feather length, and vane area. However, barb density and barbule density are traits that vary independently from the rest of feather quality measures (Table 3). None of the interactions between group and feather quality traits were significant (data not shown). This indicates that the relationship between feather quality measures was not affected by the coccidian treatment. The relationships between

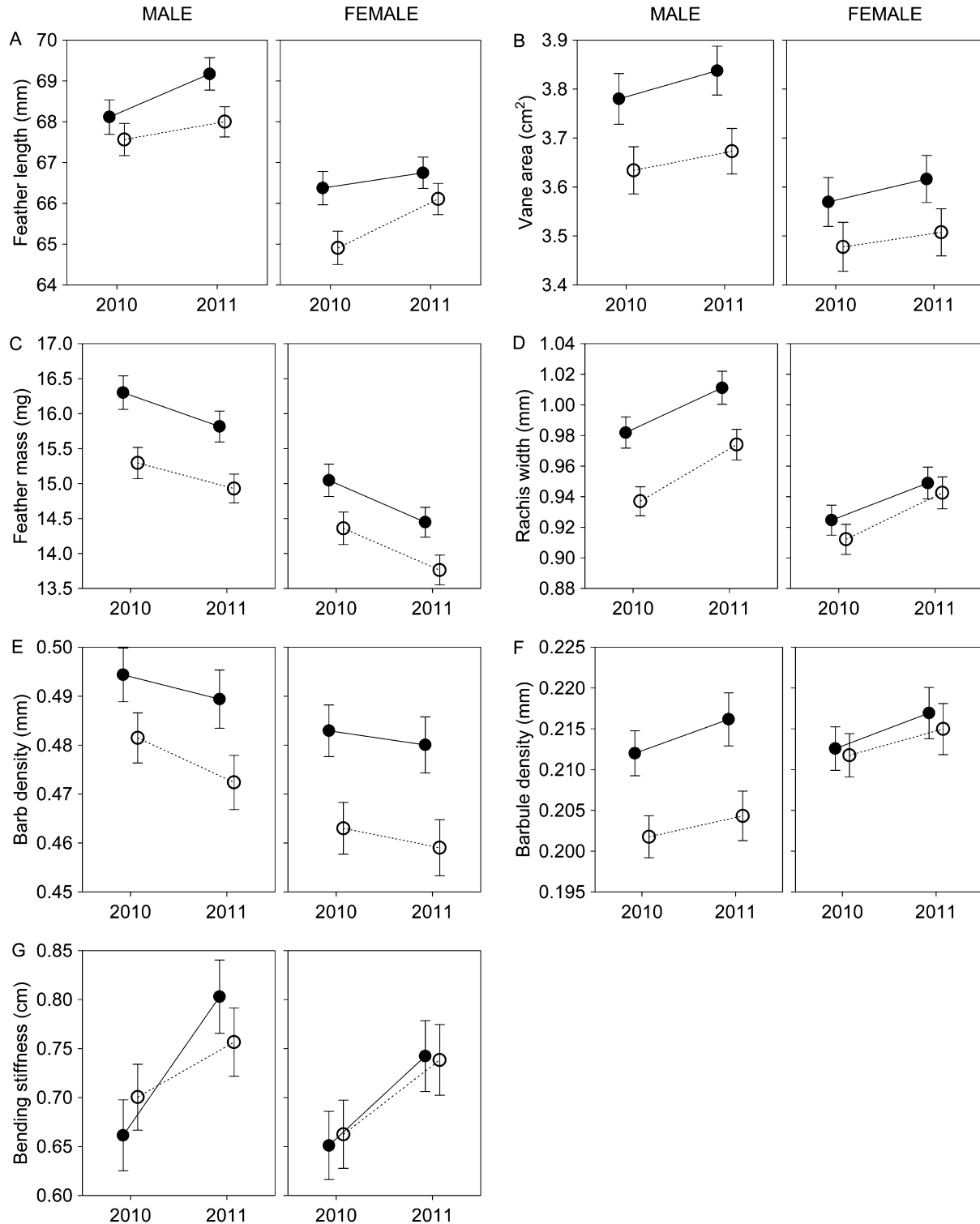


Figure 3. The effect of experimental manipulation of the coccidian infestation on the feather quality measures [feather length (A), vane area (B), feather mass (C), rachis width (D), barb density (E), barbule density (F) and bending stiffness (G)] after the first (2010) and second (2011) postnuptial moult of male and female house sparrows (Med group treated with coccidiostatic drug – black dots, continuous line; Cocc group with natural coccidian infestation – open dots, dashed line). Note that lower values on panels E and F mean higher density; for the details, see Material and Methods. Values are the mean $\pm$ SE.

Table 2. Results of repeated-measures general linear models on the effect of treatment on feather quality measurements on the primary 7 before and after the first and second postnuptial moult of the male and female house sparrows

Source	Feather length*			Vane area			Feather mass			Rachis width		
	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>
Group	7.11	1	0.010	8.04	1	0.007	14.89	1	0.0003	7.33	1	0.009
Sex	36.82	1	<0.0001	17.47	1	0.0001	31.20	1	<0.0001	22.21	1	<0.0001
Group × Sex	0.07	1	0.790	0.37	1	0.546	0.38	1	0.539	2.86	1	0.097
Subject within groups	–	52	–	–	52	–	–	52	–	–	52	–
Repeated-measures	20.84	1	<0.0001	5.01	1	0.030	52.93	1	<0.0001	58.68	1	<0.0001
Group × Repeated-measures	0.09	1	0.770	0.21	1	0.647	0.19	1	0.661	0.77	1	0.384
Sex × Repeated-measures	0.01	1	0.905	0.07	1	0.791	1.53	1	0.222	0.54	1	0.466
Repeated-measures × Subjects within groups	–	52	–	–	52	–	–	52	–	–	52	–

Source	Barb density			Barbule density			Bending stiffness		
	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>
Group	11.85	1	0.001	5.83	1	0.019	0.00	1	0.999
Sex	6.52	1	0.014	4.55	1	0.038	1.19	1	0.280
Group × Sex	0.29	1	0.594	3.51	1	0.067	0.02	1	0.898
Subject within groups	–	52	–	–	52	–	–	52	–
Repeated-measures	6.89	1	0.011	6.82	1	0.012	20.10	1	<0.0001
Group × Repeated-measures	0.43	1	0.516	0.25	1	0.620	1.53	1	0.222
Sex × Repeated-measures	0.82	1	0.370	0.02	1	0.877	0.14	1	0.709
Repeated-measures × Subjects within groups	–	52	–	–	52	–	–	52	–

Significant effects are shown in bold and all three-way interactions are nonsignificant except that marked by *.

*Group × Sex × Repeated-measures: $F = 4.65$, d.f. = 1, $P = 0.036$.

Table 3. The relationship between feather quality measures used in the analyses of aviary house sparrows

	Feather length	Vane area	Feather mass	Rachis width	Barb density	Barbule density	Bending stiffness
	After second postnuptial moult						
Feather length	After first postnuptial moult						
	–	7.45**	25.77***	0.09	0.50	0.86	13.92**
Vane area		0.386 (0.142)	0.547 (0.108)	0.045 (0.149)	0.114 (0.161)	0.155 (0.165)	0.592 (0.159)
	17.03**	–	10.60**	2.13	0.02	0.11	2.07
	0.554 (0.134)		0.362 (0.111)	0.198 (0.135)	–0.023 (0.149)	–0.050 (0.153)	0.234 (0.163)
Feather mass		22.81***	–	14.12**	0.03	0.01	0.23
	0.545 (0.114)	34.90***		0.527 (0.140)	0.030 (0.171)	–0.019 (0.176)	0.091 (0.190)
Rachis width		4.15*	10.60**	–	0.00	0.16	6.40*
	0.168 (0.145)	0.259 (0.127)	0.442 (0.136)		0.001 (0.151)	–0.062 (0.155)	–0.401 (0.158)
Barb density		0.42	1.03	0.02	–	2.88	1.30
	0.101 (0.155)	0.020 (0.140)	0.159 (0.156)	–0.020 (0.147)		–0.237 (0.140)	0.176 (0.154)
Barbule density		0.01	0.01	0.60	0.03	–	0.08
	0.014 (0.164)	–0.225 (0.144)	–0.014 (0.166)	–0.120 (0.154)	–0.026 (0.146)		0.044 (0.151)
Bending stiffness		20.37***	1.18	0.18	3.86	0.19	–
	0.682 (0.151)	0.179 (0.158)	0.194 (0.178)	–0.071 (0.168)	0.300 (0.153)	–0.066 (0.150)	

The statistical values are from general linear models (GLMs), where the effect of experimental group and sex was controlled. The effect of feather quality measures on the dependent variable was tested in separate models. Bold values represent significant correlations. The values are F and β (SE) from the GLMs. *** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$.

quality measures of feathers collected from wild house sparrows generally confirm the findings obtained in aviary birds (Table 4). However, several significant associations between feather quality traits measured on aviary birds turned to be nonsignificant in the case of feathers collected from wild birds, which may be related to the lower samples size (57 versus 22 birds in captive and wild groups, respectively).

DISCUSSION

THE EFFECT OF COCCIDIANS ON CONDITION

In the present study, we have shown that the chronic coccidian infestation significantly and negatively affects the feather growth and the production of gland oil, as revealed by the reduced wing length and small uropygial gland size in Cocc birds compared to the Med group. These results corroborate our previous findings on house sparrow and some other studies on wild birds, and indicate that coccidians drain important resources from functions that require protein (Hörak *et al.*, 2004; Hill, Doucet & Buchholz, 2005; Pap *et al.*, 2009, 2011). Body mass was not affected by the long-term exposure to coccidians, suggesting that birds can recover and are able to maintain their body mass after the initial acute stress caused by infection (Pap *et al.*, 2009). By contrast to previous studies, in the present study, we manipulated coccidian infestation for a long period that persisted for more than one annual cycle, allowing the study of the chronic effects of infestation on hosts. Interestingly, in line with our previous findings on the same host–parasite system (Pap *et al.*, 2009, 2011), we found that the start of second moulting was not affected by the coccidian manipulation, which can be explained by the adaptive allocation of resources to feather growth under nutritional stress. The re-allocation of resources during mild but chronic stress caused by coccidian infestation to moulting is likely adaptive, aiming to keep the aerodynamically costly moulting period as short as possible and to reduce its overlapping with migration or wintering (Dawson *et al.*, 2000; Hall & Fransson, 2000; de la Hera, Pérez-Tris & Tellería, 2010b). It would be insightful for future studies to explore the physiological mechanisms more thoroughly and investigate how the corticosterone stress response and the antioxidant machinery (i.e. two physiological axes tightly associated with moulting) are regulated before and during the moult in infested and medicated birds.

We found that the size of the uropygial gland significantly increased in Med compared to Cocc birds during the second postnuptial moult, which may indicate that the negative effect of coccidians on condition appears after a long-term chronic infestation. This result is in line with the significant negative effect of

Table 4. The relationship between feather quality measures used in the analyses of wild house sparrows

	Vane area	Feather mass	Rachis width	Barb density	Barbule density	Bending stiffness
Feather length	6.23* 0.500 (0.200)	2.31 0.317 (0.209)	0.13 0.084 (0.228)	0.47 −0.141 (0.205)	0.03 0.040 (0.224)	3.53 0.373 (0.199)
Vane area	–	0.11 0.073 (0.219)	0.61 0.175 (0.224)	0.06 0.052 (0.206)	0.84 −0.199 (0.217)	2.32 0.309 (0.203)
Feather mass	–	–	14.77** 0.687 (0.179)	0.39 −0.133 (0.214)	2.66 0.355 (0.218)	0.01 0.019 (0.225)
Rachis width	–	–	–	1.48 0.243 (0.200)	3.37 0.379 (0.206)	0.03 0.034 (0.216)
Barb density	–	–	–	–	0.29 0.132 (0.245)	0.07 0.064 (0.239)
Barbule density	–	–	–	–	–	0.00 0.009 (0.222)

The statistical values are from general linear models (GLMs), where the effect of sex was controlled. The effect of feather quality measures on the dependent variable was tested in separate models. Bold values represent significant correlations. The values are *F* and *β* (SE) from the GLMs. ***P* < 0.01, **P* < 0.05.

coccidians on wing length of female house sparrows only after the second moult. These findings support our expectation that, during long-term chronic infestation, the negative effect of parasites on the host's condition is cumulative. It is worth noting that, because gland oil is important in maintaining the plumage (Jacob & Ziswiler, 1982; Giraudeau *et al.*, 2010; Moreno-Rueda, 2010b, 2011; Soler *et al.*, 2012), and the amount of oil secreted is positively associated with the gland size in house sparrows (Pap *et al.*, 2010), the effect of coccidians on feather quality can be indirect. The reduced size of the uropygial gland in the infested birds may be explained by the direct effect of coccidians on the absorption of the primary components of the gland oil (e.g. ester preen waxes; Reneerkens, Piersma & Damsté, 2002) or by its negative effect on the general condition of birds which may be tightly connected to gland size (Moreno-Rueda, 2010b; P. L. Pap, C. I. Vágási, pers. observ.). To our knowledge, these results are the first to show that, besides ectoparasites and ectosymbionts (Galván *et al.*, 2008; Møller, Erritzøe & Rózsa, 2010; Soler *et al.*, 2012), endoparasitic parasites have the potential to modulate the size of the gland and feather quality in birds. However, why was gland size affected only during the premoult period? The size of the uropygial gland varies significantly over the annual cycle in male and female house sparrows (Pap *et al.*, 2010), and the volume of the gland is the largest during the end of the breeding period and the beginning of the moult. As a result of the large amount of oil produced during this period, and because of the costly gland secretion (Piault *et al.*, 2008), coccidians affected the size of the gland only during that period of the annual cycle when the gland size is the largest. The significant negative effect of coccidians on gland size at the beginning of moulting suggests that the effect of parasites on feather quality through the amount of preen oils may depend on season. Clearly, the effect of season on the relationship between coccidian infestation, gland size, and feather quality measures deserves further experimental studies.

FEATHER QUALITY

Coccidian infestation significantly reduced the general condition of the flight feathers, as revealed by shorter and lighter primaries with a smaller vane area, thin rachis, and high density of barbs and barbules of the infested relative to the Med house sparrows. Furthermore, after controlling for the confounding effect of the feather length on bending stiffness, we have shown that coccidian infestation increased the deflection under load of the flight feathers. These results generally are in line with our previous work on the house sparrow (Pap *et al.*,

2011); however, they further extend our knowledge about the effect of these parasites on the fine structure of flight feathers. To our knowledge, these results are the first to show that parasites may not only affect the moulting of wild birds (Langston & Hillgarth, 1995) and the coloration of feathers (Hill & Brawner, 1998; Hórak *et al.*, 2004), but also have a significant negative effect on feather structure and quality. These detriments may have long-lasting consequences on fitness as a result of impaired manoeuvrability and flying capacity (Swaddle *et al.*, 1996), as well as the ability of feathers to resist physical and biological abrasion and degradation. Regarding the underlying mechanism, our results are in line with experimental studies on the negative effect of corticosterone on feather growth rate and quality reported by Romero *et al.* (2005) and DesRochers *et al.* (2009). This may provide an alternative to the nutritional explanation of coccidians' effects on moulting house sparrows. Parasite infestation is known to affect the stress response of birds (Raouf *et al.*, 2006), which may reprogramme the protein uptake and hence influence feather quality of moulting birds (DesRochers *et al.*, 2009). An experimental study where the infestation and the level of stress hormones are manipulated concurrently could reveal the underlying hormonal cost of parasitism on the quality of flight feathers of moulting birds. It is interesting to note that the exogenous corticosterone hormone administration (DesRochers *et al.*, 2009) and coccidian infestation affected the feather quality measures in the same direction. The feather mass decreased and the density of barbs and barbules increased in the case of stressed and infested birds compared to the control and medicated groups. Finally, it is interesting to note that the effect of coccidians on feather quality measures was similar with the effect of high speed of moult (Vágási *et al.*, 2012), where infested and fast-moulting birds grow short and light flight and body feathers with a thin rachis and high barb and barbule density. These results suggest that, under various stressors, the same mechanisms are responsible for the reduction in feather quality. For example, the high barb and barbule density of the flight and body feathers of the birds experiencing certain stress can be explained by the low interbarb and interbarbule distances and/or by the reduced number of barbs and barbules throughout the feather vane compared to non-infested and slow-moulting house sparrows. The lack of significant effect of the moulting speed on the total number of barbs of the body feathers of the house sparrow suggests the validity of the first scenario (Vágási *et al.*, 2012). However, further studies are needed to reveal the mechanism of the development of the feather's physical properties.

Properties that describe feather quality differed similarly between experimental groups in the case of feathers grown after the first and second postnuptial moult. This indicates that, in contrast to condition measures (e.g. wing length), the negative effect of coccidians on feather quality emerges after several weeks of experimentation and remains unchanged even after more than 1 year of continuous infestation. Despite the stimulation of the immune system (Hörak *et al.*, 2004; Pap *et al.*, 2009, 2011; Lemus, Vergara & Fargallo, 2010) and the continuous drainage of nutrients by the coccidians, house sparrows improved several abrasion-independent measures of feather quality during moult, such as rachis width and barbule density, whereas feather mass and barb density significantly decreased between the two subsequent moults. This suggests that, during prolonged, chronic infestation, birds may change the structure of the flight feathers.

Finally, we found that the feather quality measures, which are expected to separately influence the resistance to wear and cyclic load (Dawson *et al.*, 2000; Weber *et al.*, 2005; DesRochers *et al.*, 2009), are not necessarily inter-correlating (for body feathers, see Aparicio *et al.*, 2003; Broggi *et al.*, 2011). This result stresses the importance of measuring several traits to honestly characterize the quality of the feather. The qualitatively similar results for the aviary and wild-living house sparrows indicate that the absence of significant associations and trade-offs between quality measures cannot be explained by the saturated nutritional supply or stressful confinement of the aviary birds. Instead, our results show that the physical properties of the flight feathers, such as rachis width, vane area, density of vane, and bending stiffness are largely independent traits. Clearly, the importance of these feather quality traits in maintaining the integrity of the feather, and resistance against wear and air pressure, deserves further study. Finally, our results show that coccidians do not affect the relationship between feather quality measures; however, under more stressful conditions, a trade-off between quality traits is expected (de la Hera *et al.*, 2009, 2010a).

In conclusion, we have shown that the long-term chronic coccidian infestations have a significant and negative effect on the condition of house sparrows. The effect of these parasites on the host depends on the annual cycle, as revealed by the increased gland size in medicated birds compared to the infested house sparrows during moulting, when the gland size is the largest. The positive effect of medication on the wing length emerged only after a prolonged (i.e. more than 1 year) reduction of natural infestation levels, highlighting the importance of multi-annual studies. By contrast, feather quality measures were signifi-

cantly affected by coccidians over both the short and long term, which demonstrates the sensitivity of flight feathers to mild but chronic parasite stress during a moult. These findings may have implications in species conservation and animal health care. We suggest that practitioners should take more care in controlling coccidian infestations in wild birds that are brought to rehabilitation centres or used for reproduction under aviary conditions, where a long-term exposure to these spontaneously emerging parasites may have substantial undesired consequences on fitness.

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REFERENCES

- Aguilar TM, Maia R, Santos ESA, Macedo RH. 2008.** Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. *Behavioral Ecology* **19**: 292–301.
- Allen PC, Fetterer RH. 2002.** Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clinical Microbiology Reviews* **15**: 58–65.
- Aparicio JM, Bonal R, Cordero PJ. 2003.** Evolution of the structure of tail feathers: implications for the theory of sexual selection. *Evolution* **57**: 397–405.
- Baeta R, Faivre B, Motreuil S, Gaillard M, Moreau J. 2008.** Carotenoid trade-off between parasitic resistance and sexual display: an experimental study in the blackbird (*Turdus merula*). *Proceedings of the Royal Society of London Series B, Biological Sciences* **275**: 427–434.

- Bonser RHC. 1995.** Melanin and the abrasion resistance of feathers. *Condor* **97**: 590–591.
- Broggi J, Gamero A, Hohtola E, Orell M, Nilsson J-Å. 2011.** Interpopulation variation in contour feather structure is environmentally determined in great tits. *PLoS ONE* **6**: e24942.
- Cotgreave P, Clayton DH. 1994.** Comparative analysis of time spent grooming by birds in relation to parasite load. *Behaviour* **131**: 171–187.
- Dawson A, Hinsley SA, Ferns PN, Bonser RHC, Eccleston L. 2000.** Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. *Proceedings of the Royal Society of London Series B, Biological Sciences* **267**: 2093–2098.
- DesRochers DW, Reed JM, Awerman J, Kluge JA, Wilkinson J, van Griethuijsen LI, Aman J, Romero LM. 2009.** Exogenous and endogenous corticosterone alter feather quality. *Comparative Biochemistry and Physiology* **152**: 46–52.
- Dolnik OV, Hoi H. 2010.** Honest signalling, dominance hierarchies and body condition in House Sparrows *Passer domesticus* (Aves: Passeriformes) during acute coccidiosis. *Biological Journal of the Linnean Society* **99**: 718–726.
- Filipiak L, Mathieu F, Moreau J. 2009.** Caution on the assessment of intestinal parasitic load in studying parasite-mediated sexual selection: the case of blackbirds coccidiosis. *International Journal of Parasitology* **39**: 741–746.
- Galván I, Barba E, Piculo R, Cantó JL, Cortés V, Monrós JS, Atiénzar F, Proctor H. 2008.** Feather mites and birds: an interaction mediated by uropygial gland size? *Journal of Evolutionary Biology* **21**: 133–144.
- Giraudeau M, Duval C, Guillon N, Bretagnolle V, Gutierrez C, Heeb P. 2010.** Effects of access to preen gland secretions on mallard plumage. *Naturwissenschaften* **97**: 577–581.
- Greiner EC. 2008.** *Isospora*, *Atoxoplasma*, and *Sarcocystis*. In: Atkinson CT, Tomas NJ, Hunter DB, eds. *Parasitic diseases of wild birds*. New York, NY: Wiley-Blackwell, 108–119.
- Hall KSS, Fransson T. 2000.** Lesser whitethroats under time-constraint moult more rapidly and grow shorter wing feathers. *Journal of Avian Biology* **31**: 583–587.
- de la Hera I, Pérez-Tris J, Tellería JL. 2009.** Migratory behaviour affects the trade off between feather growth rate and feather quality in a passerine bird. *Biological Journal of the Linnean Society* **97**: 98–105.
- de la Hera I, Pérez-Tris J, Tellería JL. 2010a.** Migratory behavior and differential resource allocation between wing and tail feathers in a passerine bird. *Auk* **127**: 647–652.
- de la Hera I, Pérez-Tris J, Tellería JL. 2010b.** Relationships among timing of moult, moult duration and feather mass in long-distance migratory passerines. *Journal of Avian Biology* **41**: 609–614.
- Hill GE, Brawner WR. 1998.** Melanin-based plumage coloration in the house finch is unaffected by coccidial infection. *Proceedings of the Royal Society of London Series B, Biological Sciences* **265**: 1105–1109.
- Hill GE, Doucet SM, Buchholz R. 2005.** The effect of coccidial infection on iridescent plumage coloration in wild turkeys. *Animal Behaviour* **69**: 387–394.
- Hörak P, Saks L, Karu U, Ots I, Surai PF, McGraw KJ. 2004.** How coccidian parasites affect health and appearance of greenfinches. *Journal of Animal Ecology* **73**: 935–947.
- Jacob J, Ziswiler V. 1982.** The uropygial gland. In: Farner DS, King JR, Parkes KC, eds. *Avian biology*, Vol. 6. New York, NY: Academic Press, 199–324.
- Jenni L, Winkler R. 1994.** *Moult and ageing of European passerines*. London: Academic Press.
- Klaassen M. 1995.** Moult and basal metabolic costs in males of two subspecies of stonechats: the European *Saxicola torquata rubicula* and the East African *S. t. axillaris*. *Oecologia* **104**: 424–432.
- Langston N, Hillgarth N. 1995.** Moult varies with parasites in Laysan albatrosses. *Proceedings of the Royal Society of London Series B, Biological Sciences* **261**: 239–243.
- Lemus JA, Vergara P, Fargallo JA. 2010.** Response of circulating T-lymphocytes to a coccidian infection: insights from a parasitization–vaccination experiment. *Functional Ecology* **24**: 638–645.
- Lindman HR. 1974.** *Analysis of variance in complex experimental designs*. San Francisco, CA: W. H. Freeman & Co.
- Lindström A, Visser GH, Daan S. 1993.** The energetic cost of feather synthesis is proportional to basal metabolic rate. *Physiological Zoology* **66**: 490–510.
- Møller AP. 1997.** Parasitism and the evolution of host life history. In: Clayton DH, Moore J, eds. *Host–parasite evolution: general principles and avian models*. Oxford: Oxford University Press, 105–127.
- Møller AP, Arriero E, Lobato E, Merino S. 2009.** A meta-analysis of parasite virulence in nestling birds. *Biological Reviews* **84**: 567–588.
- Møller AP, Erritzøe J, Rózsa L. 2010.** Ectoparasites, uropygial glands and hatching success in birds. *Oecologia* **163**: 303–311.
- Monaghan P, Metcalfe NB, Torres R. 2009.** Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters* **12**: 75–92.
- Moreno-Rueda G. 2010a.** Experimental test of a trade-off between moult and immune response in house sparrows *Passer domesticus*. *Journal of Evolutionary Biology* **23**: 2229–2237.
- Moreno-Rueda G. 2010b.** Uropygial gland size correlates with feather holes, body condition and wingbar size in the house sparrow *Passer domesticus*. *Journal of Avian Biology* **41**: 229–236.
- Moreno-Rueda G. 2011.** House sparrows *Passer domesticus* with larger uropygial glands show reduced feather wear. *Ibis* **153**: 195–198.
- Mougeot F, Pérez-Rodríguez L, Sumozas N, Terraube J. 2009.** Parasites, condition, immune responsiveness and carotenoid-based ornamentation in male red-legged partridge *Alectoris rufa*. *Journal of Avian Biology* **40**: 67–74.
- Murphy ME, King JR. 1990.** Diurnal changes in tissue glutathione and protein pools of molting white-crowned

- sparrows: the influence of photoperiod and feeding schedule. *Physiological Zoology* **63**: 1118–1140.
- Pap PL, Vágási CI, Czirják GÁ, Barta Z. 2008.** Diet quality affects postnuptial molting and feather quality of the house sparrow (*Passer domesticus*): interaction with humoral immune function? *Canadian Journal of Zoology* **86**: 834–842.
- Pap PL, Vágási CI, Czirják GÁ, Titilincu A, Pintea A, Barta Z. 2009.** Carotenoids modulate the effect of coccidian infection on condition and immune response in moulting house sparrows. *Journal of Experimental Biology* **212**: 3228–3235.
- Pap PL, Vágási CI, Czirják GÁ, Titilincu A, Pintea A, Osváth G, Fülöp A, Barta Z. 2011.** The effect of coccidians on the condition and immune profile of molting house sparrows (*Passer domesticus*). *Auk* **128**: 330–339.
- Pap PL, Vágási CI, Osváth G, Mureşan C, Barta Z. 2010.** Seasonality in the uropygial gland size and feather mite abundance in house sparrows *Passer domesticus*: natural covariation and an experiment. *Journal of Avian Biology* **41**: 653–661.
- Piault R, Gasparini J, Bize P, Paulet M, McGraw KJ, Roulin A. 2008.** Experimental support for the makeup hypothesis in nestling tawny owls (*Strix aluco*). *Behavioral Ecology* **19**: 703–709.
- Pinheiro JC, Bates DM, DebRoy S, Sarkar D, R Development Core Team. 2011.** *nlme: linear and nonlinear mixed effects models*. R package version 3.1-98. Vienna: R Development Core Team.
- Quinn GP, Keough MJ. 2002.** *Experimental design and data analysis for biologists*. Cambridge: Cambridge University Press.
- R Development Core Team. 2011.** *R: a language and environment for statistical computing*. Available at: <http://www.R-project.org/>. Vienna: R Foundation for Statistical Computing.
- Raouf SA, Smith LC, Brown MB, Wingfield JC, Brown CR. 2006.** Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows. *Animal Behaviour* **71**: 39–48.
- Reneerkens J, Piersma T, Damsté JSS. 2002.** Sandpipers (Scolopacidae) switch from monoester to diester preen waxes during courtship and incubation, but why? *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 2135–2139.
- Romero LM, Storch D, Wingfield JC. 2005.** Corticosterone inhibits feather growth: potential mechanism explaining seasonal down regulation of corticosterone during molt. *Comparative Biochemistry and Physiology A* **152**: 65–73.
- Ruiz-de-Castañeda R, Burt EH Jr, González-Braojos S, Moreno J. 2012.** Bacterial degradability of an intrafeather unmelanized ornament: a role for feather-degrading bacteria in sexual selection? *Biological Journal of the Linnean Society* **105**: 409–419.
- Sepp T, Karu U, Blount JD, Sild E, Männiste M, Hõrak P. 2012.** Coccidian infection causes oxidative damage in greenfinches. *PLoS ONE* **7**: e36495.
- Serra L. 2001.** Duration of primary moult affects primary quality in grey plovers *Pluvialis squatarola*. *Journal of Avian Biology* **32**: 377–380.
- Shawkey MD, Pillai SR, Hill GE. 2003.** Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. *Journal of Avian Biology* **34**: 345–349.
- Soler JJ, Peralta-Sánchez JM, Martín-Platero AM, Martín-Vivaldi M, Martínez-Bueno M, Møller AP. 2012.** The evolution of size of the uropygial gland: mutualistic feather mites and uropygial secretion reduce bacterial loads of eggshells and hatching failures of European birds. *Journal of Evolutionary Biology* **25**: 1779–1791.
- Swaddle JP, Witter MS, Cuthill IC, Budden A, McCowen P. 1996.** Plumage condition affects flight performance in common starlings: implications for developmental homeostasis, abrasion and moult. *Journal of Avian Biology* **27**: 103–111.
- Vágási CI, Pap PL, Vincze O, Benkő Z, Marton A, Barta Z. 2012.** Haste makes waste but condition matters: molt rate–feather quality trade-off in a sedentary songbird. *PLoS ONE* **7**: e40651.
- Weber TP, Borgudd J, Hedenström A, Persson K, Sandberg G. 2005.** Resistance of flight feathers to mechanical fatigue covaries with moult strategy in two warbler species. *Biology Letters* **1**: 27–30.