DE TTK

1949
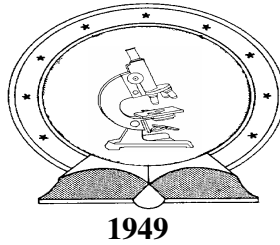
Costs of moult: feather quality, signals and physiology

A vedlés költségei: tollak minősége, jelzések és fiziológia

Egyetemi doktori (PhD) értekezés

Vágási I. Csongor

Témavezetők:
Prof. Barta Zoltán
Dr. Pap Péter László

DEBRECENI EGYETEM
Természettudományi Doktori Tanács
Juhász-Nagy Pál Doktori Iskola
Debrecen, 2013

Ezen értekezést a Debreceni Egyetem Természettudományi Doktori Tanács Juhász-Nagy Pál Doktori Iskola Biodiverzitás programja keretében készítettem a Debreceni Egyetem természettudományi doktori (PhD) fokozatának elnyerése céljából.

Debrecen, 201. . . . .

<div align="right">

…………………….
Vágási I. Csongor

</div>

Tanúsítom, hogy Vágási I. Csongor doktorjelölt 2006–2009 között a fent megnevezett Doktori Iskola Biodiverzitás programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Az értekezés elfogadását javasolom.

Debrecen, 201. . . . .

<div align="right">

…………………….
Prof. Barta Zoltán

</div>

Tanúsítom, hogy Vágási I. Csongor doktorjelölt 2006–2009 között a fent megnevezett Doktori Iskola Biodiverzitás programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Az értekezés elfogadását javasolom.

Debrecen, 201. . . . .

<div align="right">

…………………….
Dr. Pap Péter László

</div>

Costs of moult: feather quality, signals and physiology

A vedlés költségei: tollak minősége, jelzések és fiziológia

Értekezés a doktori (Ph.D.) fokozat megszerzése érdekében
a Biológia tudományágban

Írta: Vágási I. Csongor okleveles biológus

Készült a Debreceni Egyetem Juhász-Nagy Pál doktori iskolája
(Biodiverzitás programja) keretében

Témavezetők:
Prof. Barta Zoltán
Dr. Pap Péter László

A doktori szigorlati bizottság:
    elnök: Dr. Bánfalvi Gáspár ....
    tagok: Dr. Pénzes Zsolt ....
                    Dr. Végvári Zsolt ....

A doktori szigorlat időpontja: 2013. március 12.

Az értekezés bírálói:
                    Dr. ....
                    Dr. ....
                    Dr. ....

A bírálóbizottság:
    elnök: Dr. ....
    tagok: Dr. ....
                    Dr. ....
                    Dr. ....
                    Dr. ....

Az értekezés védésének időpontja: 201… . ……………… … .

Contents

Papers are referred throughout the text by their boldface roman numerals (**I–V**).

Acknowledgements

CHAPTER I

In this chapter I present the scientific background of my objectives, point out issues that are less clearly known and were addressed in my thesis, formulate my questions and predictions, and provide an outline of the major results and their discussion.

General introduction

Plumage

Birds (Aves) are feathered quadrupeds. The whole set of feathers constitutes the plumage, a corneous integumentary clothing, which is one of the most salient avian peculiarities. Several adaptations of birds are at least in part related to being feathered and capable of flight. Feather's strength-to-weight ratio is one of the highest among biological materials. This remarkable bonding of toughness with lightness stems from a highly complex architecture of feathers (Ginn & Melville 2007, Lingham-Soliar *et al*. 2010, Bodde *et al*. 2011). Beyond this general nanoscale structure, feathers come in a spectacular variety of types, forms, macrostructures, sizes and colours. In part this could endow birds with the status of favourite organisms among scientists and amateur naturalists.

Functions and structure

Plumage has variegated functions (reviewed by Jenni & Winkler 1994, Ginn & Melville 2007, Stettenheim 2000) from the trivial insulation, physical barrier (e.g. protection against mechanical forces, solar radiation, parasites, and ensuring water repellence), camouflage, extravagant visual displays and flight to the more surprising vocalization (Bostwick & Prum 2005, Clark *et al*. 2011), olfactory signalling (Hagelin 2007), carriage of drinking water (Cade & Maclean 1967), toxic warfare against ectoparasitic chewing lice (Dumbacher 1999) and ease of feather loss to elude predation (Møller *et al*. 2006).
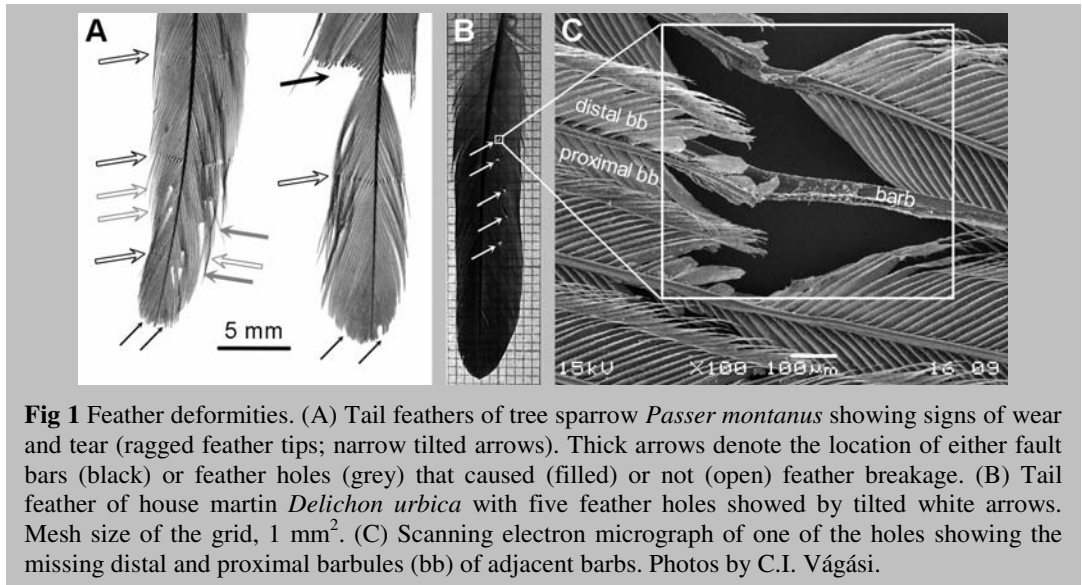
The plumage consists chiefly of three feather types, flight contour feathers (remiges and rectrices), body contour feathers (wing coverts, external layer of body feathers) and down feathers (proximal layer of body feathers). The first two classes of feathers have at least to some extent pennaceous feather parts (planar feather vanes with barbs interlocked), whilst downs are fully plumulaceous (see Prum 1999). Shallowly speaking, flight feathers function in propulsion, lift and manoeuvring, body contour feathers in streamlining, protection, camouflage, communication and insulation, and downs in insulation (Jenni & Winkler 1994, Ginn & Melville 2007).

Feathers are epidermal appendages with a ramified construction (Fig 1). The shaft is anchored into the skin by the quill (calamus), which continues above the skin in rachis, where the vanes (vexillum) are attached. Vanes are formed by complex primary and secondary branching (barbs [rami] and barbules [radii], respectively). Barbules of two adjacent barbs are interconnected by small hooks (barbicels). This highly complex raster endows the excellent toughness and lightness at one place. Spacing, number and microstructure of these components define feather's length, area, mass, strength, flexural stiffness, and in turn determine heat retention efficiency and flight capacity.

Variation in feather quality

Flight feathers are important constituents of the flight apparatus. Feathers are dead structures (i.e. no repair from the base), which implies that they continuously deteriorate after becoming unsheathed. Deteriorations include wear and breakage, which are known to impair plumage functionality. The bulk of feather is β-keratin, hence any variation in quality might stem from structural aspects that ultimately determine the mechanical properties and resistance against insults (Bodde *et al*. 2011). Deterioration results from exposure to fatiguing agents such as feather-degrading parasites, bending cycles, friction and UV-B radiation (Bergman 1982, Jenni & Winkler 1994, Borgudd 2003, Weber *et al*. 2005, Ginn & Melville 2007). Such malicious impacts could lead to different modes of feather damage: wear and tear, feather holes and fault bars, and associated feather fracture (Fig 1). These deformities can modify the strength, length, pointedness and area of the wing altering the aerodynamic conditions and morphology

required for optimal flight (Pennycuick 2008, Weber *et al*. 2010). Therefore, a standard handicapping manipulation of parental costs or flight capacity is the truncation of wing area (e.g. Love & Williams 2008, Tsurim *et al*. 2010).

Fig 1 Feather deformities. (A) Tail feathers of tree sparrow *Passer montanus* showing signs of wear and tear (ragged feather tips; narrow tilted arrows). Thick arrows denote the location of either fault bars (black) or feather holes (grey) that caused (filled) or not (open) feather breakage. (B) Tail feather of house martin *Delichon urbica* with five feather holes showed by tilted white arrows. Mesh size of the grid, 1 mm^2. (C) Scanning electron micrograph of one of the holes showing the missing distal and proximal barbules (bb) of adjacent barbs. Photos by C.I. Vágási.

First, feather wear (Fig 1A) is caused by friction and is more detrimental to flight feathers positioned at wingtips making them more rounded and ragged by time (**Paper I**). Variation in feather wear can have numerous sources. More abraded birds are those of young and very old ages, females, birds investing more in reproduction, having smaller uropygial gland, having less melanin granules deposited in the keratin matrix, that have forced short duration of moult, migrate further south or are prone to excited behaviour in captivity (Bonser 1995, Merilä & Hemborg 2000, Serra 2001, O'Hara 2002, Moreno-Rueda 2011, Sild *et al*. 2011).

Second, feather holes are minute defects in the feather vane of flight feathers (Fig 1B–C). Holes are claimed to be the feeding marks of chewing lice (Phthiraptera; Møller 1991, Vas *et al*. 2008), partly because lice graze the barbs and barbules of downy body feathers (Clayton 1990), but this cause–effect relationship in case of flight feathers is currently controversial and experimental support is lacking (**Paper I**). Feather-degrading bacteria and fungi possess keratinolytic enzymes that break down the keratin (Goldstein *et al*. 2004, Gunderson 2008, Lingham-Soliar *et al*. 2010) so initiating scratches and fracture (Shawkey *et al*. 2007). The abundance of keratinolytic

bacilli also positively correlates with feather hole burden in house sparrows *Passer domesticus* (GLM with Poisson error distribution, $\chi^2_{1,116} = 13.52$, $P = 0.0002$; Fülöp A, Czirják GÁ, Bărbos L, Pap PL & Vágási CI in prep.). Factors that add variation to feather hole incidence are: ornament size, testosterone implantation, feather melanin content, age, genetic component, wintering conditions, season, uropygial gland size, fledging date, brood size and moult (Møller 1991, Saino *et al*. 1995, Kose *et al*. 1999, Kose & Møller 1999, Møller & de Lope 1999, Møller *et al*. 2004a, Saino *et al*. 2004, Pap *et al*. 2007, Vas *et al*. 2008, Moreno-Rueda 2005, Moreno-Rueda 2010a).

Third, steroid hormones are known to suppress keratinogenesis (de Ridder *et al*. 2002, Strochlic & Romero 2008). Corticosterone (CORT) stress response seems to be responsible for the formation of fault bars (reviewed by Jovani & Diaz-Real 2012) as shown by the higher concentration of feather CORT at segments with fault bar(s) than contiguous parts without them (Bortolotti *et al*. 2009). Fault bars lie perpendicular to feather shaft (Fig 1A), result from malformation of barbules and are developed pre-dominantly during the night (Sarasola & Jovani 2006, Jovani & Diaz-Real 2012). The occurrence of fault bars depends on feather group (more common on rectrices), age (juveniles harbour more), sex, and moult, it is inversely related to condition (viz. size and abdominal fat store), melanin pigmentation, tail, wing and feather length, and positively to size of bursa of Fabricius, advancement in breeding season and tail or-nament elongation (Slagsvold 1982, Burtt 1986, Møller 1989, Møller 1994, Møller *et al*. 1996, Dawson *et al*. 2001, Blanco & de la Puente 2002, Jovani & Blas 2004, Serrano & Jovani 2005, Sarasola & Jovani 2006, Pap *et al*. 2007, Jovani *et al*. 2010).

Study motivation (**Papers I** and **II**)

Little is known about the accumulation of feather wear and holes during the annual cycle, and how the degree of wear, number of holes and fault bars are interrelated with each other and associated with indicators of feather quality and physical condition. It is also poorly understood whether a compromised moult might affect the number of fault bars.

The quality of feathers cannot be portrayed only by defects and feather losses. Feather damages are rooted in the amount and fine-scale arrangement of keratin and

melanin granules in the feather matrix (Bonser 1995, Dawson *et al*. 2000), such that feathers with lower amount of building material and constrained structure are more susceptible to failure by wearing, microcracks or rupture (Weber *et al*. 2010). Remiges of house sparrows with higher rachis diameter endure better an experimental exposure to bending cycles (Pap PL, Vágási CI, Marton A & Bărbos L unpubl. data; Fig 2). Feathers with 'damage-proof' structure better withstand the vicissitudes to which they are exposed. Thus, two feathers of different quality should be subjected to distinct damaging impact, when all other are equal, to attain similar degradation. Arguably such dependencies are even more significant if we consider that lower quality feathers degrade more by time (Dawson *et al*. 2000 and **Paper I**); so damage accumulation trajectories largely depend on the quality of feathers right after their production.
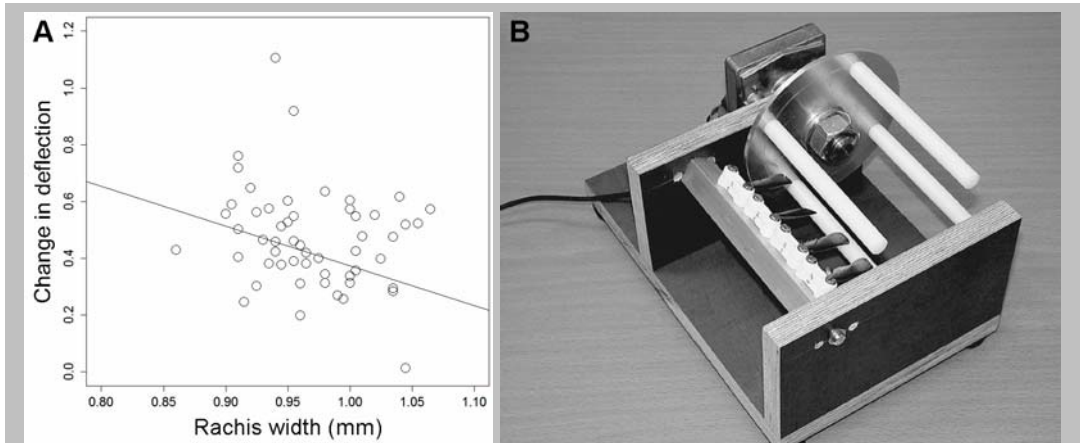

Fig 2 Feather fatigue. (A) The deflection under load (2 g) of primary feathers of house sparrows was measured twice, after collection (baseline) and again after being exposed to simulated cyclic bending (fatigued) using a feather fatigue apparatus (B; image from Weber *et al*. 2005). Change in deflection = fatigued minus baseline. Cyclic loading reduced feather stiffness (i.e. increased deflection) of all feathers (only positive values on the y-axis). Change in deflection was inversely related to rachis width, indicating that thicker rachises resist better against cyclic fatigue (general linear model after accounting for sex effect, b (SE) = –1.41 (0.56), $t_{1,52}$ = –2.51, P = 0.015).

Feather quality can be measured via (1) structural parameters (mass, length, area, rachis width and branch spacing) and/or (2) mechanical properties (flexural stiffness, Young's modulus and the second moment of rachis' cross-sectional area; see Fig 2 in Purslow & Vincent 1978). Studies that make up this thesis employed mainly the first group of measurements, and in one study the flexural stiffness. In the next few lines I give a short overview of the intercorrelations between the above feather quality traits.

Young's (elasticity) modulus, a measure of keratin quality, is highly conservative across birds, thus most variation in feather resistance is attributable to the amount of keratin rather than its material aspect (Bonser & Purslow 1995, Bachmann *et al*. 2012). Mechanical properties are tightly positively related (e.g. Dawson *et al*. 2000, Weber *et al*. 2010, Bodde *et al*. 2011, Wang *et al*. 2012) as indicated by the decrease along the shaft (from calamus towards the distal end) of rachis' bending stiffness (a measure of rigidity), second moment of the rachis (a measure of keratin quantity) and resistance against failure (Bonser & Purslow 1995, Butler & Johnson 2004, Bachmann *et al*. 2012). Volant birds have thick rachis to withstand extreme buckle under aerodynamic load (Nudds & Dyke 2010, Wang *et al*. 2012) and rachis width is strongly related to stiffness within and among species (Purslow & Vincent 1978, Dawson *et al*. 2000, Tubaro 2003). Higher elasticity towards feather tips might evolve to diminish feather wearing (Bachmann *et al*. 2012). Feather mass, vane area, length and rachis width reflect the keratin content of feathers, thus they are expected to form another cluster of interrelated traits. In fact, such nexus has been shown for some species including correlations with mechanical properties like bending stiffness (Dawson *et al*. 2000, Tubaro 2003, DesRochers *et al*. 2009, de la Hera *et al*. 2010a, de la Hera *et al*. 2010b, Weber *et al*. 2010). In conclusion, the abovementioned connections between feather quality traits suggest that the soft traits (i.e. more easily measured ones) of the first group can be used to infer hard traits of the second group (e.g. second moment).

Hypothetically we can expect a positive coupling among feather quality traits if all aspects are commonly dependent on the bearer's phenotype (e.g. nutritional or physiological state). Alternatively, a conflict of investment might arise, where allocations into traits or trait groups are traded off against each other (e.g. Weber *et al*. 2005, Moreno & Soler 2011). If traits form separated clusters, a combination of previous two (i.e. positive within and negative between cluster relations) or no association is predicted (decoupling).

Study motivation (**Papers I** and **III**)

Although unfolding the associations among feather quality traits may shed light on contingent constraints in the evolution of feather structure, this was

rarely addressed. We tested the associations between several flight feather quality measures correlatively in great tits and among parasitized and experimentally medicated house sparrows across multiple moulting seasons.

Although body feathers serve several functions, their geometry is much more overlooked from both proximate and ultimate perspective. From a proximate perspective, age is the major predictor; juveniles grow less sophisticated feathers (Svensson 1992, Jenni & Winkler 1994, Butler *et al*. 2008, Moreno & Soler 2011). This could be driven by the short development time of nestlings (Butler *et al*. 2008). Certain parasites are adapted to exploit the host's body feathers (Clayton 1990, Burtt & Ichida 1999). Moult speed was also suspected to explain variation in body feather structure (Nilsson & Svensson 1996, Dawson *et al*. 2000, Broggi *et al*. 2011) and microstructure-related spectral properties of colourful body feather patches that serve as secondary sexual ornaments or social signals (Serra *et al*. 2007, Griggio *et al*. 2009).

Study motivation (**Papers II** and **IV**)

We tested experimentally for the first time the proposition that moult rate might compromise body feather structure. We also studied the potential contribution of body condition as modulator of moult costs. Within the same experiment, we measured the colouration of two different ornaments as well.

Plumage signals

Conditions prevailing during feather synthesis determine how feathers are structured and this ultimately affects not only their durability, but showiness (spectral properties) of colourful plumage advertisements as well. Plumage colouration can be either pigmentary or structural. The former depend largely on the melanin or carotenoid pigments deposited in the feather matrix, while the latter determined by the nanometer-scale arrangement of keratin, pigmented layers and their interface (Hill & McGraw 2006). However, the picture can be more intricate, inasmuch pigments and microstructure interplay to determine feather's reflectance (Shawkey & Hill 2005, Hill & McGraw 2006, Galván 2011, Kemp *et al*. 2012). Ornamental feathers can be struc-

tured and coloured only when growing, so these are fixed traits in the strict sense. This encouraged the integration of feather development and pigmentation lines of research (Badyaev & Young 2004).

A number of models were postulated to explain the evolution, maintenance and honesty of sexual and social signals (reviewed by Emlen *et al*. 2012). Briefly, common to all is the assumption that signals indicate genetic or phenotypic quality not necessarily encompassing condition-dependence. Cheaters are disqualified either because they cannot afford to invest in ornament production with high signalling value or cannot bear the consequences of maintaining a full-sized signal (Emlen *et al*. 2012). A large pile of studies addressed these theoretical considerations using carotenoid-based and gaudy structural colouration of birds and other vertebrates. It has been shown that the costs of signals depend on several circumstances experienced during production including nutritional state, energy capital (condition), parasite load, immune responsiveness, hormonal profile and oxidative physiology (reviewed by Hill & McGraw 2006). Melanised and depigmented white ornaments, however, were much less scrutinized making them ripe for similar investigations.

Evidence about melanin-based signalling suggests the existence of maintenance (e.g. Veiga 1996, Nakagawa *et al*. 2008) and production costs as well, but the mechanistic basis of the latter have remained unclear. Several hypotheses have been put forward to find production costs for melanised plumage traits (reviewed by Hill & McGraw 2006, McGraw 2008). Briefly, some studies have focused on the nutritional condition during moult. Juvenile male house sparrows with higher blood protein levels grew larger badges (Veiga & Puerta 1996) and those supplemented with an essential precursor of melanin, the phenylalanine, grew blacker badges (Poston *et al*. 2005), while zebra finches *Taeniopygia guttata* fed with extra calcium, which is cofactor of melanin-producing enzymes, grew larger black signals (McGraw 2007; but see Stewart & Westneat 2010). Galván & Alonso-Alvarez (2008) demonstrated the inhibitory effect of an antioxidant, the glutathione, on the melanised breast stripe of great tits *Parus major*. Buchanan *et al*. (2001) showed that testosterone implants enhanced both the metabolic rate and badge size in male house sparrows. Roulin *et al*. (2008) demonstrated that CORT-implanted juvenile barn owls *Tyto alba* developed plumage with

less phaeomelanin. All of these factors can rely on condition-dependence, as presumably prime quality birds have better access to food resources, endure more the elevated metabolic costs of testosterone-dependent ornament production, and have lower stress-induced CORT profiles. However, other studies could not detect nutritional influence (e.g. Gonzalez *et al*. 1999, Stewart & Westneat 2010), while the link between immune capacity and colouration mediated by hormones was partially supported and is more complicated than previously thought (e.g. Buchanan *et al*. 2003).

Another honesty-reinforcing mechanism was envisaged by Serra *et al*. (2007) and Griggio *et al*. (2009) who argued that signals might convey information about how the bearer timed its activities prior to and during the moult and hypothesized that an accelerated moult speed adversely affects the value of the carotenoid-based and structural UV-blue ornaments ('moult constraint' hypothesis).

Study motivation (**Paper IV**)

The validity of the 'moult constraint' hypothesis about the reinforcement of melanised and depigmented plumage ornament honesty was not tested or remained controversial, respectively. We manipulated moult rate and followed its effect on the size and brightness of melanised badge and depigmented wingbar of male house sparrows.

Avian moult

Adaptations to overcome feather erosion abound in birds because accumulating damages bear high fitness costs. Feather maintenance is orchestrated by several mechanisms activated during and/or after feather synthesis: steroid downregulation (Anderson 2006, Romero 2002), 'adaptive fault bar allocation' (Jovani & Blas 2004, Pap *et al*. 2007, Jovani *et al*. 2010 and references therein), melanin pigmentation (Bonser 1995, Goldstein *et al*. 2004; but see Gunderson *et al*. 2008), behavioural defence (preening, dusting, bathing and sunning [Cotgreave & Clayton 1994, Saranathan & Burtt 2007; reviewed by Clayton *et al*. 2010]), oily secretion of the uropygial gland (Jacob & Ziswiler 1982, Shawkey *et al*. 2003, Giraudeau *et al*. 2010, Moreno-Rueda

2010a, Moreno-Rueda 2011, Soler *et al*. 2012; but see Cristol *et al*. 2005, Czirják *et al*. 2013, Moyer *et al*. 2003) and bill shape (Clayton *et al*. 2010). Damages can also be left behind and feather quality resettled by the (annual) replacement of feathers (i.e. moult).

Moult (or shedding) is the process by which the avian integument or a part of it is regularly exchanged (Burtt 1986, Jenni & Winkler 1994, Ginn & Melville 2007). Moulting strategies had undergone a radiation in extent, timing, duration, sequence, location, number per year at all taxonomic levels and also within species (Jenni & Winkler 1994, Barta *et al*. 2006, Ginn & Melville 2007, Barta *et al*. 2008, de la Hera *et al*. 2009a, de la Hera *et al*. 2010a, de la Hera *et al*. 2010b). This diversity is still un-explained and subject of population-level and phylogenetic comparative analyses (Holmgren & Hedenström 1995, Svensson & Hedenström 1999, de la Hera *et al*. 2009b, Rohwer *et al*. 2009, de la Hera *et al*. 2010c, de la Hera *et al*. 2011). Although moult occurs infrequently, its prime adaptive value lies in being the very single mean of comprehensive and in-depth feather functionality retrieval. In fact, old feathers are replaced with a new generation of functionally superior feathers (e.g. Williams & Swaddle 2003, Pap *et al*. 2007). The structural, material and mechanical properties of feathers are determined once, at their development, hence moult has large footprint on future fitness landscapes.

Despite the paramount importance of moult in avian ecology and evolution, it is odd that ornithologists have somehow overlooked this stage of the bird's annual cycle in comparison with reproduction and migration. For instance, currently large-scale phylogenetic comparative analyses on moult evolution, which are based on a large number of avian species, cannot be conducted (de la Hera *et al*. 2009b, Rohwer *et al*. 2009, Bridge 2011) because even basic knowledge on moult (e.g. duration) is unavail-able for most of the species or suffers from precision and sample size problems (e.g. Jenni & Winkler 1994, Perrins 1998, Ginn & Melville 2007). Jenni & Winkler (1994) had pointed out nearly two decades ago the main caveats in the study of avian moult: "There are, however, surprisingly few studies on the effect of moult on plumage func-tion." and "… ecological and behavioural explanations of the timing, extent and rate of moult and their effects on other events in the life of a bird lag behind corresponding

studies of reproduction." Despite the long afterlife of this influential book, moult is still a black box (Bridge 2011). Modern analytical procedures that are now ready for proper computation combined with museum and ringer's data could poise this derogatorily latent topic to emerge (Newton 2009).

Costs of moult: putting moult into a life-history context

Moult was rarely placed into a broad eco-evolutionary frame or it was reduced to subsidiary notions within case-studies. Conducting a shallow survey using Web of Science and Google Scholar, almost no review paper was retrieved (keywords: moult*, shed*, renew*, exchang*, feather, plumage, integument and life-history) despite a focus on all the major biological review periodicals. If we are generous, exceptions are regulation issues by Dawson (2008), tangential discussion by Ricklefs & Wikelski (2002) and a review with reference to aquatic mammals by King (1970). Thus understanding the implication of moult in life history modulation requires settling questions like: what are the drivers of variation in moult pattern and feather quality? How perturbations of moulting sequence are mirrored in feather quality? Does moult interfere with other exigencies and survival-enhancing traits (e.g. physiology)?

I argue that moult has crucial importance in life-history evolution and at least three lines of evidence can be mentioned to support this: (1) timing of moult and its duration affect subsequent state and behaviour ('carry-over' effect), (2) moult is costly and (3) moult is tightly coupled with other physiological processes. The first line is encompassed by the 'moult constraint' hypothesis (MCH; Nilsson & Svensson 1996, Dawson *et al.* 2000) as follows. Breeding has been shown to carry survival costs, but how are these costs realized? The MCH suggests that a compromised moult carries over these costs; a mechanism proposed first by Bensch *et al.* (1985) and Siikamäki *et al.* (1994), but tested only later on. Because of immediate costs of both breeding and moult, these events are adaptively separated in time, hence prolonged breeding evokes delay in the timing of moult (Dawson *et al.* 2000, Dawson 2008, Verhulst & Nilsson 2008 and references therein). Because moult has to be finished before embarking to migration or preparing to winter, postponed moult onset results in shorter moult dura-

tion (i.e. accelerated moult speed). However, such acceleration is penalized by lower quality flight and body feathers produced (Nilsson & Svensson 1996, Dawson *et al*. 2000, Hall & Fransson 2000, Serra 2001, Dawson 2004, de la Hera *et al*. 2009a, de la Hera *et al*. 2010c, Weber *et al*. 2010, Broggi *et al*. 2011) and subsequent lower survival and future reproductive performance (Nilsson & Svensson 1996, Siikamäki 1998, Wiggins *et al*. 1998).

Study motivation (**Paper II**)

So far, studies involved only migratory species that have temporally tighter, more constrained moulting. Also, the contribution of body condition to moult rate–feather quality trade-off is barely known. Finally, although body feather structure is central to the 'moult constraint' hypothesis, it was never tested experimentally. We filled these gaps in a manipulation study on house sparrows.

Second, moult was considered a buffering, low-budget period because "only" somatic growth occurs scattered over a long time. However, a burgeoning number of studies demonstrate that moult incurs costs. Plumage constitutes up to 30% of the dry mass of birds representing thus a considerable protein investment (Weber *et al*. 2010). Given the high caloric content of feathers (Murphy & King 1982) and the very low efficiency of their production (Murphy & King 1984, Hoye & Buttemer 2011), the energetic cost of feather production is comparable to that of reproduction (Lindström *et al*. 1993, Klaassen 1995) especially for smaller species (Hoye & Buttemer 2011). In agreement with the metabolic cost of moult, birds have higher body temperature during than after moult (Newton 1968, Vágási *et al*. unpublished). Although resource limitation of moult was frequently heralded as negligible (Murphy *et al*. 1998; see also Ginn & Melville 2007), empirical and theoretical work suggests the contrary since the need for high-quality food (Murphy & King 1982, Murphy & King 1986, Cherel *et al*. 1994, Barta *et al*. 2008) and amino acid supply (e.g. cysteine reserved in antioxidant glutathione; Murphy & King 1990) is essential. Further, juvenile white-crowned sparrows *Zonotrichia leucophrys gambelii* moulting faster their body feathers had lower fat stores and had higher foot intake rate when subjected to induced moult (Bonier *et al*. 2007). In line with these observations, moult is traded off against body mass (Mo-

reno-Rueda 2010b) and birds in prime body condition (i.e. higher resource asset) engage earlier in moult (Langston & Hillgarth 1995, Svensson & Nilsson 1997, Remisiewicz *et al*. 2010), have higher feather growth rate (Grubb 2006, Gienapp & Merilä 2010, Marzal *et al*. 2013) and moult faster (Møller *et al*. 1995). Besides energetic and material costs, moult also entails aerodynamic and thermoregulation costs due to missing flight and body feathers, respectively (Hedenström & Sunada 1999, Hedenström 2003, Williams & Swaddle 2003, Ginn & Melville 2007; but see Lind 2001 and Lind & Jakobsson 2001 for compensatory aptness). Flight costs are also known to increase allometrically (Carrascal & Polo 2006, Rohwer *et al*. 2009).

Study motivation (**Paper V**)

Little is known about the dependence of moult on nutritional state and even much less about whether the interference of moult with other demanding functions (e.g. immunity) is a resource-based one. For this, we manipulated diet composition and activated the immune system, and followed the pattern of moult and possible effects on feather quality.

Third, trade-offs (i.e. allocation in one demand shortens resources disposable to a competing exigence) form the backbone of life-history theory (Stearns 1989, Roff 2002). The physiological network and its regulators and subnetworks (sensu Cohen *et al*. 2012) are increasingly recognised as the pivotal mediators of life-history evolution through the 'pace-of-life syndrome' (Sheldon & Verhulst 1996, Zera & Harshman 2001, Wikelski & Ricklefs 2001, Ricklefs & Wikelski 2002, Romero 2004, Monaghan *et al*. 2009, Sadd & Schmid-Hempel 2009, Selman *et al*. 2012). Moult interacts dynamically with most of the components of this network, which makes moult a prominent life-history trait. Despite this broadband interference with physiology, only the hormonal and photoperiodic regulation of moult was under considerable scientific siege (Dawson 2008). Moult seems to be in conflict with immune investment (Nava *et al*. 2001, Sanz *et al*. 2004, Martin 2005, Moreno-Rueda 2010b, Männiste & Hõrak 2011, Ellis *et al*. 2012; but see Moreno *et al*. 2001, Hegemann *et al*. 2012). Keratin production also depends on the antioxidant glutathione, which is the major intracellular cysteine reservoir (Murphy & King 1990). Given that moult might deplete the glu-

tathione pool and has energetic costs, feather synthesis should trigger oxidative stress (Vieira & Gomes 2010); however this deserves manipulative investigation of wild-living species. Allocation trade-off between moult and stress response is also apparent as only species with extended moulting schedule can afford to sustain stress respon-siveness (Cornelius *et al*. 2011, Li *et al*. 2011). Even house sparrows that benefit from high stress responsiveness at the expansion front in Kenya reduce their stress hormone titres during moult (Liebl & Martin 2012). Furthermore, parasites are recognized as major selective agents acting on their avian hosts. They induce fitness costs by usurp-ing part of host's limited resources, stimulating the costly defence machinery orches-trated by the activation of metabolism (free radical production), and immune and anti-oxidant system, and triggering stress response. In line with these, Marzal *et al*. (2013) found that house martins *Delichon urbica* infested with blood parasites have lower feather growth rate at their wintering grounds and this negative effect is carried to the breeding ground in form of reduced reproductive output. Lyasian albatrosses *Phoe-bastria immuntabilis* with higher nematode infestation initiated moult later and re-placed fewer feathers (Langston & Hillgarth 1995). Nevertheless, surprisingly little is known about how parasites might affect the moulting schedule and feather quality of the hosts. Intestinal parasites are good candidates for influencing plumage aspects be-cause they divert vital resources directly from host's alimentary tube when the protein requirements of feather production are at the ceiling.

Study motivation (**Papers II**, **III** and **V**)

We tested the prediction that high-quality birds cope better with challenges imposed by moult especially when moult is constrained. We also manipulated parasite levels throughout 15 months spanning two moulting periods and sought for infestation costs in terms of moult pattern and feather quality. Fi-nally, we studied experimentally the putative trade-off between moult and hu-moral immune activity and their common dependence on protein resources.

Objectives and major results

Hereby, I first formulate the objectives, questions and predictions of the studies, and also briefly mention the core method used. After then, the new scientific findings brought about are outlined. Note that the detailed methods and all of the results could be found in **Papers I–V** included in Chapter II.

Paper I   *Predictors and feather quality correlates of feather damages*

(i) Objectives

Damaged feathers may impair flight ability and ultimately reduce fitness. The gradual shortening of wing length and increasing dullness of flight feather colour after moult is caused by wearing. Although pervasive among birds, still less attention has been paid to understand which factors are responsible for the intensity of feather abrasion. Impairment of flight ability could also result from the appearance of feather damages. Such abnormalities are the tiny feather holes on the flight feather vanes. Although this deformity is used as proxy for feather quality, the relationship between feather hole abundance and feather quality has not been explicitly addressed. Further, it is also unclear which factors other than feather quality add variation to feather hole numbers (e.g. age, sex, season, feather deformities). We conducted a correlative study on resident great tits *Parus major*, where birds were sampled monthly throughout three consecutive annual cycles. Our aims were to examine:

   (1) certain intrinsic and extrinsic sources of variation in the degree of feather wear and incidence of feather holes;

   (2) whether different feather deformities (feather wear, feather holes and fault bars) are associated; and

   (3) the possible link between feather quality variables (e.g. rachis width, barbule density) and feather hole load.

(ii) Results

(1) Breeding may incur significant costs in terms of flight feather usage. Primaries of females are more abraded than that of males during the breeding season. (2) The fre-

quency of feather abnormalities may depend on the condition and feather quality of the birds. (3) Juveniles grew feathers that deteriorate faster and hold higher feather hole loads. (4) We have also found that feather abnormalities were positively interrelated: great tits with feather holes had more abraded primaries, and those with fault bars on the flight feathers had higher incidence of feather holes.

Paper II   *Moult rate and body condition interplay to influence feather quality*

(i) Objectives

The trade-off between current reproductive effort and residual reproductive value is of outstanding concern in life-history theory. However, the mechanisms that potentially mediate such long-lasting effects remain an evolutionary conundrum. The 'moult constraint' hypothesis suggests that the costs of breeding are expressed via compromised moult, which can lead to plumage malfunctions and hence curtailed future fitness. The hypothesis, however, was not tested on less time-constrained sedentary species. Furthermore, no experimental studies have explored whether the accelerated moult rate can represent one of the proximal underpinnings of altered body feather structure. Body condition might also play a role: birds with greater energy capital might better fuel the demands of moult to grow better-quality feathers, even if moult is compromised. We tested the 'moult constraint' hypothesis by experimentally adjusting the moult rate through a photoperiod treatment. We aimed at testing the following predictions:

(1) the speed of moult should have no effect on feather attributes if the longer moulting period of sedentary birds permits cost-spreading;

(2) based on correlative results of Broggi *et al*. (2011) showing that northern great tits, which face moulting time constraints grew shorter, more densely structured body feathers with fewer plumulaceous barbs, we predict that the body feather structure of fast-moulting experimental sparrows should resemble that of northern great tits; and

(3) an interaction between treatment and body condition is predicted if good-quality birds can cope better with difficulties caused by faster moult rate.

(ii) Results

(1) The faster moult of experimental birds was detrimental for flight and body feather quality. (2) The experimental birds harboured more fault bars, which might indicate that accelerated moulting was stressful. (3) Rapid moulting resulted in shorter and smaller feathers with a higher barbule density and a lower proportion of plumulaceous barbs; these traits are supposed to reduce insulation capacity. (4) Body condition was positively associated with feather traits either irrespective of the treatment (primaries' rachis width and mantle feather length) or only in the experimental group (wing, tail and primary lengths).

Paper III   *Parasite's detrimental effect on feather quality*

(i) Objectives

A large body of evidence shows a causal relation between the presence of parasites and fitness of their avian hosts. Nevertheless, our knowledge about parasites' effect on moult is scarce. Parasites (e.g. intestinal coccidians) can affect moulting and thus the quality of feathers produced by usurping resources, increasing metabolism, triggering immune response, elevating CORT levels and inducing oxidative stress. After feathers are synthesized, their functionality can be sustained e.g. by coating them with uropygial gland secretions. We confined adult house sparrows in large outdoor aviaries, medicated them (coccidians purged) or kept naturally infested and followed their condition, uropygial gland size, moult and feather quality of the primary flight feathers during two subsequent annual moults (over a period of 15 months). We sought to answer the following questions:

    (1) do the presence of coccidians decrease two aspects of condition that are known to affect feather quality, namely body mass and gland size;

    (2) do coccidians impair the quality of flight feathers at short-term too or only after a chronic (i.e. long-term) infestation; and

    (3) are allocations into different structural feather traits traded off (e.g. feather length with feather mass per unit area), and are these possible trade-offs coupled or decoupled by coccidian infestation.

(ii) Results

(1) The long-term coccidian infestations had a significant negative effect on the condition and uropygial gland size, but only when gland is at its circannual peak. (2) Medication had a positive effect on wing length, but only at long-term, highlighting the importance of multi-annual studies. (3) On the contrary, feather quality measures were significantly negatively affected by coccidians at both short- and long-term.

Paper IV   *Moult rate as cost of plumage ornament production*

(i) Objectives

Signalling theory posits that communication systems are susceptible to cheaters' invasion unless a significant and quality-differential production cost of the signal exists. A wealth of studies dealt with production and maintenance costs of extravagant carotenoid-based or gaudy iridescent structural colouration. Several mechanisms that might ensure reliable signalling were tested. Among them, moult rate was proposed as a candidate costly mechanism by negatively affecting ornament elaboration. However, no studies examined to date whether melanised ornaments suffer a moult rate cost, and previous results on depigmented white ornaments do not support the honesty-reinforcing effect of moult rate. Using the same photoperiod-based manipulation of moult speed and set of birds as in **Paper II**, this study aimed at testing whether accelerated plumage replacement of male house sparrows adversely affects:

　　(1) the size and/or colouration of the melanin-based badge; and

　　(2) the size and/or colouration of structural white wing-bars

(ii) Results

(1) The 'moult constraint' hypothesis was largely supported: experimental moult rate acceleration adversely affected the melanin-based badge size and the depigmented wing-bar brightness. (2) The fact that the pigmentation pattern of badge feathers did not differ between groups advocate that the number of melanised badge feathers was affected. (3) Our results ascertain moult constraint as an underpinning of the costly expression of depigmented plumage areas.

Paper V   *Nutritional costs of moult and possible trade-off with immunity*

(i) Objectives

Moult and immune response are two highly energy- and nutrition-demanding attributes of birds. It seems thus reasonable to assume a resource-based trade-off between them. This trade-off may be manifested as a decrease in moult speed and feather quality during an immune challenge, or inversely, the depression of immune response during intense somatic growth. We subjected adult male house sparrows to nutritional and immunological treatments in a factorial design. Our aims were to test:

(1) the possible dependence of moult pattern, feather quality and antibody responsiveness against sheep red blood cells on nutritional state; and

(2) an expected trade-off between moult/feather quality and humoral immunity, and whether this conflict differs between low- and high-quality diet groups.

(ii) Results

(1) House sparrows having access to low-quality food experienced a prolonged moult and produced low-quality feathers. (2) Furthermore, we have shown that the food-quality had no effect on the induced humoral immune response of the house sparrows during the energy- and nutrition-demanding period of moult. (3) Finally, the activation of humoral immunity had no effect on moult.

General discussion

In this section I am going to elaborate on the implications of my findings putting emphasis on the ultimate perspective. Note that the specific discussions of the found results are provided in **Papers I–V** included in Chapter II.

Feather quality and its fitness consequences

Flight feathers are exposed throughout millions of cycles to large aerodynamic forces and must therefore be stiff to maintain function. However, feather quality is rarely the highest possible because damages are inevitable. Damages can alter the shape and area of the wings (i.e. wing loading [body mass / wing area]) or the stiffness of feathers. In general, such changes in the flight apparatus can deeply reduce flight ability and consequently increase flight costs and predation risk. The following evidences point in this direction.

Wing wear has been shown to have multiple fitness costs in virtually all flight-capable taxa through the reduction in flight performance (Combes *et al*. 2010), increase in wingbeat frequency (Hedenström *et al*. 2001), decreased take-off speed (Swaddle *et al*. 1996) and elevated mortality risk (Cartar 1992). The pointed wings of European starlings *Sturnus vulgaris* become rounded by wear (Swaddle & Lockwood 2003). Winter-moulting migrants have to undergo their autumn journey with abraded feathers, and thus rounded not pointed wings. Abraded, rounded wings could have adverse effects on them because migratory behaviour selects for wing pointedness (Mönkkönen 1995, Lockwood *et al*. 1998, Pérez-Tris & Tellería 2001). Accordingly, simulated wing wear reprogrammed pre-migratory fuelling and postponed migration initiation of Western sandpipers *Calidris mauri* (O'Hara 2002).

Concerning the fitness consequences of fault bars, all effects are rooted in the fact that feathers are predisposed to fracture along fault bars (loss of distal feather part; Fig 1A; see also Sarasola & Jovani 2006). Because truncation reduces wing area and, in turn, upsets the optimal wing loading, detriment in fitness might ensue owing to impaired aerodynamics, increased flight energetics or costs of compensatory reduction of

mass (Hedenström & Sunada 1999, Senar *et al*. 2002). In fact, the scant evidence on free-ranging birds indicates that fault bars incur both reproductive and survival costs (Bortolotti *et al*. 2002, Pap *et al*. 2007, Møller *et al*. 2009). Evidence on natural or manipulated wing gaps lend further support for this notion (e.g. Slagsvold & Dale 1996, Lind 2001, Love & Williams 2008). Things are even worse because costs of wing area loss are not only paid by the carrying individual as cross-generational effects were also found. Verspoor *et al*. (2007) clipped some feathers of starling mothers and found that the flight capacity of their sons was negatively affected by treatment. Recognizing these fitness deficits of wing loss, one might expect that natural selection would favour mechanisms that can ameliorate such negative effects. First, Jovani & Blas (2004) suggested that natural selection could perpetuate mechanisms by which birds can adaptively position fault bars into flight feathers with lower flight requirements (coined 'adaptive fault bar allocation' hypothesis). Upcoming studies on distant taxa have bolstered the generality of this hypothesis (Serrano & Jovani 2005, Sarasola & Jovani 2006, Pap *et al*. 2007, Jovani *et al*. 2010). Second, not only the positioning of fault bars, but the likely bilateral asymmetry that breakages are causing should be selected against. In agreement, Balmford *et al*. (1997) showed using the phylogenetic comparative approach that species that spend much time on wings and meet stronger aerodynamic requirements have more symmetric wings and tails.

Feather holes might also be associated in a negative way with various measures of fitness from mating success, breeding phenology and performance, secondary sex characters until moult speed, flight ability and survival (Møller 1991, Møller *et al*. 1995, Barbosa *et al*. 2002, Møller *et al*. 2004b, Garamszegi *et al*. 2005, Pap *et al*. 2005, Møller 2010, Moreno-Rueda & Hoi 2012; but see Pap *et al*. 2007).

Importantly, we showed that feather damages are positively interrelated and also influenced by feather quality (**Paper I**), which can exacerbate their negative fitness pay-offs. We found that one of the major predictors of feather damage is age: juvenile birds have lower quality feathers and this age pattern was consistently true for each feather deformity (i.e. wear, fault bars and feather holes; Pap *et al*. 2007, **Paper I**). This could arise because nestlings have accelerated growth rate and assemble all the vital functions simultaneously (brain, skeleton, musculature, feathers, physiological

networks), which can give birth to trade-off and might be stressful. Indeed, experimentally accelerated growth has been documented to evoke varied negative effects (e.g. Alonso-Alvarez *et al*. 2007), stressful rearing conditions result in more feather holes (Pap *et al*. 2007), which could be stress-induced microcracks (**Paper I**), and the larger-sized male nestlings seem to be more sensitive with this respect (Fig 1B in **Paper I**; see also Vespoor *et al*. 2007). The considerably lower feather quality of juveniles combined with their possible adverse effects on flight might explain the generally lower breeding success (Curio 1983; reviewed by Forslund & Pärt 1995) and reduced survival expectancies of juveniles, which are quite general phenomena among birds. These possible constraints are largely unexplored and their importance in age-related performance merits future experimental investigations.

Not only damages, but the gradual weakening of feathers (e.g. different structural failures [Bodde *et al*. 2011] or getting less stiff at bending) can also be detrimental for flight ability (Weber *et al*. 2010). This accelerates the negative effects of low feather quality because such feathers degrade faster and more by time (Dawson *et al*. 2000, **Paper I**) and also become less stiff (Pap PL, Vágási CI, Marton A & Bărbos L unpubl. data; Fig 2). All these changes in feather aspects (both damages and structural / material malformations) might alter wing area and shape and reduce aerodynamic force, which are very important determinants of flight capacity (Pennycuick 2008, Weber *et al*. 2010). As such impairments reduce the escape success from a predatory attack (Metcalfe & Ure 1995) and increase flight expenses (Weber *et al*. 2010), they provide a substrate for natural selection. Indeed, European starlings with apparently undamaged, but used pre-moult feathers performed worse in escape flight than after moult was completed (Williams & Swaddle 2003), while those with feather damage had reduced take-off speed and manoeuvrability (Swaddle *et al*. 1996). If birds with functionally inferior feathers or pruned wing area reduce body mass to compensate for the higher wing loading and enhance predator evasion chances, they might assume elevated risk of starvation during short and cold winter days with food shortage (Macleod *et al*. 2005).

Body feather integrity is also impacted by intrinsic and extrinsic factors and feathers with modified structure might not adequately insulate. Body feather loss
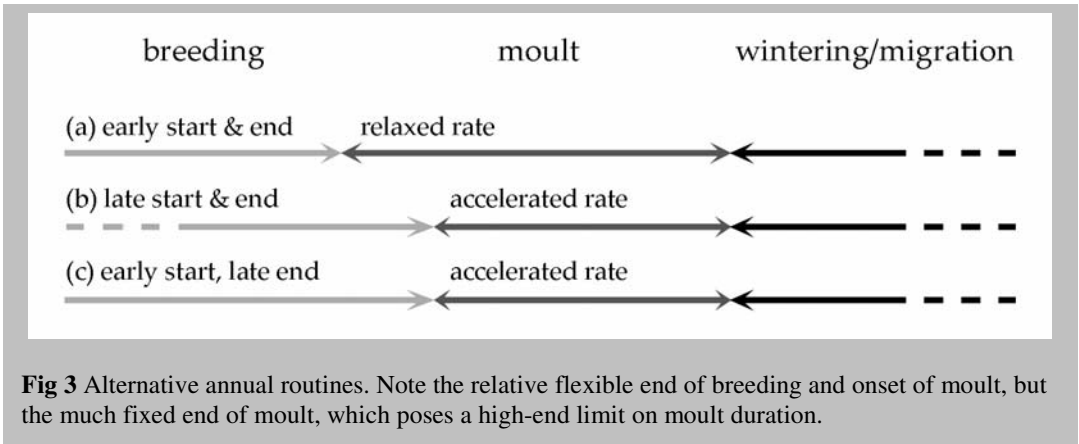
caused by parasites (see Fig 2 in Clayton 1990) could impair heat conservation capacity and consequently increase energetic expenses (Booth *et al*. 1993). Blue tits *Cyanistes caeruleus* forced to breed later had higher thermoregulation costs during the next winter and lower breeding success the next year probably owing to compromised moult of body feathers (Nilsson & Svensson 1996) as demonstrated by correlative and experimental studies (Broggi *et al*. 2011, **Paper II**). Finally, if birds with less insulative feathers use heat-conserving postures more often, they might be exposed to higher predation risk (Carr & Lima 2012). Cost of altered body feather structure might also be revealed via less colourful sexual ornaments (Shawkey & Hill 2005, Griggio *et al*. 2009, Galván 2011, **Paper IV**).

Fitness pay-offs of moult

Timing and duration of moult

The structural and material properties of feathers are determined during the moult and the repair of already grown feathers is impossible. Thus conditions during moult and the quality of feathers are intimately connected. Based on the above detailed fitness costs of low feather quality, we can expect that compromised moult has fitness detriments through feather quality. There is accumulating evidence showing that costs of constrained moult are carried further to the next life-stages and these might ultimately lead to impaired reproductive output or survival prospects (e.g. Nilsson & Svensson 1996). Siikamäki *et al*. (1994) followed pied flycatchers *Ficedula hypoleuca* with different original or manipulated clutch sizes and found that females with large clutches begin to moult later and proposed for the first time that such tight moult schedule might have disadvantages in terms of feather quality that ultimately decreases residual fitness. Wiggins *et al*. (1998) showed that female collared flycatchers *Ficedula albicollis* with experimentally delayed breeding onset had smaller clutches the next season, which they supposed to stem from constrained moult or migration. Collared flycatchers with simulated moult–breeding overlap had reduced fitness both in terms of survival and reproduction (Hemborg & Lundberg 1998). Brommer *et al*. (2003) suggested that the reproductive investment of Ural owls *Strix uralensis* might manifest in

increased partial moult asymmetry, which they showed to negatively affect survival. Finally, body feathers might also contribute in linking current to future performance given their pivotal role in thermoregulation and energetic expenses attributable to it. Broggi *et al*. (2011) found that great tits at the northern edge of their European distribution that underwent a shorter and faster moult have body feather features that convey lower heat retention capacity. In agreement, birds from these populations expend more energy when subject to thermal challenges (Broggi *et al*. 2004), which might explain the sink population attributes (e.g. lower survival rate) of northern great tits (Broggi *et al*. 2011).

Fig 3 Alternative annual routines. Note the relative flexible end of breeding and onset of moult, but the much fixed end of moult, which poses a high-end limit on moult duration.

The timing and duration of moult have purported fitness consequences, but this topic was much overlooked. Timing and duration of moult are highly correlated such that birds ceasing early their breeding activity are not constrained during their moult and thus can attain a relaxed moulting rate (Fig 3, case (a)). In contrary, birds that finish their breeding late either due to late onset of breeding or having multiple broods experience a constrained moult and in turn are forced to moult faster (i.e. reduce duration) because moult should be undertaken before migration or winter commence (see studies above; Fig 3, cases (b) and (c)). That timing of moult is moulded by breeding phenology and moulting time is constrained by breeding is supported by the fact that (i) in species with natural moult–breeding overlap those birds overlap the two demands more likely that have lower reproductive effort or enjoy better feeding conditions (e.g. Siikamäki 1998, Hemborg 1999), (ii) experimentally delayed breeders overlap chick provisioning with moult more often than controls (Siikamäki 1998) and (iii)

females with large original or manipulated clutches initiate their moult later (Sii-kamäki *et al*. 1994).

From another perspective, however, taking the costs of incomplete plumage during moult (e.g. aerodynamic cost due to gaps in the wing or worse thermoregulation) it pays to undertake plumage renewal as rapidly as possible. Best support come from studies showing that birds in prime condition usually have higher feather growth rate, earlier moult initiation or shorter moult (Møller *et al*. 1995, de la Hera *et al*. 2009a, Marzal *et al*. 2013, **Paper II** and **V**). So, this prompts the question how can this dilemma, evoked by opposed direction of selection advantages of slow and fast moult, be solved? Simultaneous moult of all flight feathers is very uncommon among birds; only waterfowl are making exception. This suggests that intense feather synthesis and eventual loss of flight capacity poses too large costs. Fast moult could constrain the whole-body protein synthesis and thus lesser amount of keratin per unit feather volume could be synthesized during feather development (Murphy & Taruscio 1995), which may result in decreased feather quality (see the 'moult constraint' hypothesis in Introduction and **Paper II**) and could cause feathers to abrade faster, finally leading to fitness shrinkage. Prolongation of moult is also a bad job as it maintains the costs of incomplete and abraded plumage longer. This path is usually followed only by low quality or nutritionally deprived birds (Langston & Hillgarth 1995, Møller *et al*. 1995, Svensson & Nilsson 1997, Grubb 2006, Gienapp & Merilä 2010, Remisiewicz *et al*. 2010, Marzal *et al*. 2013, **Paper V**). According to this argument, a stabilizing selection is expected to shape moulting schedule that maximizes lifetime fitness (Fig 4). This is achieved by a lower-end constrain imputed by large costs of fast moult and a less severe upper-end cut represented by prolonged costs of incomplete plumage and time limit (migration/wintering). The optimum, i.e. balance between the costs and benefits of fast and slow moult, could vary among individuals (e.g. according to condition; **Paper II**) and among species according to their specific annual routine and life-history (de la Hera *et al*. 2009a, Weber *et al*. 2010). In other words, different individuals or species might meet different upward or downward selection forces, which can be manifested in different stabilized optima within or between species. This in part might explain the diversification of moult strategies. Knowing the purported ef-

fects of climate change on timing of activities and the potential effect of moult on fitness and thus population demography, it would be fruitful to incorporate the study of moult pattern into monitoring and management actions by conservation practitioners.
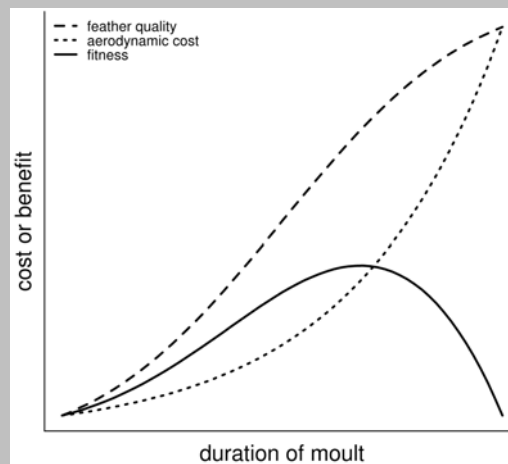

Fig 4 Schematic model of moulting pattern. The advantage of shedding plumage as fast as possible (i.e. short duration) is the reduction of aerodynamic costs (dotted line), however with sacrificing feather condition (broken line). Prolongation of moult (i.e. long duration) is also costly, but its penalization is less severe; birds have to bear the aerodynamic costs of missing feathers longer, but can undergo an uncompromised moulting schedule and grow high-quality feathers. This is represented also by the slightly skewed fitness pay-off of moult duration (continuous line).

In conclusion, major activities of the annual cycle cannot be treated in isolation because of their linkage through direct functional or carry-over effects (Barta *et al*. 2006, Barta *et al*. 2008). Although the survival costs of reproduction and their possible physiological underpinnings (e.g. suppressed immunity) are widely documented, non-physiological mechanism might also modulate such costs. Feather quality is one promising candidate for two reasons. Breeding activity has a disproportionately high effect on feather degradation (**Paper I**) on the one hand, and breeding investment can compromise moult and thus feather quality on the other hand. In **Papers II** and **IV** we simulated the second situation, i.e. the natural variation in annual schedules as depicted on Fig 3 such that case (a) was represented by the control photoperiod and cases (b) or (c) by experimental photoperiod (i.e. birds that enter moult late in the season and experience short and fast shortening days). Based on our results, we conclude that the longer moult duration characterized to sedentary lifestyle does not equate to be released from the moult rate–feather quality trade-off (**Paper II**) or the production

costs of plumage ornaments (**Paper IV**). In **Paper II**, we also report experimental results for body condition-dependent costs of somatic growth during adulthood. Therefore, feather traits could be honest indicators of the bearer's condition (see also correlative results in **Paper I**). These results have implications for the evolution of feather trait diversity among avian taxa and selective forces acting on annual routines. This latter study is the first, as far as we are aware, to test the effects of moult speed on body feather structure using an experimental approach.

Physiological costs of moult and its sensitivity to infestation

Our results refute the hypothesis that moult and immunity are competing demands. However, moult might be differentially associated with different branches of the immune system. Martin (2005) and Moreno-Rueda (2010b) showed that T-cell-mediated immunity is traded off against moult, while we studied moult's interference with antibody-mediated humoral immune response against sheep red blood cells (SRBCs; **Paper V**). Scarce evidence suggests that humoral immunity is not very expensive metabolically (Eraud *et al*. 2005) and nutritionally (**Paper V**), which might explain that its induction did not compromised moult. Notwithstanding that Sanz *et al*. (2004) found that SRBC-immunized pied flycatchers initiated their moult later, these birds faced multiple demands by overlapping moult and breeding. In other studies, we explored how different measures of innate and acquired immunity are regulated during and outside the moulting season and found that no simple answer exists as it depends on the physiological marker in question (Pap *et al*. 2010a, Pap *et al*. 2010b; see also Hegemann *et al*. 2012). Univocal to all is that markers of humoral immunity were relatively high during the moulting season (immunoglobulin, Pap *et al*. 2010a; SRBC antibody response, natural antibodies and complement, Pap *et al*. 2010b), which corroborates our experimental findings.

Parasites can affect moulting and thus the quality of feathers produced e.g. by usurping nutrients on which moult depends (**Paper V**) or triggering certain immune branches that are traded off with moult (Moreno-Rueda 2010b). Some of the most prevalent and abundant avian parasites are coccidians, which are microscopic, unicel-

lular organisms that inhabit the epithelium of host's intestine (Greiner 2008). Coccidians reduce general absorption and drain of essential nutrients (e.g. amino acids) and stimulate the immune system (Allen & Fetterer 2002). Thus, coccidian infestation during moult can induce a range of costs, including prolonged moult, reduced feather quality, and increased post-moult mortality resulting from reduced predator escape ability. According to this hypothesis, we found that a large number of feather quality traits were sensitive to infestation, but moult pattern was not influenced. This suggests that coccidiosis does not affect the regulation or baseline pattern of moult, but influences the synthesis of keratin. It is interesting to note that the effect of coccidians on feather quality measures (**Paper III**) was similar with the effect of high speed of moult (**Paper II**), that is infested and fast moulting birds grow short and light flight and body feathers with thin rachis and high barb and barbule density. These results suggest that under various stress conditions the same mechanisms are responsible for the reduction in feather quality. In conclusion, parasites might shorten lifespan not only through physiological survival-related functions (i.e. immune and stress response, and oxidative status), but also by compromising feather quality of avian hosts. Our findings may have implications for species conservation and animal health care, as well. We suggest for bird keepers to pay more attention to the control of coccidian infestations of wild birds 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 fitness consequences.

Signalling costs

Plumage signals, either sexual or social, evolved because of fitness benefits of signallers and, usually, receivers as well. The latter chiefly get information based on costly signals that impose quality-dependent differential costs on the signaller and hence preclude deception. However, internal mechanisms that ensure signal reliability (i.e. signal value represents true quality) are relatively less known in case of melanin-based and depigmented (white) ornaments. In **Paper IV**, we addressed this question by manipulating moult rate and seeking its ornament production costs.

Regarding melanin-based signalling, studies have focused on the nutritional and physiological aspects (see Introduction). We experimentally tested the 'moult speed constraint' hypothesis as a proximate mechanism and found that moult speed might ensure the honesty of eumelanin-based ornaments since only slow-moulting birds are able to grow large badges. Moult speed could explain variation in badge size and/or coloration because there is a rate-limiting factor in the biochemical pathway of endogenous melanin synthesis, the activity of the enzyme tyrosinase (Hearing & Tsukamoto 1991, Ito *et al*. 2000) that catalyzes several biochemical reactions in the whole course of eumelanin synthesis (McGraw 2006). If the process of eumelanin synthesis is of lower efficiency (e.g. reduced tyrosinase activity), a smaller or, alternatively, similar-sized but less intense black ornament (i.e. fewer pigment granules deposited per feather) is expected to develop (Stoehr 2006). We demonstrated in **Paper IV** that the size of black ornaments was affected by treatment without any effect on colouration of pigmentation pattern of melanised feathers (e.g. relative length of black part of the badge feathers). This means that instead of a tyrosinase rate limitation there is a threshold effect in the distribution of active, pigment-producing melanosomes. Those feathers that are positioned at the bottom periphery of the badge had no melanosomes activated at all in the faster moulting group, thus fewer badge feathers were coloured to black but with similar intensity (see also McGraw 2007).

Depigmented white signals were thought to be 'cheap-to-produce', though empirical evidence is accumulating that the expression of these signals is also costly (e.g. Török *et al*. 2003, Hanssen *et al*. 2006, McGlothlin *et al*. 2007). However, the mechanism responsible for these apparent production costs is unclear. In **Paper IV**, we showed that faster moult rate results in reduced brightness of a white signal. It is possible that the birds which had undergone a rapid moult were unable to either impute large vacuoles and/or precisely structure the spongy layer in the ways required to produce a bright white feather patch (Prum 2006).

This study did not test explicitly a condition-dependent model of ornamentation, however, we can make speculative inferences on how honest signalling may be guaranteed by moult rate. The timing of moult may be connected to condition in two non-exclusive ways. Lower quality males often acquire a mate later in the season (e.g.

Møller 1988), thus early breeders are usually in better condition (Verhulst *et al*. 1995, Griggio *et al*. 2009). Alternatively, birds that undertake high parental investments in current reproduction relative to their condition may run out of time and/or deplete their finite resources (Badyaev & Vleck 2007), reflecting a trade-off between breeding and moult (e.g. Siikamäki *et al*. 1994). For instance, Griffith (2000) found that male house sparrows with experimentally increased breeding effort expressed smaller-sized badges in the subsequent moult. Our findings emphasize that, through a cascade effect (delayed breeding is followed by delayed moult), moult couples the pre- and post-moult events. Moult speed may mediate the hidden costs of late breeding and/or high parental activity (Badyaev & Vleck 2007) not only through compromised feather quality but also by diminishing plumage ornamentation. Given that both the melanin-based badge and the depigmented wing-bar are subjects to intra- and intersexual selection, the coupling of successive breeding attempts by moult could explain the strong relationship between the condition-dependent ornamentation and annual (Møller 1988) or lifetime reproductive success (Jensen *et al*. 2004). These together highlight the selective advantage attained by birds which breed and fledge their nestlings earlier in the season (Serra *et al*. 2007, Griggio *et al*. 2009).

Concluding remarks

The studies in this thesis support the view that moult is a demanding life-cycle stage of the avian annual routine because (i) it depends on nutritional state and condition, (ii) its rate is inversely related to feather quality and (iii) it is highly sensitive to intestinal parasite infestation. However, it is apparently not in conflict with the humoral arm of the immune system. Moult might have multifaceted fitness consequences because (i) the quality of feathers is established during moult, (ii) birds are unable to repair feather damages but only during moult and (iii) the suffered damages and the low quality of flight and both ornamental and non-ornamental body feathers might lead to reduced performance. Hence, both the costs of moult and compromised feather quality pose substantial viability selection pressures. The potential of these characters to evolve is insured by the heritable component of several feather traits, timing of moult and feather growth rate (Grant & Grant 1995, Larsson 1996, Jensen *et al*. 2003, Gienapp & Merilä 2010). Consequently, species that have different life histories, thrive in different environmental conditions and have diverse annual routines may be exposed to species-specific selective forces, which act upon the degree of investment into moult (i.e. afford different costs), the rate of feather synthesis and condition-dependent feather quality, and this could have led to the perplexingly high diversity in feather shape, structure and their material properties (e.g. Wang *et al*. 2012). Further studies are needed to shed more light on the determinants and evolution of moult pattern and feather quality, and their fitness consequences. These causal factors should be addressed by both cross-sectional and longitudinal data, and data gathered on the field, during experiments or by conducting phylogenetic comparative analysis.

The way forward

Answers beget some more questions. Studying the costs and evolution of moult pattern and feather attributes is not an exception; their study is obviously not closed with this thesis. We have ongoing experiments, already collected samples for within and among species comparisons, and future plans dedicated to this topic as well. First, in a recently finished experiment we sought the feather maintenance function of the uropygial gland and its oily secretions. Two sets of house sparrows underwent their annual post-breeding moult either without (surgical removal) or with gland (sham operation) and we collected feather samples to explore the abundance of feather-degrading bacteria and the quality of feathers. Second, the survival cost (e.g. oxidative stress) of the adult-life somatic growth was not examined yet. Also, despite the known dependence of feather synthesis on amino acid cysteine, of which intracellular reservoir is the antioxidant glutathione, it is poorly known how antioxidant status might affect moult and feather quality and vice versa. We are currently running a set of experiments to examine the potential reciprocal conflict between two survival-enhancing groups of traits, moult and feather quality on the one side, oxidative stress and antioxidants on the other side. Third, we handle a large set of feather samples collected from a wide variety of bird species and intend to test some evolutionary hypothesis about the selection forces acting on feather traits and their constraints such as (i) how feather traits fit the slow–fast 'pace-of-life' syndrome or (ii) how parasites and physiology affect feather traits. Fourth, using the phylogenetic comparative approach, we search for morphological (wing area and aspect ratio) predictors of risk-taking behaviour when approached by a predator (Møller *et al*. 2013). Finally, using the correlative approach, we test the possible relationship between feather-degrading bacteria load and feather quality and the possible mediator effect of the uropygial gland secretions in free-ranging breeding and non-breeding house sparrows.

Összefoglaló

A madarak legszembetűnőbb ismertetője a tollazat, amely meghatározza hordozója küllemét. Emellett sokrétű hatása van a madarak életére tudva, hogy (i) számos fiziológiai adaptáció a tollal való borítottság és ezzel kapcsolatos repülési képesség eredménye, valamint (2) a tollazat számos funkciója összefügg a túlélési és élettartam alatti szaporodási sikerrel. A tollak elhalt epidermális struktúrák, melyek minősége csupán lecseréléssel javítható. A tollazat szabályos időközönkénti felújítását nevezzük vedlésnek. Ebből következik, hogy a tollak szerkezeti és anyagi tulajdonságai a vedléskor való növesztésük alatt alakulnak ki és ezután állapotuk kötelezően hanyatlik. Gyenge minőségű vagy megrongálódott tollak képtelenek maradéktalanul ellátni szerepüket, aminek a fentiek értelmében negatív következményei lehetnek az egyed rátermettségére. Meglepő tehát, hogy a sokrétű sikerességgel kapcsolatos hatásai ellenére a madarak vedlése és tollminősége egy hiányosan kutatott ágazata az ornitológiának és madárökológiának.

Értekezésemben a madarak vedlésének és a jó minőségű tollazat fejlesztésének költségeit keresem, különös tekintettel a tollminőséget jelző változók összefüggéseire, a vedlés forrásigényére, kondíciótól és fertőzöttségtől való függésére, illetve immuntevékenységgel való csereviszonyára, valamint a tollazati jelzések növesztésére. Ezeket a kérdéseket 5 vizsgálat keretében tanulmányoztam, melyeknek rövid összefoglalója olvasható lentebb.

I. cikk   *Tollazati hibákat magyarázó tényezők és tollminőség*

Az evező- és faroktollak minőségének romlása ronthatja hordozóik repülési képességét, ami növeli a predációs kockázatot, és így végső soron csökkentheti a rátermettséget. A tollak kopása egy nyilvánvaló jele (Fig 1A) a tollak rongálódásának, mégis ennek okairól keveset tudunk. Nem ismerjük például a tollkopás éves ciklus alatti változását és összefüggését a tollminőséget jelző más paraméterekkel. A toll-lyukak képezik a tollazati hibák egy másik típusát (Fig 1B–C); ezek helyén a tollágacskák hiányoznak. Kiváltó okaként leggyakrabban a tolltetveket említik, bár ennek a vélt ok–

okozati összefüggésnek egyelőre elmaradt a kísérleti bizonyítása. Bár használják a toll-lyukakat tollminőséget jelző elváltozásként, tollminőséggel való kapcsolata ismeretlen.

Vizsgálatunkban 3 teljes éves ciklus alatt követtük vadon élő széncinegék tollminőségének változását havi mintavételezéssel. Rögzítettük a befogott egyedek (n = 964) korát, nemét, biometriai adatait, 8. elsőrendű evezőjük tollszárának vastagságát és kondíció indexet számoltunk (tömeg és tarsus közötti I. típusú OLS regresszió reziduumai). Emellett feljegyeztük az elsőrendű evezők kopásának mértékét (0–3 skála) és megszámoltuk az összes evezőtollak toll-lyukainak és növekedési hibáinak számát (Fig 1A). Kevert általánosított lineáris modellekkel elemeztük adatainkat.

A kopás mértéke nem-lineárisan emelkedett az éves ciklus során, leginkább megkopott tollakkal a költő madarak rendelkeztek. Ezen kívül a fiatal madarak kopottabbak voltak a teljes éves ciklus során, míg a tojók csak a költési időszakban. A toll-lyukak jelenléte pozitívan, a tömeg pedig fordítottan függött össze a kopással. A toll-lyukak száma nem növekedett a vedléstől eltelt hónapokkal. A fiatalok toll-lyuk terhelése magasabb volt, főképp a fiatal hímeké. A vastagabb tollszárú, hosszabb szárnyú és növekedési hibától mentes madarak kevesebb lyukat hordoztak.

A költéskor aránytalanul felerősödő tollkopásért feltehetően a szülők magas erőfeszítése okolható, amit a tojók magasabb kopása is alátámaszt tudva, hogy a tojók nagyobb szerepet vállalnak a tojások és utódok gondozásában. A fiatalok egyöntetűen alacsonyabb tollminősége minden bizonnyal a gyors egyedfejlődés folyománya, amikor egyidejűleg fejlődik ki számos vitális funkciójuk szemben a felnőtt madarak elnyúlt vedlésével, amikor látszólag mindössze keratinszintézis zajlik. Adataink megerősítik, hogy a tollazati hibák jó tollminőség jelzők és akár a madarak kondíciójáról is információt nyújthatnak. A tollhibák negatív hatása a madarak teljesítményére erőteljesebb lehet, mint előzőleg gondoltuk, ugyanis egymással pozitívan összefüggnek.

II. cikk   *Vedlési sebesség és kondíció hatása a tollazat minőségére*

Az életmenet elmélet egyik alappillére a jelenlegi és jövőbeni siker közötti fordított kapcsolat, azonban ennek mechanizmusa nem ismert maradéktalanul. A „vedlési

kényszer" hipotézis egy lehetséges magyarázatot kínál. Eszerint a vedlést megelőző költési erőfeszítés negatív hatással lehet a vedlés időzítésére és időtartamára, ami a gyenge minőségű tollazaton keresztül fejti ki negatív hatását a fitnessre. A hipotézis a következő megfigyelésekre épül. Egyrészt, a költés és vedlés általában időben el van választva, lévén mindkettő költséges, így az időben kitolt költészárás kései vedléskezdéshez vezet. Másrészt, a késleltetett vedléskezdést a madarak magasabb vedlési rátával kompenzálják, ugyanis a vedlés fotoperiódus által szabályozott és a vonulás/telelés előtt be kell fejeződnie. A hipotézis kimondja, hogy gyors vedlési ütem csökkenti a tollak minőségét, aminek negatív hatása lehet a jövőbeni túlélési és szaporodási sikerre. Bár napvilágot látott a hipotézis néhány tesztje, a vizsgálatok főképp korrelatívak, vonuló fajok képezték a modell szervezeteket, nem terjesztették ki testtollakra és a kondíció hatása szintén ismeretlen.

Öreg hím házi verebeket tartottunk fogságban vedlési idejük alatt ($n = 50$; 25 / beltéri röpde). Elsőrendű evezőik vedlési állapotát és tömegüket rögzítettük tíznaponta. Dominanciasorban elfoglalt helyüket meghatároztuk és kontrolláltunk rá az elemzésekben. Két kísérleti csoporttal dolgoztunk: (i) kezelt – kísérletesen gyorsított fotoperiódus; (ii) kontroll – természetessel megegyező fotoperiódus (napi 8, illetve 3 perc nappalhossz rövidülés). A tollminőség elemzése kiterjedt az evező- (5-ös és 7-es elsőrendű evezők, legbelsőtől számolva) és testtollakra (háttollak) egyaránt. Evezőtollak paraméterei: szárny- és farokhossz, evezők hossza, tömege, tollszárának vastagsága és növekedési hibáik száma. Testtollak paraméterei: ágacskák sűrűsége, ágak száma, pihés ágak aránya, hossz és felület. Általános és általánosított lineáris modellekkel elemeztük adatainkat.

A kezelésnek önmagában nem volt hatása a szárny, farok és evezők hosszára, azonban interakcióban volt a kondícióval (log-tömeg és log-tarsus közötti II. típusú SMA regresszió); a lassan vedlő (LV) madaraknál nem volt hatása a kondíciónak, míg a gyorsan vedlő (GYV) csoportban csak a jó kondíciójú madarak voltak képesek az LV madarakhoz hasonló szárny-, farok- és tollhosszúságot elérni. A GYV verebek tollain több növekedési hibát találtunk. A testtollak minősége szinte kizárólag csak a kezeléstől függött; a GYV madarak tollain nagyobb volt az ágacskák sűrűsége, alacsonyabb volt a pihés ágak aránya, valamint tollaik rövidebbek és kisebbek voltak.

A gyors vedlési sebesség hátrányosan befolyásolta úgy az evező-, mint a testtollak minőségét, ami a későbbiekben túlélési és szaporodási veszteséget eredményezhet. Ezek szerint az állandó fajok hosszabb vedlési időszaka nem jelent vedlési költségek alóli mentességet. A gyors vedléssel járó növekedési hibák arra utalnak, hogy a magas vedlési ráta stresszes, ugyanis a kortikoszteron stresszhormon fékezi a keratin szintézisét. Eredményeink támogatják a vedlési kényszer hipotézis egyik premisszáját, miszerint a testtollak minősége is közvetítheti a kurrens költési befektetés költségeit, és első ízben kínálnak kísérletes bizonyítékot erre. Mivel a GYV verebek testtollai alacsonyabb hőszigetelési kapacitással bírhatnak, egy állandó faj esetében ez komoly termoregulációs költségeket vonhat maga után a hideg téli periódusban. Ezen eredményekkel megegyeznek kék- és széncinegén végzett vizsgálatok, amiben a kompromittált vedlésű madarak télen több tömeget veszítenek az éjszaka folyamán, és több energiát égetnek. Végezetül, eredményeink azt sugallják, hogy a kondíciónak nagyon fontos szerepe van a vedlési költségek megnyilvánulásában: míg kényelmes vedlési feltételek mellett a verebek jó minőségű tollakat fejlesztettek, addig csak a jó minőségű madarak voltak képesek elviselni a megemelkedett költségeket gyors tollnövesztés esetén. Ennek tág evolúciós következményei lehetnek.

III. cikk   *Parazita kokcídiumok káros hatása a tollminőségre*

A paraziták széleskörű negatív hatással vannak gazdáik életfolyamataira, aminek következtében befolyásolják ezek túlélési és szaporodási sikerét. Habár a tollazatnak kimagasló szerepe van a rátermettség meghatározásában, a paraziták tollazatra kifejtett hatása hiányosan ismert. A tollazat minőségében megmutatkozó hatásuk joggal várható, ugyanis esszenciális forrásokat vonnak el a gazdától, növelik annak metabolikus rátáját, aktiválják az immuntevékenységet és oxidatív stressz állapotot válthatnak ki. Ezek mind kapcsolatban állhatnak a vedlés folyamatával és ily módon a növesztett tollak milyenségével. A kokcídiumok egysejtű bélparaziták, melyeknek fentebb említett gazdára kifejtett hatásai jól ismertek.

Öreg tojó és hím házi verebeket ($n = 60$, ivararány 1:1) tartottunk kültéri röpdékben 15 hónapon keresztül, ami két teljes vedlési időszakot ölelt át. A madarak felénél

a vizsgálat teljes ideje alatt alacsony szinten tartottuk a fertőzést (gyógyított), míg másik felénél meghagytuk a természeteshez közeli fertőzöttséget (fertőzött). A madarakat 4 alkalommal mértük és mintáztuk, mindkét vedlési időszak előtt és után. Kondíciójukat, biometriai változóikat és vedlésüket az előző vizsgálatokhoz hasonlóan mértük. További kondíció-jelző változóként mértük a faroktőmirigy térfogatát is. Az eltávolított 7-es elsőrendű evezőn mértünk hosszt, zászló felületet, tömeget, tollszár vastagságot, ág és ágacska sűrűséget, valamint teher alatti elhajlást, ami a tollak rigiditását jelzi. Általános lineáris modellekkel elemeztük adataink.

A gyógyított verebek faroktőmirigye nagyobb volt, mint fertőzött társaiké, amikor a mirigyméret éves ciklusának csúcsán volt, de nem azon kívül. Tojók esetében a fertőzés hosszú távon (második vedlés után) negatívan befolyásolta a szárnyhosszat. A kezelés nem volt hatással a kondícióra és a vedléskezdésre, de hatással volt a tollak minőségére mindkét vedlési időszakban. A fertőzött madarak rövidebb, kisebb zászlófelületű, könnyebb, vékonyabb tollszárú, magasabb ág és ágacska sűrűségű, illetve elhajlással szemben kevésbé ellenálló evezőtollakat növesztettek.

Eredményeink alapján elmondható, hogy a kokcídiumok hatása jelentős a gazdák kondíciójára és tollnövesztésére. Ráadásul, ismerve a mirigy tollkarbantartási szerepét, a paraziták hatása a tollazatra nagyobb lehet, mint elsőre gondolnánk. Némely hatás mindössze hosszú távon való jelentkezése mutat rá a több ciklust átfogó kísérletek fontosságára. Bár ismert volt a paraziták káros hatása a vedlésre és a tollazati díszek színezetére, tudomásunk szerint ez az első vizsgálat, amiben az evezőtollak minőségére is ezt kimutatták. Mivel a gyenge minőségű tollazat rontja a madarak repülési képességét, jelentős túlélési (predációs kockázat) költségeket vonhat maga után.

IV. cikk   *Vedlési sebesség és tollazati díszek létrehozási költsége*

A madarak tollazati díszeinek színét leggyakrabban a karotinoid- és melaninpigmentek adják. A pigment-alapú tollazati szignálok mellett ismertek pigmenthiányos fehér szignálok is. A tollazati díszek révén történő kommunikáció egyik központi dogmája, hogy e szignálok által közvetített információk rendszerében könnyen elterjedhetnek a csaló jelzők (valós minőséghez képest aránytalanul fejlett szignál),

amennyiben ezek létrehozása nem jár költségekkel, amelyek ráadásul nagyobbak a gyengébb kondíciójú egyedek számára (hátrányelv). A melanin-alapú és pigment-hiányos fehér jellegek létrehozási költségéért és ily módon minőségjelző értékéért felelős mechanizmusok kevéssé ismertek.

A tollazati másodlagos nemi jellegek vedléskor alakulnak ki, ezért erre az időszakra jellemző körülmények kulcsfontosságúak. Jelen vizsgálatban azt a hipotézist teszteltük kísérletesen miszerint a gyors vedlési ráta költségei megmutatkoznak a melanin-alapú és fehér strukturális tollazati szignálok fejlettségében. A melaninok *de novo* szintetizálódnak a melanoszómákban és szintézisük rátáját a tirozináz enzim aktivitása limitálja, így gyorsan fejlődő tollakba kevesebb melanin pigment raktározható. A melanin pigmentek mennyisége pedig szorosan összefügg az adott folt méretével és/vagy feketeségével. A pigment-hiányos jelzések fehér színéért felelős tollazati mikroszerkezet precizitása lehet hátrányosan érintve a gyors tollnövesztés által.

Ez a munka a II. vizsgálat társa, általános módszereik teljes mértékben megegyeznek. Ami eltérő, az a jelzések mérése; kísérlet kezdése előtt és lejárta után, standard megvilágítás és kamera-beállítások mellett, lefényképeztük a verebek fekete torokfoltját és fehér szárnycsíkját, majd ezek méretét (mm^2) és akromatikus fényességét (feketeség illetve fehérség) mértük. Általános lineáris modellekkel elemeztük adatainkat.

A kezelés nem volt hatással sem a madarak tömegére, sem a verekedések számára, tehát a gyorsított fotóperiódus nem jelentett stresszesebb környezetet legalábbis kondíció és szociális viselkedés szempontjából. Az evezőtollak és szignáltollak vedlésének kezdési ideje nem különbözött a csoportok között, ellenben a vedlés időtartama közel két héttel rövidebb volt a kezelt csoport madarainál úgy az evezőtollak, mint a szignáltollak esetében. A gyorsabban vedlő kezelt madarak kisebb fekete torokfoltot és kevésbé fényes fehér szárnycsíkot növesztettek, mint kontroll fajtársaik. Bár a torokfolt feketesége és a szárnycsík méretében nem volt csoport-hatás, a kezelt madarak e változók esetében is gyengébben teljesítettek.

Számos okunk van azt feltételezni, hogy a vedlési ráta hozzájárulhat a melanin-alapú szignálok egyedek közti varianciájához. (i) Kéthetes különbség a vedlés időtartamában számos madárfaj esetében, köztük a házi verébnél is gyakran előfordul. (ii)

Természetes körülmények között a szűkösebb források feltehetően felerősítik ezt a hatást. (iii) A melaninok biokémiai szintézisében a legtöbb reakció katalizálásáért felelős tirozináz enzim aktivitása és/vagy hatékonysága befolyásolhatja a melanizáltságot.

A pigment-hiányos szignálokat jellemzően létrehozási költségektől menteseknek tekintik a szakirodalomban, éppen a pigmentek szintetizálásának költségei alól való felszabadulás miatt, és inkább fenntartási költségekkel magyarázzák megbízhatóságukat. Azonban ennek a feltételezésnek ellent mondanak az újabb és saját eredményeink is. A fehér strukturális díszek reflektancia spektrumát a keratin mátrix alkotói (levegővel telt vakuólumok, szivacsos réteg) és ezek finom léptékű elrendezése is meghatározza. Elképzelhető, hogy a gyorsan vedlő madarak képtelenek (i) elegendő számú és méretű vakuólumot beépíteni a tollaik mikroszerkezetébe és/vagy (ii) precíz mikrostruktúrát létrehozni.

Vizsgálatunk kiegészíti az előző munkákat és kiemeli a gyakran elhanyagolt vedlési időszak fontosságát, ugyanis a vedléskor jelentkező kényszerek okozta költségek összekapcsolhatják a vedlés előtti reprodukciós eseményt a jövőbeni szaporodással. Végezetül, eredményeink rámutatnak a korai költés szelekciós előnyeire.

V. cikk   *A vedlés forrásigénye és csereviszonya az immuntevékenységgel*

A madarak egyik keveset vizsgált forrás- és energiaigényes időszaka a vedlés. Ismerve a tollak magas proteintartalmát és szintézisük alacsony hatásfokát, a vedlés metabolikus költsége a szaporodáséval vetekedik, főképp a kistestű fajok esetében (testarányos költségek magasabbak). Ehhez hasonlóan a gazdaszervezetet idegen szervezetek támadásától védő immunrendszer is költséges. Emiatt joggal várható a két befektetés között egy negatív csereviszony, amit recens sejtes immunválaszt indukáló vizsgálatok is alátámasztanak (kezelt madarak lassabban vedlettek). Vizsgálatunkban a humorális immunág hasonló összefüggéseit kerestük és azt feltételeztük, hogy (i) alacsony minőségű táplálék a vedlést/tollminőséget és/vagy immunkapacitást rontja, illetve (ii) az antigénnel kezelt madarak lassabban vedlenek és/vagy gyengébb minőségű evezőtollakat növesztenek.

Öreg hím házi verebeket (*n* = 56) tartottunk 4 kültéri röpdében teljes vedlési időszakuk alatt és két kétszintes kezelésnek vetettük alá faktoriális elrendezésben. Egyrészt manipuláltuk a fogyasztott táplálék minőségét (alacsony/magas fehérjetartalom, ezentúl AF/MF), másrészt mindkét csoportban a madarak felét immunkezeltük (birka vörösvérsejttel/fiziológiás sóoldattal oltottuk, ezentúl SRBC/PBS). Heti rendszerességgel mértük a kondíciójukat (tömeg) és elsőrendű evezőik vedlési állapotát. A vedlés végén eltávolítottuk a 2-es és 7-es elsőrendű evezőtollakat (legbelsőtől számozva) és minőségüket hossz, tömeg és tollszár-vastagság alapján jellemeztük. Az immunválaszt részben az SRBC antigénre adott antitest válasszal (hemagglutinciós teszt), részben az össz-immunoglobulinok (Ig) koncentrációjával mértük.

Az AF madarak kondíciója a teljes kísérlet alatt alacsonyabb volt. Habár a táplálékkezelés nem volt hatással az SRBC-re adott antitestválasz erősségére, az AF madarak Ig-koncentrációja 1 héttel az immunkezelés után alacsonyabb volt. Az immunkezelés nem volt hatással a tömegre. Az MF verebek vedlési rátája gyorsabb volt és összességében 2 héttel korábban cserélték ki evezőtollaikat, ugyanis képesek voltak egyszerre több tollat növeszteni. Továbbá az MF csoportban átlagosan hosszabb és súlyosabb tollakat növesztettek a verebek. Az SRBC kezelés nem változtatta meg a vedlés folyamatát és a tollminőségre sem volt hatással.

Annak ellenére, hogy a vedléskori metabolikus ráta akár 100%-al megugorhat, nem találtunk csereviszonyt a vedlés és humorális immunválasz között. Ez feltehetően részben annak tudható be, hogy a humorális immunitás kevésbé költséges, amit a jelen vizsgálat is támogat, hiszen csak hosszas fehérjehiány után csökkent az Ig szintje. Továbbá, egybecsengenek ezzel fiziológiai éves ciklusos vizsgálataink is, amiben azt találtuk, hogy széncinegék Ig szintje vedléskor a legmagasabb, valamint a házi verebek természetes antitestjei és komplement rendszere viszonylag magas szintet mutat vedlési időszakban. Ellenben sikerült bizonyítani a vedlés magas forrásigényét: az AF madarak kettős költséget szenvedtek el, ugyanis részben hosszabban vedlettek, másrészt rosszabb minőségű tollakat növesztettek; mindkettő fitness-költségekkel járhat.

References

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. *Clin Microbiol Rev* 15: 58–65.

Anderson TR (2006). *Biology of the Ubiquitous House Sparrow: From Genes to Populations*. Oxford University Press, New York, USA.

Bachmann T, Emmerlich J, Baumgartner W, Schneider JM & Wagner H (2012). Flexural stiffness of feather shafts: geometry rules over material properties. *J Exp Biol* 215: 405–415.

Badyaev AV & Vleck CM (2007). Context-dependent development of sexual ornamentation: implications for a trade-off between current and future breeding efforts. *J Evol Biol* 20: 1277–1287.

Badyaev AV & Young RL (2004). Complexity and integration in sexual ornamentation: an example with carotenoid and melanin plumage pigmentation. *J Evol Biol* 17: 1317–1327.

Balmford A, Jones IL & Thomas ALR (1997). On avian asymmetry: evidence of natural selection for symmetrical tails and wings in birds. *Proc R Soc Lond B* 252: 245–251.

Barbosa A, Merino S, de Lope F & Møller AP (2002). Effects of feather lice on flight behavior of male Barn Swallows (*Hirundo rustica*). *Auk* 119: 213–216.

Barta Z, Houston AI, NcNamara JM, Welham RK, Hedenström A, Weber TP & Feró O (2006). Annual routines of non-migratory birds: optimal moult strategies. *Oikos* 112: 580–593.

Barta Z, NcNamara JM, Houston AI, Weber TP, Hedenström A & Feró O (2008). Optimal moult strategies in migratory birds. *Phil Trans R Soc Lond B* 363: 211–229.

Bergman G (1982). Why are the wings of *Larus f. fuscus* so dark? *Ornis Fennica* 59: 77–83.

Blanco G & de la Puente J (2002). Multiple elements of the black-billed magpie's tail correlate with variable honest information on quality in different age/sex classes. *Anim Behav* 63: 217–225.

Bodde SG, Meyers MA & McKittrick J (2011). Correlation of the mechanical and structural properties of cortical rachis keratin of rectrices of the Toco Toucan (*Ramphastos toco*). *J Mech Behav Biomed Mater* 4: 723–732.

Bonier F, Martin PR, Jensen JP, Butler LK, Ramenofsky M & Wingfield JC (2007). Pre-migratory life history stages of juvenile arctic birds: costs, constraints, and trade-offs. *Ecology* 88: 2729–2735.

Bonser RHC & Purslow PP (1995). The Young's modulus of feather keratin. *J Exp Biol* 198: 1029–1033.

Bonser RHC (1995). Melanin and the abrasion resistance of feathers. *Condor* 97: 590–591.

Booth DT, Clayton DH & Block BA (1993). Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proc R Soc Lond B* 253: 125–129.

Borgudd J (2003). *Mechanical properties of bird feathers – influence of UV-radiation and mechanical fatigue*. MSc thesis, Lund University, Lund, Sweden.

Bortolotti GR, Dawson RD & Murza GL (2002). Stress during feather development predicts fitness potential. *J Anim Ecol* 71: 333–342.

Bortolotti GR, Marchant T, Blas J & Cabezas S (2009). Tracking stress: localisation, deposition and stability of corticosterone in feathers. *J Exp Biol* 212: 1477–1482.

Bostwick KS & Prum RO (2005). Courting bird sings with stridulating wing feathers. *Science* 309: 736.

Bridge EL (2011). Mind the gaps: what's missing in our understanding of feather molt. *Condor* 113: 1–4.

Broggi J, Gamero A, Hohtola E, Orell M & Nilson J-Å (2011). Interpopulation variation in contour feather structure is environmentally determined in Great Tits. *PLoS ONE* 6: e24942.

Broggi J, Orell M, Hohtola E & Nilson J-Å (2004). Metabolic response to temperature variation in the great tit: an interpopulation comparison. *J Anim Ecol* 73: 967–972.

Brommer JE, Pihlajamäki O, Kolunen H & Pietiäinen H (2003). Life-history consequences of partial moult asymmetry. *J Anim Ecol* 72: 1057–1063.

Buchanan KL, Evans MR & Goldsmith AR (2003). Testosterone, dominance signalling and immunosuppression in the house sparrow, *Passer domesticus*. *Behav Ecol Sociobiol* 55: 50–59.

Buchanan KL, Evans MR, Goldsmith AR, Bryant DM & Rowe LV (2001). Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? *Proc R Soc Lond B* 268: 1337–1344.

Burtt EH Jr. & Ichida JM (1999). Occurrence of feather-degrading bacilli in the plumage of birds. *Auk* 116: 364–372.

Burtt EH Jr. (1986). An analysis of physical, physiological, and optical aspects of avian coloration with emphasis on wood-warblers. *Ornithol Monogr* 38: 1–126.

Butler LK, Rohwer S & Speidel MG (2008). Quantifying structural variation in contour feathers to address functional variation in life history trade-offs. *J Avian Biol* 39: 629–639.

Butler M & Johnson AS (2004). Are melanized feather barbs stronger? *J Exp Biol* 207: 285–293.

Cade TJ & Maclean GL (1967). Transport of water by adult sandgrouse to their young. *Condor* 69: 323–343.

Carr JM & Lima SL (2012). Heat-conserving postures hinder escape: thermoregulation–predation trade-off in wintering birds. *Behav Ecol* 23: 434–441.

Carrascal LM & Polo V (2006). Effects of wing area reduction on winter body mass and foraging behaviour in coal tits: field and aviary experiments. *Anim Behav* 72: 663–672.

Cartar RV (1992). Morphological senescence and longevity: an experiment relating wing wear and life span in foraging wild bumble bees. *J Anim Ecol* 61: 225–231.

Cherel Y, Charrassin JB & Challet E (1994). Energy and protein requirements for molt in the king penguin *Aptenodytes patagonicus*. *Am J Physiol* 266: R1182–R1188.

Clark CJ, Elias DO & Prum RO (2011). Aeroelastic flutter produces hummingbird feather songs. *Science* 333: 1430–1433.

Clayton DH (1990). Mate choice in experimentally parasitized rock doves: lousy males lose. *Am Zool* 30: 251–262.

Clayton DH, Koop JAH, Harbison CW, Moyer BR & Bush SE (2010). How birds combat ectoparasites. *Open Ornithol J* 3: 41–71.

Cohen AA, Martin LB, Wingfield JC, McWilliams SR & Dunne JA (2012). Physiological regulatory netweorks: ecological roles and evolutionary constraints. *Trends Ecol Evol* 27: 428–435.

Combes SA, Crall JD & Mukherjee S (2010). Dynamics of animal movement in an ecological context: dragonfly wing damage reduces flight performance and predation success. *Biol Lett* 6: 426–429.

Cornelius JM, Perfito N, Zann R, Breuner CW & Hahn TP (2011). Physiological trade-offs in self-maintenance: plumage molt and stress physiology in birds. *J Exp Biol* 214: 2768–2777.

Cotgreave P & Clayton DH (1994). Comparative analysis of time spent grooming by birds in relation to parasite load. *Behaviour* 131: 171–187.

Cristol DA, Armstrong JL, Whitaker JM & Forsyth MH (2005). Feather-degrading bacteria do not affect feathers on captive birds. *Auk* 122: 222–230.

Curio E (1983). Why de young birds reproduce less well? *Ibis* 125: 400–404.

Czirják GÁ, Pap PL, Vágási CI, Giraudeau M, Mureşan C, Mirleau P & Heeb P (2013). Preen gland removal increases plumage bacterial load, but not that of feather-degrading bacteria. *Naturwissenschaften* 100: 145–151.

Dawson A (2004). The effects of delaying the start of moult on the duration of moult, primary feather growth rates and feather mass in Common Starlings *Sturnus vulgaris*. *Ibis* 146: 493–500.

Dawson A (2008). Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. *Phil Trans R Soc Lond B* 363: 1621–1633.

Dawson A, Hinsley SA, Ferns PN, Boser RHC & Eccleston L (2000). Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. *Proc R Soc Lond B* 267: 2093–2098.

Dawson RD, Bortolotti GR & Murza GL (2001). Sex-dependent frequency and consequences of natural handicaps in American Kestrels. *J Avian Biol* 32: 351–357.

de la Hera I, Díaz JA, Pérez-Tris J & Tellería JL (2009b). A comparative study of migratory behaviour and body mass as determinants of moult duration in passerines. *J Avian Biol* 40: 461–465.

de la Hera I, Hedenström A, Pérez-Tris J & Tellería JL (2010a). Variation in the mechanical properties of flight feathers of the blackcap *Sylvia atricapilla* in relation to migration. *J Avian Biol* 41: 342–347.

de la Hera I, Pérez-Tris J & Tellería JL (2009a). Migratory behaviour affects the trade-off between feather growth rate and feather quality in a passerine bird. *Biol J Linn Soc* 97: 98–105.

de la Hera I, Pérez-Tris J & Tellería JL (2010b). Migratory behaviour and differential resource allocation between wing and tail feather in a passerine bird. *Auk* 127: 647–652.

de la Hera I, Pérez-Tris J & Tellería JL (2010c). Relationship among timing of moult, moult duration and feather mass in long-distance migratory passerines. *J Avian Biol* 41: 609–614.

de la Hera I, Schaper SV, Díaz JA, Pérez-Tris J, Staffan B & Tellería JL (2011). How much variation in the molt duration of passerines can be explained by the growth rate of tail feathers? *Auk* 128: 321–329.

de Ridder E, Pinxten R, Mees V & Eens M (2002). Short- and long-term effects of male-like concentrations of testosterone on female European starlings (*Sturnus vulgaris*). *Auk* 119: 487–497.

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. *Comp Biochem Physiol A* 152: 46–52.

Dumbacher JP (1999). The evolution of toxicity in pitohuis: I. Effects of homobatrachotoxin on chewing lice (Order Phthiraptera). *Auk* 116: 957–963.

Ellis VA, Merrill L, Wingfield JC, O'Loghlen AL & Rothstein SI (2012). Changes in immunocompetence and other physiological measures during molt in brown-headed cowbirds (*Molothrus ater*). *Auk* 129: 231–238.

Emlen DJ, Warren IA, Johns A, Dworkin I & Lavine LC (2012). A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* 337: 860–864.

Eraud C, Duriez O, Chastel O & Faivre B (2005). The energetic cost of humoral immunity in the Collared Dove, *Streptopelia decaocto*: is the magnitude sufficient to force energy-based trade-offs? *Funct Ecol* 19: 110–118.

Forslund P & Pärt T (1995). Age and reproduction in birds – hypotheses and tests. *Trends Ecol Evol* 10: 374–378.

Galván I & Alonso-Alvarez C (2008). An intracellular antioxidant determines the expression of a melanin-based signal in a bird. *PLoS ONE* 3: e3335.

Galván I (2011). Feather microstructure predicts size and colour intensity of a melanin-based plumage signal. *J Avian Biol* 42: 473–479.

Garamszegi LZ, Heylen D, Møller AP, Eens M & de Lope F (2005). Age-dependent health status and song characteristics in the barn swallow. *Behav Ecol* 16: 580–591.

Gienapp P & Merilä J (2010). Genetic and environmental effects on a condition-dependent trait: feather growth in Siberian jays. *J Evol Biol* 23: 715–723.

Ginn HB & Melville DS (2007). *Moult in Birds*. BTO Guide 19. Page Bros, Norwich, UK.

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.

Goldstein G, Flory KR, Browne BA, Majid S, Ichida JM & Burtt EH Jr. (2004). Bacterial degradation of black and white feathers. *Auk* 121: 656–659.

Gonzalez G, Sorci G, Møller AP, Ninni P, Haussy C & de Lope F (1999). Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). *J Anim Ecol* 68: 1225–1234.

Grant PR & Grant BR (1995). Predicting microevolutionary responses to directional selection on heritable variation. *Evolution* 49: 241–251.

Greiner EC (2008). *Isospora*, *Atoxoplasma*, and *Sarcocystis*. In: Atkinson CT, Tomas NJ & Hunter DB (eds) *Parasitic diseases of wild birds*, pp 108–119. Wiley-Blackwell, New York, USA.

Griffith SC (2000). A trade-off between reproduction and a condition-dependent sexually selected ornament in the house sparrow *Passer domesticus*. *Proc R Soc Lond B* 267: 1115–1119.

Griggio M, Serra L, Licheri D, Campomori C & Pilastro A (2009). Moult speed affects structural feather ornaments in the blue tit. *J Evol Biol* 22: 782–792.

Grubb TC Jr. (2006). *Ptilochronology: Feather Time and the Biology of Birds*. Oxford University Press, New York, USA.

Gunderson AR (2008). Feather-degrading bacteria: a new frontier in avian and host–parasite research? *Auk* 125: 972–979.

Gunderson AR, Frame AM, Swaddle JP & Forsyth MH (2008). Resistance of melanized feathers to bacterial degradation: is it really so black and white? *J Avian Biol* 39: 539–545.

Hagelin JC (2007). The citrus-like scent of crested auklets: reviewing the evidence for an avina olfactory ornament. *J Ornithol* 148 (Suppl 2): S195–S201.

Hall KSS & Fransson T (2000). Lesser Whitethroats under time-constraint moult more rapidly and grow shorter wing feathers. *J Avian Biol* 31: 583–587.

Hanssen SA, Folstad I & Erikstad KE (2006). White plumage reflects individual quality in female eiders. *Anim Behav* 71: 337–343.

Hearing VJ & Tsukamoto K (1991). Enzymatic control of pigmentation in mammals. *FASEB J* 5: 2902–2909.

Hedenström A & Sunada S (1999). On the aerodynamics of moult gaps in birds. *J Exp Biol* 202: 67–76.

Hedenström A (2003). Flying with holey wings. *J Avian Biol* 34: 324–327.

Hedenström A, Ellington CP & Wolf TJ (2001). Wing wear, aerodynamics and flight energetics in bumblebees (*Bombus terrestris*): an experimental study. *Funct Ecol* 15: 417–422.

Hegemann A, Matson KD, Versteegh MA & Tieleman BI (2012). Wild skylarks seasonally modulate energy budgets but maintain energetically costly inflammatory immune responses throughout the annual cycle. *PLoS ONE* 7: e36358.

Hemborg C & Lundberg A (1998). Costs of overlapping reproduction and moult in passerine birds: an experiment with the pied flycatcher. *Behave Ecol Sociobiol* 43: 19–23.

Hemborg C (1999). Sexual differences in moult–breeding overlap and female reproductive costs in pied flycatchers, *Ficedula hypoleuca*. *J Anim Ecol* 68: 429–436.

Hill GE & McGraw KJ (eds) (2006). *Bird Coloration*. Vol. 1 and 2. Harvard University Press, Cambridge, USA.

Holmgren N & Hedenström A (1995). The scheduling of molt in migratory birds. *Evol Ecol* 9: 354–368.

Hoye BJ & Buttemer WA (2011). Inexplicable inefficiency of avian molt? Insights form an opportunistically breeding arid-zone species, *Lichenostomus penicillatus*. *PLoS ONE* 6: e16230.

Ito S, Wakamatsu K & Ozeki H (2000). Chemical analysis of melanins and its application to the study of the regulation of melanogenesis. *Pigment Cell Res* 13 (Suppl 8): 103–109.

Jacob J & Ziswiler V (1982). The uropygial gland. In: Farner DS, King JR and Parkes KC (eds) *Avian Biology*. Vol. 6. Academic Press, New York, USA, pp. 199–324.

Jenni L & Winkler R (1994). *Moult and Aging of European Passerines*. Academic Press, London, UK.

Jensen H, Sæther B-E, Ringsby TH, Tufto J, Griffith SC & Ellegren H (2003). Sexual variation in heritability and genetic correlations of morphological traits in house sparrows (*Passer domesticus*). *J Evol Biol* 16: 1296–1307.

Jensen H, Sæther B-E, Ringsby TH, Tufto J, Griffith SC & Ellegren H (2004). Lifetime reproductive success in relation to morphology in the house sparrow *Passer domesticus*. *J Anim Ecol* 73: 599–611.

Jovani R & Blas J (2004). Adaptive allocation of stress-induced deformities on bird feathers. *J Evol Biol* 17: 294–301.

Jovani R & Diaz-Real J (2012). Fault bars timing and duration: the power of studying feather fault bars and growth bands together. *J Avian Biol* 43: 97–101.

Jovani R, Blas J, Stoffel MJ, Bortolotti LE & Bortolotti GR (2010). Fault bars and the risk of feather damage in cranes. *J Zool* 281: 94–98.

Kemp DJ, Herberstein ME & Grether GF (2012). Unraveling the true complexity of costly color signaling. *Behav Ecol* 23: 233–236.

King JK (1970). Pelage and molting in wild mammals with special reference to aquatic forms. *Quart Rev Biol* 45: 16–54.

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.

Kose M & Møller AP (1999). Sexual selection, feather breakage and parasites: the importance of white spots in the tail of the barn swallow (*Hirundo rustica*). *Behav Ecol Sociobiol* 45: 430–436.

Kose M, Mänd R & Møller AP (1999). Sexual selection for white tail spots in the barn swallow in relation to habitat choice by feather lice. *Anim Behav* 58: 1201–1205.

Langston N & Hillgarth N (1995). Moult varies with parasites in Laysan Albatrosses. *Proc R Soc Lond B* 261: 239–243.

Larsson K (1996). Genetic and environmental effects on the timing of wing moult in the barnacle goose. *Heredity* 76: 100–107.

Li D, Wu J, Zhang X, Ma X, Wingfield JC, Lei F, Wang G & Wu Y (2011). Comparison of adrenocortical responses to acute stress in lowland and highland Eurasian tree sparrows (*Passer montanus*): similar patterns during the breeding, but different during the prebasic molt. *J Exp Zool A* 315: 512–519.

Liebl AL & Martin LB (2012). Exploratory behaviour and stressor hyper-responsiveness facilitate range expansion of an introduced songbird. *Proc R Soc Lond B* 279: 4375–4381.

Lind J & Jakobsson S (2001). Body building and concurrent mass loss: flight adaptations in tree sparrows. *Proc R Soc Lond B* 268: 1915–1919.

Lind J (2001). Escape flight in moulting Tree Sparrows (*Passer montanus*). *Funct Ecol* 15: 29–35.

Lindström A, Visser GH & Daan S (1993). The energetic cost of feather synthesis is proportional to basal metabolic rate. *Physiol Zool* 66: 490–510.

Lingham-Soliar T, Bonser RHC & Wesley-Smith J (2010). Selective biodegradation of keratin matrix in feather rachis reveals bioengineering. *Proc R Soc Lond B* 277: 1161–1168.

Lockwood R, Swaddle JP & Rayner JMV (1998). Avian wingtip shape reconsidered: wingtip shape indices and morphological adaptations to migration. *J Avian Biol* 29: 273–292.

Love OP & Williams TD (2008). The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. *Am Nat* 172: E135–E149.

Macleod R, Barnett P, Clark JA & Cresswell W (2005). Body mass change strategies in blackbirds *Turdus merula*: the starvation–predation risk trade-off. *J Anim Ecol* 74: 292–302.

Männiste M & Hõrak P (2011). Effects of immune activation and glucocorticoid administration on feather growth in greenfinches. *J Exp Zool A* 315: 527–535.

Martin LB II. (2005). Trade-offs between molt and immune activity in two populations of house sparrows (*Passer domesticus*). *Can J Zool* 83: 780–787.

Marzal A, Reviriego M, Hermosell IG, Balbontín J, Bensch S, Relinque C, Rodríguez L, Garcia-Longoria L & de Lope F (2013). Malaria infection and feather growth rate predict reproductive success in house martins. *Oecologia* in press.

McGlothlin JW, Duffy DL, Henry-Freeman JL & Ketterson ED (2007). Diet quality affects an attractive white plumage pattern in dark-eyed juncos (*Junco hyemalis*). *Behav Ecol Sociobiol* 61: 1391–1399.

McGraw KJ (2006). Mechanics of melanin-based coloration. In: Hill GE & McGraw KJ (eds) *Bird Coloration. Vol. I. Mechanisms and Measurements*, pp. 243–294. Harvard University Press, Cambridge, USA.

McGraw KJ (2007). Dietary mineral content influences the expression of melanin-based ornamental coloration. *Behav Ecol* 18: 137–142.

McGraw KJ (2008). An update on the honesty of melanin-based color signals in birds. *Pigment Cell Melanoma Res* 21: 133–138.

Merilä J & Hemborg C (2000). Fitness and feather wear in the Collared Flycatcher *Ficedula albicollis*. *J Avian Biol* 31: 504–510.

Metcalfe NB & Ure SE (1995). Diurnal variation in flight performance and hence potential predation risk in small birds. *Proc R Soc Lond B* 261: 395–400.

Møller AP & de Lope F (1999). Senescence in a short-lived migratory bird: age-dependent morphology, migration, reproduction and parasitism. *J Anim Ecol* 68: 163–171.

Møller AP (1988). Badge size in the house sparrow *Passer domesticus*. Effects of intra- and intersexual selection. *Behav Ecol Sociobiol* 22: 373–378.

Møller AP (1989). Viability costs of male tail ornaments in a swallow. *Nature* 339: 132–135.

Møller AP (1991). Parasites, sexual ornaments, and mate choice in the barn swallow. In: Loye JE and Zuk M (eds) *Bird–parasite interactions: ecology, evolution, and behaviour.* Oxford University Press, New York, USA, pp. 328–343.

Møller AP (1994). *Sexual Selection and the Barn Swallow*. Oxford University Press, Oxford, UK.

Møller AP (2010). Host–parasite interactions and vectors in the barn swallow in relation to climate change. *Global Change Biol* 16: 1158–1170.

Møller AP, de Lope F & Saino N (2004b). Parasitism, immunity, and arrival date in a migratory bird, the barn swallow. *Ecology* 85: 206–2019.

Møller AP, Erritzøe J & Nielsen JT (2009). Frequency of fault bars in feathers of birds and susceptibility to predation. *Biol J Linn Soc* 97: 334–345.

Møller AP, Kimball RT & Erritzøe J (1996). Sexual ornamentation, condition, and immune defence in the house sparrow *Passer domesticus*. *Behav Ecol Sociobiol* 39: 317–322.

Møller AP, Magnhagen C, Ulfstrand A & Ulfstrand S (1995). Phenotypic quality and molt in the barn swallow, *Hirundo rustica*. *Behav Ecol* 6: 242–249.

Møller AP, Martinelli R & Saino N (2004a). Genetic variation in infestation with a directly transmitted ectoparasite. *J Evol Biol* 17: 41–47.

Møller AP, Nielsen JT & Erritzøe J (2006). Losing the last feather: feather loss as an antipredator adaptation in birds. *Behav Ecol* 17: 1046–1056.

Møller AP, Vágási CI & Pap PL (2013). Risk-taking and the evolution of mechanisms for rapid escape from predators. *J Evol Biol* (in press).

Møller AP, Vágási CI & Pap PL (2013). Risk-taking and the evolution of mechanisms for rapid escape from predators. *J Evol Biol* in press.

Monaghan P, Metcalfe NB & Torres R (2009). Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol Lett* 12: 75–92.

Mönkkönen M (1995). Do migrant birds have more pointed wings?: a comparative study. *Evol Ecol* 9: 520–528.

Moreno J & Soler JJ (2011). Sources of distinctiveness of juvenile plumage in Western Palearctic passerines. *Biol J Linn Soc* 102: 440–454.

Moreno J, Sanz JJ, Merino S & Arriero E (2001). Daily energy expenditure and cell-mediated immunity in pied flycatchers while feeding nestlings: interaction with moult. *Oecologia* 129: 492–497.

Moreno-Rueda G & Hoi H (2012). Female house sparrows prefer big males with a large wing bar and fewer feather holes caused by chewing lice. *Behav Ecol* 23: 271–277.

Moreno-Rueda G (2005). Is the white wing-stripe of male House Sparrows *Passer domesticus* an indicator of the load of Mallophaga? *Ardea* 93: 109–114.

Moreno-Rueda G (2010a). Uropygial gland size correlates with feather holes, body condition and wingbar size in the house sparrow *Passer domesticus*. *J Avian Biol* 41: 229–236.

Moreno-Rueda G (2010b). Experimental test of a trade-off between moult and immune response in house sparrows *Passer domesticus*. *J Evol Biol* 23: 2229–2237.

Moreno-Rueda G (2011). House Sparrows *Passer domesticus* with larger uropygial glands show reduced feather wear. *Ibis* 153: 195–198.

Moyer BR, Rock AN & Clayton DH (2003). Experimental test of the importance of preen oil in rock doves (*Columba livia*). *Auk* 120: 490–496.

Murphy ME & King JR (1982). Amino acid composition of the plumage of the white-crowned sparrow. *Condor* 84: 435–438.

Murphy ME & King JR (1984). Sulfur amino acid nutrition during molt in the white-crowned sparrow. 1. Does dietary sulfur amino acid concentration affect the energetics of molt as assayed by metabolized energy? *Condor* 86: 314–323.

Murphy ME & King JR (1986). Composition and quantity of feather sheaths produced by white-crowned sparrows during the postnuptial molt. *Auk* 103: 822–825.

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. *Physiol Zool* 63: 1118–1140.

Murphy ME & Taruscio TG (1995). Sparrows increase their rate of tissue and whole-body protein synthesis during the annual molt. *Comp Biochem Physiol A* 111: 385–396.

Murphy ME, King JR & Lu J (1988). Malnutrition during the postnuptial molt of White-crowned Sparrows: feather growth and quality. *Can J Zool* 66: 1403–1413.

Nakagawa S, Lee J-W, Woodward BK, Hatchwell BJ & Burke T (2008). Differential selection according to the degree of cheating in a status signal. *Biol Lett* 4: 667–669.

Nava MP, Veiga JP & Puerta M (2001). White blood cell counts in house sparrows (*Passer domesticus*) before and after moult and after testosterone treatment. *Can J Zool* 79: 145–148.

Newton I (1968). The temperatures, weights, and body composition of molting bullfinches. *Condor* 70: 323–332.

Newton I (2009). Moult and plumage. *Ringing Migr* 24: 220–226.

Nilsson J-Å & Svensson E (1996). The cost of reproduction: a new link between current reproductive effort and future reproductive success. *Proc R Soc Lond B* 263: 711–714.

Nudds RL & Dyke GJ (2010). Narrow primary feather rachises in *Confuciusornis* and *Archaeopteryx* suggest poor flight ability. *Science* 328: 887–889.

O'Hara PD (2002). *The Role of Feather Wear in Alternative Life-history Strategies of a Long-distance Migratory Shorebird, the Western Sandpiper*. PhD thesis, Simon Fraser University, Burnaby, Canada.

Pap PL, Barta Z, Tökölyi J & Vágási CI (2007). Increase of feather quality during moult: a possible implication of feather deformities in the evolution of partial moult in the great tit *Parus major*. *J Avian Biol* 38: 471–478.

Pap PL, Czirják GÁ, Vágási CI, Barta Z & Hasselquist D (2010b). Sexual dimorphism in immune function changes during the annual cycle in house sparrows. *Naturwissenschaften* 97: 891–901.

Pap PL, Tökölyi J & Szép T (2005). Frequency and consequences of feather holes in Barn Swallows *Hirundo rustica*. *Ibis* 147: 169–175.

Pap PL, Vágási CI, Tökölyi J, Czirják GÁ & Barta Z (2010a). Variation in haematological indices and immune function during the annual cycle in the Great Tit *Parus major*. *Ardea* 98: 105–112.

Pennycuick CJ (2008). *Modelling the Flying Bird*. Elsevier, Amsterdam, The Netherlands.

Pérez-Tris J & Tellería JL (2001). Age-related variation in wing shape of migratory and sedentary Blackcaps *Sylvia atricapilla*. *J Avian Biol* 32: 207–213.

Perrins CM (1998). *The Complete Birds of the Western Palearctic on CD-ROM*. version 1.0. Oxford University Press, Oxford, UK.

Poston JP, Hasselquist D, Stewart IRK & Westneat DF (2005). Dietary amino acids influence plumage traits and immune responses of male house sparrows, *Passer domesticus*, but not as expected. *Anim Behav* 70: 1171–1181.

Prum RO (1999). Development and evolutionary origin of feathers. *J Exp Zool B* 285: 291–306.

Prum RO (2006). Anatomy, physics, and evolution of structural colors. In: Hill GE & McGraw KJ (eds) *Bird Coloration. Vol. I. Mechanism and Measurements*, pp 295–353. Harvard University Press, Cambridge, USA.

Purslow PP & Vincent JFV (1978). Mechanical properties of primary feathers from the pigeon. *J Exp Biol* 72: 251–260.

Remisiewicz M, Tree AJ, Underhill LG & Taylor PB (2010). Rapid or slow moult? The choice of a primary moult strategy by immature Wood Sandpipers *Tringa glareola* in southern Africa. *J Ornithol* 151: 429–441.

Ricklefs RE & Wikelski M (2002). The physiology/life-history nexus. *Trends Ecol Evol* 17: 462–468.

Roff DA (2002). *Life History Evolution*. Sinauer, Sunderland, USA.

Rohwer S, Ricklefs RE, Rohwer VG & Copple MM (2009). Allometry of the duration of molt in birds. *PLoS Biol* 7: e1000132.

Romero LM (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen Comp Endocrinol* 128: 1–24.

Romero LM (2004). Physiological stress in ecology: lessons from biomedical research. *Trends Ecol Evol* 19: 249–255.

Roulin A, Almasi B, Rossi-Pedruzzi A, Ducrest A-L, Wakamatsu K, Miksik I, Blount JD, Jenni-Eiermann S & Jenni L (2008). Corticosterone mediates the condition-dependent component of melanin-based coloration. *Anim Behav* 75: 1351–1358.

Sadd BM & Schmid-Hempel P (2009). Principle of ecological immunology. *Evol Appl* 2: 113–121.

Saino N, Møller AP & Bolzern AM (1995). Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behav Ecol* 6: 397–404.

Saino N, Szép T, Ambrosini R, Romano M & Møller AP (2004). Ecological conditions during winter affect sexual selection and breeding in a migratory bird. *Proc R Soc Lond B* 271: 681–686.

Sanz JJ, Moreno J, Merino S & Tomás G (2004). A trade-off between two resource-demanding functions: post-nuptial moult and immunity during reproduction in male pied flycatchers. *J Anim Ecol* 73: 441–447.

Saranathan V & Burtt EH Jr. (2007). Sunlight on feathers inhibits feather-degrading bacteria. *Wilson J Ornithol* 119: 239–245.

Sarasola JH & Jovani R (2006). Risk of feather damage explains fault bar occurrence in a migrant hawk, the Swainson's hawk *Buteo swainsoni*. *J Avian Biol* 37: 29–35.

Selman C, Blount JD, Nussey DH & Speakman JR (2012). Oxidative damage, ageing, and life-history evolution: where now. *Trends Ecol Evol* 27: 570–577.

Senar JC, Domènech J & Uribe F (2002). Great tits (*Parus major*) reduce body mass in response to wing area reduction: a field experiment. *Behav Ecol* 13: 725–727.

Serra L (2001). Duration of primary moult affects primary quality in Grey Plovers *Pluvialis squatarola*. *J Avian Biol* 32: 377–380.

Serra L, Griggio M, Licheri D & Pilastro A (2007). Moult speed constrains the expression of a carotenoid-based sexual ornament. *J Evol Biol* 20: 2028–2034.

Serrano D & Jovani R (2005). Adaptive fault bar distribution in a long-distance migratory, aerial forager passerine? *Biol J Linn Soc* 85: 455–461.

Shawkey MD & Hill GE (2005). Carotenoids need structural colours to shine. *Biol Lett* 1: 121–124.

Shawkey MD, Pillai SR & Hill GE (2003). Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. *J Avian Biol* 34: 345–349.

Shawkey MD, Pillai SR, Hill GE, Siefferman LM & Roberts SR (2007). Bacteria as an agent for change in structural plumage color: correlational and experimental evidence. *Am Nat* 169: S112–S121.

Sheldon BC & Verhulst S (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol Evol* 11: 317–321.

Siikamäki P (1998). Limitation of reproductive success by food availability and breeding time in pied flycatchers. *Ecology* 79: 1789–1796.

Siikamäki P, Hovi M & Rätti O (1994). A trade-off between current reproduction and moult in the Pied Flycatcher—an experiment. *Funct Ecol* 8: 587–593.

Sild E, Sepp T & Hõrak P (2011). Behavioural trait covaries with immune responsiveness in a wild passerine. *Brain Behav Immun* 25: 1349–1354.

Slagsvold T & Dale S (1996). Disappearance of female pied flycatchers in relation to breeding stage and experimentally induced molt. *Ecology* 77: 461–471.

Slagsvold T (1982). Sex, size, and natural selection in the Hooded Crow *Corvus corone cornix*. *Ornis Scand* 13: 165–175.

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 in European birds. *J Evol Biol* 25: 1779–1791.

Stearns SC (1989). Trade-offs in life-history evolution. *Funct Ecol* 3: 259–268.

Stettenheim PR (2000). The integumentary morphology of modern birds—An overview. *Am Zool* 40: 461–477.

Stewart IRK & Westneat DF (2010). Dietary calcium negatively affects the size of a status signal in juvenile male house sparrows (*Passer domesticus*). *Auk* 127: 549–557.

Stoehr AM (2006). Costly melanin ornaments: the importance of taxon? *Funct Ecol* 20: 276–281.

Strochlic DE & Romero LM (2008). The effects of chronic and physiological stress on feather replacement in European starlings (*Sturnus vulgaris*). *Comp Biochem Physiol A* 149: 68–79.

Svensson E & Hedenström A (1999). A phylogenetic analysis of the evolution of moult strategies in Western Palearctic warblers (Aves: Sylviidae). *Biol J Linn Soc* 67: 263–276.

Svensson E & Nilsson J-Å (1997). The trade-off between molt and parental care: a sexual conflict in the blue tit? *Behav Ecol* 8: 92–98.

Svensson L (1992) *Identification Guide to European Passerines*. 4th edn. Stockholm, Sweden.

Swaddle JP & Lockwood R (2003). Wingtip shape and flight performance in the European Starling *Sturnus vulgaris*. *Ibis* 145: 457–464.

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. *J Avian Biol* 27: 103–111.

Török J, Hegyi G & Garamszegi LZ (2003). Depigmented wing patch size is a condition-dependent indicator of viability in male collared flycatchers. *Behav Ecol* 14: 382–388.

Tsurim I, Kotler BP, Gilad A, Elazary S & Abramsky Z (2010). Foraging behavior of an urban bird species: molt gaps, distance to shelter, and predation risk. *Ecology* 91: 233–241.

Tubaro PL (2003). A comparative study of aerodynamic function and flexural stiffness of outer tail feathers in birds. *J Avian Biol* 34: 243–250.

Vas Z, Csörgő T, Møller AP & Rózsa L (2008). The feather holes on the barn swallow *Hirundo rustica* and other small passerines are probably caused by *Brueelia* spp. lice. *J Parasitol* 94: 1438–1440.

Veiga JP & Puerta M (1996). Nutritional constraints determine the expression of a sexual trait in the house sparrow, *Passer domesticus*. *Proc R Soc Lond B* 263: 229–234.

Veiga JP (1996). Permanent exposure versus facultative concealment of sexual traits: an experimental study in the house sparrow. *Behav Ecol Sociobiol* 39: 345–352.

Verhulst S & Nilsson J-Å (2008). The timing of bird's breeding seasons: a review of experiments that manipulated the timing of breeding. *Phil Trans R Soc Lond B* 363: 399–410.

Verhulst S, van Balen JH & Tinbergen JM (1995) Seasonal decline in reproductive success of the Great Tit: variation in time or quality? *Ecology* 76: 2392–2403.

Verspoor JJ, Love OP, Rowland E, Chin EH & Williams TD (2007). Sex-specific development of avian flight performance under experimentally altered rearing conditions. *Behav Ecol* 18: 967–973.

Vieira JLF & Gomes ALS (2010). Oxidative stress at different stages of the molting cycle of captive *Coturnix coturnix*. *Res J Biol Sci* 5: 610–614.

Wang X, Nudds RL, Palmer C & Dyke GJ (2012). Size scaling and stiffness of avian primary feathers: implications for the flight of Mesozoic birds. *J Evol Biol* 25: 547–555.

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. *Biol Lett* 1: 27–30.

Weber TP, Kranenbarg S, Hedenström A, Waarsing JH & Weinans H (2010). Flight feather shaft structure of two warbler species with different moult schedules: a study using high-resolution X-ray imaging. *J Zool* 280: 163–170.

Wiggins DA, Pärt T & Gustafsson L (1998). Timing of breeding and reproductive costs in collared flycatchers. *Auk* 115: 1063–1067.

Wikelski M & Ricklefs RE (2001). The physiology of life histories. *Trends Ecol Evol* 16: 479–481.

Williams EV & Swaddle JP (2003). Moult, flight performance and wingbeat kinematics during take-off in European starlings *Sturnus vulgaris*. *J Avian Biol* 34: 371–378.

Zera AJ & Harshman LG (2001). The physiology of life history trade-offs in animals. *Annu Rev Ecol Syst* 32: 95–126.

house sparrow   © Zsoldos Márton   great tit

CHAPTER II

This chapter lines up the original reprints, which are meant to show the methodology, results and their discussion in details.

List of papers

I. <u>Vágási CI</u>, Pap PL, Tökölyi J, Székely E & Barta Z (2011). Correlates of variation in flight feather quality in the Great Tit *Parus major*. *Ardea* 99: 53–60. [IF$_{2011}$ 0.6]

II. <u>Vágási CI</u>, 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. [IF$_{2011}$ 4.09]

III. Pap PL, <u>Vágási CI</u>, Bărbos L & Marton A (2013). Chronic coccidian infestation compromises flight feather quality in house sparrows, *Passer domesticus*. *Biol J Linn Soc* 108: 414–428. [IF$_{2011}$ 2.19]

IV. <u>Vágási CI</u>, Pap PL & Barta Z (2010). Haste Makes Waste: Accelerated Molt Adversely Affects the Expression of Melanin-based and Depigmented Plumage Ornaments in House Sparrows. *PLoS ONE* 5: e14215. [IF$_{2010}$ 4.41]

V. Pap PL, <u>Vágási CI</u>, Czirják GÁ & Barta Z (2008). Diet quality affects postnuptial molting and feather quality of the house sparrow (*Passer domesticus*): interaction with humoral immune function? *Can J Zool* 86: 834–842. [IF$_{2008}$ 1.38]

Papers are referred throughout the text by their boldface roman numerals (**I–V**).

Own contribution to papers included in the thesis

	I	II	III	IV	V
Original idea	*	*	*	*	
Study design and data collection	*	*	*	*	*
Data analysis	*	*		*	
Manuscript preparation	*	*	*	*	*

Paper I

Correlates of variation in flight feather quality in the Great Tit *Parus major*

<u>Csongor I. Vágási</u>, Péter L. Pap, Jácint Tökölyi, Edina Székely
& Zoltán Barta (2011) *Ardea* 99: 53–60 [IF$_{2011}$ 0.6]

Summary

The most important function of moult in birds is the renewal of deteriorated feathers. Worn and holey remiges may incur fitness costs, yet little is known about the factors responsible for the degree of feather wear and sources of variation in feather hole incidence. Here, we report results on variation in feather quality based on a study of three consecutive annual cycles of a Great Tit *Parus major* population. We found that month, age, sex, weight and the presence of feather holes are the main sources of variation in degree of wear. Juveniles and individuals with lower feather quality (measured through rachis diameter) and with fault bars present had higher feather hole loads. Feather abrasion peaked in the breeding season and was higher in females probably due to higher workload. The lower feather quality of juveniles compared to adults probably arises because of fast ontogeny in contrast to adults' prolonged moult. Our results indicate that feather deformities are positively interrelated and can be used as proxies of feather quality. Our findings on the correlates of feather holes seem partially contradictory with the widespread view that holes are feeding marks of chewing lice. We propose that feather holes are instead minor feather handicaps, at least in Great Tits.

Correlates of variation in flight feather quality in the Great Tit *Parus major*

Csongor I. Vágási[1,2,*], Péter L. Pap[2], Jácint Tökölyi[1], Edina Székely[1] & Zoltán Barta[1]

The most important function of moult in birds is the renewal of deteriorated feathers. Worn and holey remiges may incur fitness costs, yet little is known about the factors responsible for the degree of feather wear and sources of variation in feather hole incidence. Here, we report results on variation in feather quality based on a study of three consecutive annual cycles of a Great Tit *Parus major* population. We found that month, age, sex, weight and the presence of feather holes are the main sources of variation in degree of wear. Juveniles and individuals with lower feather quality (measured through rachis diameter) and with fault bars present had higher feather hole loads. Feather abrasion peaked in the breeding season and was higher in females probably due to higher workload. The lower feather quality of juveniles compared to adults probably arises because of fast ontogeny in contrast to adults' prolonged moult. Our results indicate that feather deformities are positively interrelated and can be used as proxies of feather quality. Our findings on the correlates of feather holes seem partially contradictory with the widespread view that holes are feeding marks of chewing lice. We propose that feather holes are instead minor feather handicaps, at least in Great Tits.

Key words: annual cycle, fault bar, feather deformities, feather hole, feather wear, flight feather quality, *Parus major*

[1]Behavioural Ecology Research Group, Dept Evolutionary Zoology, University of Debrecen, Egyetem tér 1, H–4032 Debrecen, Hungary; [2]Dept Taxonomy and Ecology, Babeş-Bolyai University, Clinicilor street 5–7, RO–400006 Cluj Napoca, Romania;
*corresponding author (csongor.vagasi@vocs.unideb.hu)

One of the primary functions of complete moult in birds is the renewal of old, deteriorated flight feathers, since damaged feathers may impair flight performance and ultimately reduce fitness. The gradual shortening of wing length and increasing dullness of flight feather colour following moult is a widespread and well-known phenomenon in birds (e.g. used for age classification; Svensson 1992). Yet less attention has been paid towards understanding which factors are responsible for the intensity of feather abrasion (but see Merilä & Hemborg 2000, Serra 2001). In a Collared Flycatcher *Ficedula albicollis* population Merilä & Hemborg (2000) found that age, sex and reproductive effort are the main factors that predict the degree of abrasion of the primaries. In several Grey Plover *Pluvialis squatarola* wintering populations, birds with a prolonged moulting period had less abraded feathers compared to individuals with fast moulting (Serra 2001). However, these investigations were conducted in only one season (breeding and wintering, respectively), thus information about the pattern of annual accumulation of feather wear is scarce. Similarly, the suspected dependence of feather deformities (such as feather holes and fault bars) on physical condition and feather quality and their predicted positive effects on feather wear are topics largely neglected. Although flight feathers abrade mainly at their tip (in our population 3–4 mm when heavily abraded, but without breakages), which consist of less than 5% of the total surface of the feathers, the abovementioned topics are worth investigating, because the reduced wing area (i.e. increased wing load) and the altered wingtip shape may influence the

flight performance of birds. For instance, studies on European Starlings *Sturnus vulgaris* demonstrated that birds with shortened and more rounded wingtips (which characterize the birds just before their annual complete moult) had a lower take-off angle and reduced flight speed (Swaddle *et al.* 1996, Swaddle & Lockwood 2003, Williams & Swaddle 2003). These capacities are predictors of escape success from a pursuing predator (Kullberg *et al.* 1998) in that they decrease the likelihood of escape when reduced. Therefore, understanding which factors contribute to feather usage may open an avenue to study the fitness consequences of worn feathers.

Impairment of flight ability could also result from the appearance of feather deformities during the period between two moults. Such abnormalities are the feather holes (with a diameter of approximately 0.5–1 mm) which appear on the vanes of flight feathers and are the consequences of missing barbs / barbules (Fig. 1 in Vas *et al.* 2008). Although this deformity is used as a proxy for feather quality (Pap *et al.* 2005, 2007), the relationship between feather hole abundance and feather quality has not been addressed. Considering the proximal causes of feather hole incidence, Kose & Møller (1999) found that the length of the outermost tail feather, a secondary sexual character in male Barn Swallows *Hirundo rustica*, negatively correlates with the occurrence of holes, suggesting that feather deformities are condition-dependent. Holes are thought to be the feeding traces of chewing lice (e.g. Møller 1991), but the available indirect results are ambiguous (Pap *et al.* 2005, Vas *et al.* 2008). Moreover, the causes and consequences of feather holes have only been investigated in Barn Swallows (e.g. Kose & Møller 1999, Barbosa *et al.* 2002, Pap *et al.* 2005, Møller 2010), a passerine which forages on the wing and is a long-distance migrant. It is also unclear which factors create variation in the frequency of feather holes.

Here, we report correlative results for a resident Great Tit *Parus major* population. Great Tits start breeding from mid April and nestlings generally fledge until early July. Between June and September, adults perform their annual complete post-breeding moult (primaries are replaced from the 1st innermost towards the 10th outermost), while juveniles replace only the tertials, rectrices, and their body feathers and wing coverts during the partial post-juvenile moult (Cramp & Perrins 1993, Pap *et al.* 2007, 2010). Birds were followed throughout three consecutive annual cycles by monthly sampling, allowing us to compare the breeding and the non-breeding season on a fine time-scale. Our aims were to examine (1) the main sources of variation in feather wear and incidence of feather holes; (2) whether different feather deformities (feather wear, feather holes and fault bars) are associated; (3) the possible causal link between 'true' feather quality variables (e.g. rachis width, barbule density; see Dawson *et al.* 2000) and feather hole load. We predicted that, during the annual cycle breeding activity will greatly deteriorate the quality of flight feathers due to increased locomotion in an abrasive environment and because there is less time available for self maintenance (i.e. preening). We also expect sex differences in feather usage since parental effort in tits is biased towards females (Cramp & Perrins 1993). Further, if feather deformities are dependent on feather quality (which is dependent on condition; see Pap *et al.* 2008), then they are expected to be positively related to each other. Provided that the condition of juveniles is generally lower and they develop the whole plumage in an extremely short period, we predicted that their feathers are of inferior quality compared to adults.

METHODS

Study population and procedures
We studied a Great Tit population situated in a 40-ha orchard of various old fruit-trees between March 2004 and May 2007 near Stana village, Transylvania, central Romania (46°89'N, 23°14'E). During three consecutive annual cycles, we captured Great Tits ($n = 964$, recaptures $n = 448$) with mist nets (Ecotone, Poland). After capture, we sexed and aged birds following Svensson's (1992) criteria. Here, 'juveniles' are birds before, whereas 'adults' are those after their first complete post-breeding moult. Note that in this categorisation, the 'juveniles' group contains both first- and second-year birds. The latter are reproductively active, but still prior to their first complete moult. We decided to choose this age division because, although second-year birds do breed, they differ from adults with respect to flight feather quality as they retain their primaries and secondaries during the first years' partial post-juvenile moult (see above; Pap *et al.* 2007). We were able to determine the sex and age of all individuals, because we present data only for the period outside the aforementioned moulting period, namely from September of one year to May of the next year (see below why we restricted the analyses to this period). We collected standard physical measurements: wing length (± 0.5 mm with a ruler), tarsus length (± 0.01 mm with a digital calliper), weight (± 0.1 g with a Pesola spring balance), and the rachis diameter of the 8th primary

(±0.01 mm, measured with digital calliper at the tip of the 8th primary covert; 1st primary being the innermost), which is a proxy for feather quality (see Dawson *et al.* 2000). We calculated a condition-index using the residuals of the regression between tarsus length and weight.

Feather quality measurements

We categorized the degree of flight feather wear after Prater *et al.* (1977) as follows: 0 = unworn (i.e. immaculate feather tip), 1 = slightly, 2 = moderately, and 3 = very abraded (i.e. a considerably shorter feather, even with breakage at the tip). We scored all flight feathers separately, although in the analyses we only used the primaries, because of their greater function in flight compared to secondaries (Barta *et al.* 2006, Merilä & Hemborg 2000), and since they are significantly more worn than other flight feathers (pers. obs.). We computed a primary wear index by summing the abrasion score of nine fully developed primaries (the outermost primary is vestigial). Feather wear was not examined in adults and second-year juveniles between June and August when they were performing the annual complete post-breeding moult. Although newly fledged first-year juveniles shed only the tertials and rectrices during the partial post-juvenile moult, their feather wear data were also excluded if gathered in the above mentioned period, because at this time the wear index was on average zero with almost no variation. Thus we analysed the period between the end of the moult in the given year and beginning of the moult in the next year (i.e. September–May in our population). In order to test the assumption that feather wear depends on feather quality, we counted the number of fault bars (Fig. 1 in Sarasola & Jovani 2006) and feather holes (Fig. 1 in Vas *et al.* 2008) on the whole wing. Variation in the intensity of feather deformities can indicate individual condition, yet this has rarely been tested (but see Bortolotti *et al.* 2002, Pap *et al.* 2007). To provide more robust data, we assessed feather wear, the number of feather holes and fault bars on both wings, and then used the mean values in the subsequent analyses. In contrast to feather wear, feather holes are more evenly distributed across the different wing feathers (see Pap *et al.* 2007), therefore we used the number of holes on the whole wing. Another reason for using this parameter was the low occurrence of holes in the case of adults (see also Pap *et al.* 2007).

In order to evaluate the quality of the primaries more precisely, we collected the innermost primaries of both left and right wings in a subset of birds (*n* = 82), which were analysed later in the laboratory. From every

pair of primaries a digital photograph was taken under standard light conditions and camera settings. From these recordings we measured the rachis diameter at the base of the feather vane. In case of feathers with at least one feather hole, we first digitalized the contour of the vanes and the line of the rachis; thereafter we geometrically transformed the points of the contour and rachis to set the naturally curvilinear rachis to be straight. Subsequently, the relative longitudinal position of the hole was measured, where the proximal (shaft) end of the vane was 0 and the distal end (tip) was 1. Photographs taken under microscope were used to quantify the density of barbules (measured at 3 barbs along a portion of 530 pixels length and then averaged). For these purposes (relative hole position, rachis width and barbule density measurement) we used the ImageJ software (Rasband 2008). All digital measurements were done by the same person (ES).

Statistical analyses

We performed two separate analyses for the two dependent variables (primary feather wear and whole wing feather hole number). The data were analysed by fitting generalized linear mixed effect models (GLMMs) with Poisson distribution of error terms and logarithmic link function. The identity (ID) of individuals, study year and ringer (CIV, PLP and JT) were entered as random factors, sex and age as factors, while month, biometrical variables and condition were included as covariates. The distributions of fault bars and feather holes among individuals were highly skewed even after data transformations, thus we converted them into binomial variables (present / absent) when analysing the effect of holes and fault bars on feather wear and the effect of fault bars on feather hole numbers. Initially we entered all explanatory variables and their second order interactions into the models and then used stepwise backward removal of those variables with $|z|$ scores < 2. Thus, only those explanatory variables were retained in the final minimal model that fulfilled the $|z|$ > 2 criterion. We used this conservative criterion for significance testing instead of the actual probabilities because the derivation of these probabilities is not well founded theoretically (Pinheiro & Bates 2000, Crawley 2007). All analyses were carried out with the 'lmer' function ('lme4' package; Bates 2008) in the R statistical environment version 2.11.1 (R Development Core Team 2010).

RESULTS

Feather wear

Time of year (expressed in months) significantly explained the abrasion of primaries (Table 1). Birds had unworn feathers after their complete annual moult (adults) or fledging (juveniles), as expected. Feather wear score increased non-linearly during the annual cycle reaching its maximum during the breeding season, before the onset of post-breeding moult (Fig. 1A). The effect of age was significant during the whole annual cycle, since adults had less worn primary feathers than juveniles (Fig. 1A; Table 1). The pattern of seasonal change differed between the age classes, as indicated by the significant month × age interaction. This is caused by the absence of wear in adults as opposed to juveniles in September, but adults become nearly as worn as juveniles until May (Fig. 1A). Nonetheless, restricting the analysis to May revealed that adults had less worn primaries than juveniles (GLMM, estimate = –0.28, SE = 0.07, z = –3.83). Males and females differed in their degree of primary wear: females had less abraded feathers (Table 1) over the whole annual cycle, but their wearing trajectories diverged during the annual cycle as shown by the significant month × sex interaction (Fig. 1A; Table 1). Consequently, females become more abraded until May (May estimate = 0.15, SE = 0.07, z = 2.02).

Weight was negatively related to feather wear, indicating that the flight feathers of birds with higher body mass were less intensively abraded (Table 1). Wing length (estimate = –0.01, SE = 0.01, z = –0.26), tarsus length (estimate = –0.03, SE = 0.04, z = –0.79) and condition (estimate = 0.04, SE = 0.05, z = 0.89) did not explain any variation in the intensity of primary abrasion. Birds with higher rachis diameter had marginally more worn primaries (estimate = 0.87, SE = 0.49, z = 1.78). In the presence of feather holes, feather abrasion was more extensive (Table 1) and females with feather holes present tended to have more worn feathers (hole × sex: estimate = 0.13, SE = 0.08, z = 1.75). The presence of fault bars (estimate = 0.03, SE = 0.06, z = 0.52) was not related to the degree of wear.

Feather holes

The variation in the number of feather holes among individual Great Tits did not depend on month (estimate = 0.01, SE = 0.02, z = 0.43), thus the number of feather holes remained unchanged during the whole annual cycle (Fig. 1B). Juveniles had significantly higher hole loads than adults throughout the annual
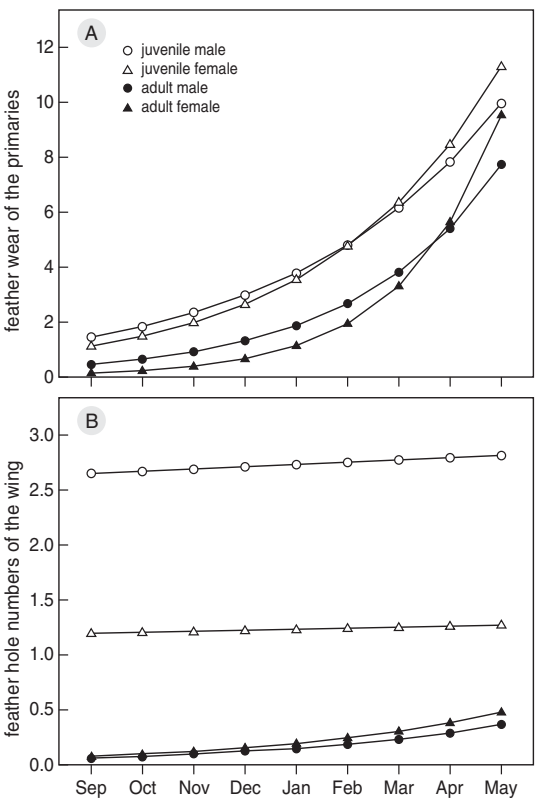

Figure 1. Seasonal variation of (A) feather wear of primaries and (B) feather hole numbers of the whole wing in juveniles and adults. The data plotted are estimates predicted by the model and transformed back to the original variable scale; lines are fits from the model.

Table 1. Estimated parameter values with SE and z-values of explanatory variables in the generalized linear mixed effect model to explain the intensity of feather wear of the primary feathers. The final model is presented.

Source	Estimate	SE	z
Intercept	1.49	0.38	3.95
Month	0.24	0.02	14.29
Age	–1.27	0.21	–6.13
Sex	–0.30	0.15	–2.06
Month × age	0.11	0.03	3.63
Month × sex	0.05	0.02	1.98
Age × sex	–1.03	0.40	–2.61
Weight	–0.07	0.02	–4.12
Feather holes	0.15	0.04	3.69

The variances of the random effects in the final model:
birds' ID = 0.11, ringer = 0.03, year = 0.03.

cycle (Fig. 1B; Table 2). The significant age × sex interaction shows that juvenile males had significantly more feather holes than juvenile females, while the sex of adults had no effect (Fig. 1B).

Rachis diameter and wing length were significantly negatively related to the number of feather holes (Table 2), while weight (estimate = 0.04, SE = 0.04, $z = 1.06$), tarsus length (estimate = –0.14, SE = 0.13, $z = –1.08$) and condition (estimate = 0.03, SE = 0.04, $z = 0.67$) were unrelated to the number of holes in the wing. If fault bars were present, the incidence of feather holes was significantly higher (Table 2).

On the removed flight feathers, holes were more often present in the distal (65 out of 77 holes) than proximal part of the feather vane (12 out of 77 holes) and this differed significantly from a uniform distribution ($\chi^2_6 = 29.2$, $P < 0.001$; Fig. 2). Feathers with thinner rachis had more holes (Spearman's rank correlation, $r_S = –0.28$, $P = 0.0005$; Fig. 3), while the density of barbules did not correlate with hole frequency ($r_S = –0.12$, $P = 0.13$). Results pertaining to rachis diameter corroborated the results based on data collected during field inspection (see above and cf. Table 2).

DISCUSSION

The fine scale time effect revealed that the abrasion rate of feathers became disproportionately accentuated during the annual cycle, reaching a peak in the breeding season. This non-linear increase can be ascribed to
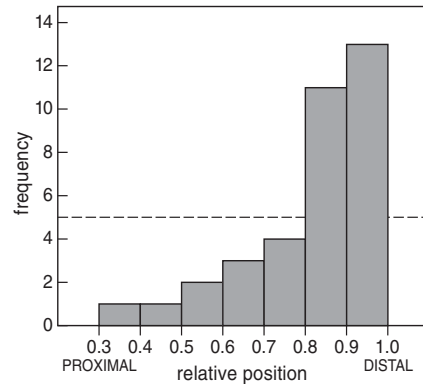
Figure 2. Frequency distribution of the relative feather hole position (0 = proximal end, shaft of the primary, 1 = distal end, tip of the primary). The dashed line represents the expected value calculated from the χ^2-test.
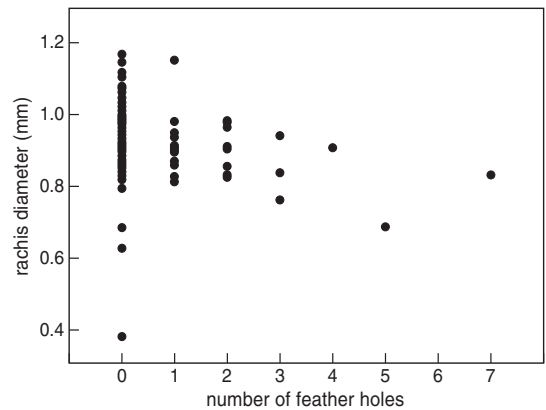

Figure 3. The relationship between rachis diameter and the number of feather holes measured on Great Tit feathers.

Table 2. Estimated parameter values with standard errors and z-values of explanatory variables in the generalized linear mixed effect model to explain the intensity of feather hole incidence of the whole wing. Here only the final model is presented (month was also included owing to its interaction with age).

Source	Estimate	SE	z
Intercept	7.36	2.63	2.79
Age	–3.99	0.37	–10.65
Sex	–0.79	0.19	–4.15
Month	0.01	0.02	0.43
Month × age	0.22	0.05	3.94
Age × sex	1.08	0.30	3.64
Rachis diameter	–4.84	1.12	–4.33
Wing length	–0.09	0.03	–2.47
Fault bars	0.32	0.14	2.32

The variances of the random effects in the final model: birds' ID = 3.73, ringer = 0.003, year = 0.03.

the higher workload and elevated locomotor activity during reproduction (Drent & Daan 1980). During peak chick provisioning, parents perform hundreds of daily trips between nest and feeding sites, meanwhile airborne particles, dense vegetation, and the cavity entrance and inner wall may abrade the primaries. Breeding-related costs can also explain the finding that females became more intensively worn during breeding. Parental duties of tits (Paridae) are female biased in terms of nest building, incubation inside an abrasive cavity and chick feeding rate (Cramp & Perrins 1993, Sanz *et al.* 2000). Moreover, the abrasion rate was marginally higher in females with feather holes present. This could be due to the non-uniform distribution of holes within individual primaries. Since the majority

of feather holes were situated close to the tip of the primaries, this may result in the breakage of the barbs positioned toward the tip (i.e. enhancing the abrasion status) mainly in the highly demanding period of reproduction.

Age was another significant predictor: juveniles bore feathers of lower quality, a pattern that was consistently true for each feather deformity (degree of abrasion, number of holes and fault bars; Pap *et al.* 2007). Juveniles have a short developmental period when all vital functions develop simultaneously, and these may compromise each other owing to resource-based trade-offs. Besides, the primaries and secondaries are retained during the partial post-juvenile moult, being shed only the next year. In comparison, adults grow their flight feathers during the prolonged complete post-breeding moult, when apparently only somatic growth occurs. These results corroborate our previous results showing a positive relationship between feather hole load and number of brood mates and relative laying date (Pap *et al.* 2007).

Our study provides evidence that the number of feather holes can be used as a proxy of flight feather quality (measured through rachis diameter; see Dawson *et al.* 2000) at least in juveniles where their prevalence is adequately high. We also showed that feather deformities are positively interrelated. These associations arise probably through the joint dependence of these variables on feather quality, which is affected by body condition during moult (Pap *et al.* 2008). This, in effect, means that birds in poor condition during moult grow lower quality remiges, which became more rapidly and intensely damaged. These possible long-term effects could have a pronounced impact on Great Tits, as feather deformities increase the breakage of feathers (Kose & Møller 1999, Sarasola & Jovani 2006) and are known to impair flight capacity (Barbosa *et al.* 2002, Swaddle & Lockwood 2003) by altering the wing load or the aerodynamic conditions.

According to a widespread paradigm, feather holes are feeding traces of Ischnoceran chewing lice (Phthiraptera), a conclusion based on correlative data involving mainly Barn Swallows (e.g. Møller 1991). Comparing sister taxa, Vas *et al.* (2008) suggested that feather holes in Barn Swallows and other passerines are probably caused by *Brueelia* spp., as only host species infected with these lice had holey feathers. However, our results are diametrically opposed to this, as hole prevalence was 62.2%, while we found no *Brueelia* spp. and no lice at all ($n = 23$ Great Tits examined visually combined with fumigation; C. Adam, P.L. Pap and C.I. Vágási, pers. obs.). Similarly, Shumilo &

Lunkaschu (1972) inspecting 51 Great Tits found only *Menacanthus sinuatus* (Amblycera) and *Philopterus pallescens* (Ischnocera) with low prevalence (7.8% and 5.9%, respectively). In addition, we found that feather holes were situated disproportionately closer to the tip of the primaries, which questions the chewing lice origin of feather holes, since we expect ectoparasites to feed preferentially closer to the base of the remiges, where aerodynamic forces are the lowest, i.e. the risk of accidental falling is negligible. Although keratin remains were found in the stomachs of lice, these could derive from already broken feather fragments and/or body feathers. Moreover, among Barn Swallows, the number and distribution of feather holes strongly correlates with that of chewing lice (Møller 1991). Such correlations could also be found if highly parasitized birds are of inferior condition (Møller *et al.* 2004), and hence have lower-quality feathers (Pap *et al.* 2008) that have more handicaps (e.g. holes). Furthermore, the distribution pattern of fault bars among tits is also highly skewed (see Pap *et al.* 2007 for the same population), although bars are known to be caused by stressors acting during feather production rather than by ectoparasites. Finally, if chewed by lice, we would expect the number of holes to increase during the annual cycle, while this did not happen in our study. Based on our results, we propose an alternative explanation. Feather holes are possibly feather handicaps that emerge due to minor breaks on feather vanes occurring where the strength of barbs / barbules is locally compromised. Breaks could arise due to the failure of feathers to resist high mechanic stress (cyclic loads; Weber *et al.* 2010) on the tip of flight feathers (e.g. during breeding) or because barbs / barbules are weakened by feather-degrading bacteria. The first mechanical assumption is supported by our results (see above). For the second, there is evidence that colony-forming feather-degrading bacteria can cause breaks on barbs / barbules (Shawkey *et al.* 2007), which may result in formation of feather holes. However, this scenario remains to be tested, as bacteria are less likely to occur on wing feathers compared to ventral contour feathers (Burtt & Ichida 1999). Furthermore, Great Tits are found to have a reduced mean number of bacilli phylotypes (Saag *et al.* 2008), and it is still unknown, whether keratinolytic bacteria spores are able to develop on the feathers of living birds (Muza *et al.* 2000), because preen oils have a sanitation function in inhibiting bacteria growth (Shawkey *et al.* 2003). Altogether, we suggest more caution regarding the origin of feather holes, until well-designed, rigorous experimental studies are performed.

To summarize, we have shown that breeding may incur significant costs in terms of flight feather usage. Primaries of females are more abraded than those of males during the breeding season. The frequency of feather abnormalities may depend on the condition and feather quality of the birds. We have also found that feather abnormalities were positively interrelated: Great Tits with feather holes had more abraded primaries, and those with fault bars on flight feathers had a higher incidence of feather holes. These results may serve as starting-point for future studies aiming to explore how condition-dependent feather quality may be translated to age-specific reproductive output, among individual differences in fitness-related traits and the evolution of repair or mitigating mechanisms (e.g. moult, melanin-pigment allocation) that reduce the possible adverse effects of feather deformities.

ACKNOWLEDGEMENTS

REFERENCES

Barbosa A., Merino S., de Lope F. & Møller A.P. 2002. Effects of feather lice on flight behavior of male barn swallows (*Hirundo rustica*). Auk 119: 213–216.

Barta Z., Houston A.I., McNamara J.M., Welham R.K., Hedenström A., Weber T.P. & Feró O. 2006. Annual routines of non-migratory birds: optimal moult strategies. Oikos 112: 580–593.

Bates D. 2008. lme4: Linear mixed-effects models using S4 classes. URL: http://lme4.r-forge.r-project.org.

Bortolotti G.R., Dawson R.D. & Murza G.L. 2002. Stress during feather development predicts fitness potential. J. Anim. Ecol. 71: 333–342.

Burtt E.H., Jr. & Ichida J.M. 1999. Occurrence of feather-degrading bacilli in the plumage of birds. Auk 116: 364–372.

Cramp S. & Perrins C.M. 1993. The birds of the western Palearctic, vol. 7. Oxford University Press, Oxford.

Crawley M.J. 2007. The R book. John Wiley & Sons, Chichester, UK.

Dawson A., Hinsley S.A., Ferns P.N., Bonser R.H.C. & Eccleston L. 2000. Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. Proc. R. Soc. Lond. B 267: 2093–2098.

Drent R.H. & Daan S. 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68: 225–252.

Kose M. & Møller A.P. 1999. Sexual selection, feather breakage and parasites: the importance of white spots in the tail of the barn swallow (*Hirundo rustica*). Behav. Ecol. Sociobiol. 45: 430–436.

Kullberg C., Jakobsson S. & Fransson T. 1998. Predator-induced take-off strategy in great tits (*Parus major*). Proc. R. Soc. Lond. B 265: 1659–1664.

Merilä J. & Hemborg C. 2000. Fitness and feather wear in the Collared Flycatcher *Ficedula albicollis*. J. Avian Biol. 31: 504–510.

Møller A.P. 1991. Parasites, sexual ornaments, and mate choice in the barn swallow. In: Loye J.E. & Zuk M. (eds) Bird–parasite interactions: ecology, evolution, and behaviour. Oxford University Press, New York, pp 328–343.

Møller A.P. 2010. Host–parasite interactions and vectors in the barn swallow in relation to climate change. Glob. Change Biol. 16: 1158–1170.

Møller A.P., de Lope F. & Saino N. 2004. Parasitism, immunity, and arrival date in a migratory bird, the barn swallow. Ecology 85: 206–219.

Muza M.M., Burtt E.H., Jr. & Ichida J.M. 2000. Distribution of bacteria on feathers of some Eastern North American birds. Wilson Bull. 112: 432–435.

Pap P.L., Tökölyi J. & Szép T. 2005. Frequency and consequences of feather holes in Barn Swallows *Hirundo rustica*. Ibis 147: 169–175.

Pap P.L., Barta Z., Tökölyi J. & Vágási C.I. 2007. Increase of feather quality during moult: a possible implication of feather deformities in the evolution of partial moult in the great tit *Parus major*. J. Avian Biol. 38: 471–478.

Pap P.L., Vágási C.I., Czirják G.Á. & Barta Z. 2008. Diet quality affects postnuptial molting and feather quality of the house sparrow (*Passer domesticus*): interaction with humoral immune function? Can. J. Zool. 86: 834–842.

Pap P.L., Vágási C.I., Tökölyi J., Czirják G.Á. & Barta Z. 2010. Variation in haematological indices and immune function during the annual cycle in the Great Tit *Parus major*. Ardea 98: 105–112.

Pinheiro J.C. & Bates D.M. 2000. Mixed-effects models in S and S-PLUS. Springer-Verlag, New York.

Prater A.J., Marchant J.H. & Vuorinen J. 1977. Guide to the identification and aging of Holarctic waders. BTO Guide 17, BTO, Tring, UK.

R Development Core Team 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. URL http://www.R-project.org.

Rasband W.S. 2008. ImageJ, release version 1.37. National Institutes of Health, Bethesda, Maryland. URL http://rsbweb.nih.gov/ij.

Saag P., Mänd R., Tilgar V., Mägi M. & Kilgas P. 2008. Feather degrading bacteria as a factor potentially affecting breeding performance in great tits *Parus major*. 12th International Behavioral Ecology Congress, Ithaca, p. 259.

Sanz J.J., Kranenbarg S. & Tinbergen J.M. 2000. Differential response by males and females to manipulation of partner contribution in the great tit (*Parus major*). J. Anim. Ecol. 69: 74–84.

Sarasola J.H. & Jovani R. 2006. Risk of feather damage explains fault bar occurrence in a migrant hawk, the Swainson's hawk *Buteo swainsoni*. J. Avian Biol. 37: 29–35.

Serra L. 2001. Duration of primary moult affects primary quality in Grey Plovers *Pluvialis squatarola*. J. Avian Biol. 32: 377–380.

Shawkey M.D., Pillai S.R. & Hill G.E. 2003. Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. J. Avian Biol. 34: 345–349.

Shawkey M.D., Pillai S.R., Hill G.E., Siefferman L.M. & Roberts S.R. 2007. Bacteria as an agent for change in structural plumage color: correlational and experimental evidence. Am. Nat. 169: S112–S121.

Shumilo R.P. & Lunkaschu M.I. 1972. Mallophaga from wild terrestrial birds of the Dnester-Prut region. Moldavian Academy of Sciences. (In Russian)

Svensson L. 1992. Identification guide to European passerines, 4th edn. Stockholm.

Swaddle J.P. & Lockwood R. 2003. Wingtip shape and flight performance in the European Starling *Sturnus vulgaris*. Ibis 145: 457–464.

Swaddle J.P., Witter M.S., Cuthill I.C., Budden A. & McCowen P. 1996. Plumage condition affects flight performance in Common Starlings: implications for developmental homeostasis, abrasion and moult. J. Avian Biol. 27: 103–111.

Vas Z., Csörgő T., Møller A.P. & Rózsa L. 2008. The feather holes on the barn swallow *Hirundo rustica* and other small passerines are probably caused by *Brueelia* spp. lice. J. Parasitol. 94: 1438–1440.

Weber T.P., Kranenbarg S., Hedenström A., Waarsing J.H. & Weinans H. 2010. Flight feather shaft structure of two warbler species with different moult schedules: a study using high-resolution X-ray imaging. J. Zool. 280: 163–170.

Williams E.V. & Swaddle J.P. 2003. Moult, flight performance and wingbeat kinematics during take-off in European starlings *Sturnus vulgaris*. J. Avian Biol. 34: 371–378.

SAMENVATTING

Vogels ruien om oude, versleten veren te vervangen door nieuwe. Versleten slagpennen met gaten kunnen ten koste gaan van de kans op overleving en voortplanting. Toch is er maar weinig bekend over de factoren die verantwoordelijk zijn voor de mate van slijtage aan veren en wat de veroorzakers zijn van gaten in veren. Deze studie beschrijft mogelijke oorzaken van variatie in veerkwaliteit bij de Koolmees *Parus major* en is gebaseerd op drie achtereenvolgende jaarcycli. De auteurs laten zien dat variatie in de mate van veerslijtage voornamelijk samenhangt met de tijd van het jaar, de leeftijd, het geslacht en het gewicht van de vogel en met de aanwezigheid van gaten in de veren. Juveniele vogels en individuen met een lage veerkwaliteit en met zogenaamde fault bars (veerafwijkingen) hebben daarbij meer gaten in hun veren dan andere vogels. Veerslijtage vond vooral plaats tijdens het broedseizoen en was het grootst bij vrouwtjes, hoogstwaarschijnlijk als gevolg van hun grotere werkdruk. De auteurs verklaren het verschil in veerkwaliteit tussen juveniele en adulte vogels door het versnelde ruiproces dat bij juveniele vogels optreedt. Verschillende maten van gebreken en slijtage in de veren correleerden met elkaar en kunnen dus gebruikt worden als een indicator voor veerkwaliteit. Het idee dat gaten in veren een mate voor veerkwaliteit zijn, staat in contrast met de huidige gedachte dat deze gaten vraatsporen zijn van veerluizen. De auteurs stellen echter dat deze gaten, in het geval van de Koolmees, kleine misvormingen zijn die bij de ontwikkeling van de veren zijn ontstaan. (KvO)

Paper II

Haste Makes Waste but Condition Matters: Molt Rate–Feather Quality Trade-Off in a Sedentary Songbird

Csongor I. Vágási, Péter L. Pap, Orsolya Vincze, Zoltán Benkő,
Attila Marton & Zoltán Barta (2012) *PLoS ONE* 7: e40651 [IF$_{2011}$ 4.09]

Summary

Background: The trade-off between current and residual reproductive values is central to life history theory, although the possible mechanisms underlying this trade-off are largely unknown. The 'molt constraint' hypothesis suggests that molt and plumage functionality are compromised by the preceding breeding event, yet this candidate mechanism remains insufficiently explored.

Methodology/Principal Findings: The seasonal change in photoperiod was manipulated to accelerate the molt rate. This treatment simulates the case of naturally late-breeding birds. House sparrows *Passer domesticus* experiencing accelerated molt developed shorter flight feathers with more fault bars and body feathers with supposedly lower insulation capacity. However, the wing, tail and primary feather lengths were shorter in fast-molting birds if they had an inferior body condition, which has been largely overlooked in previous studies. The rachis width of flight feathers was not affected by the treatment, but it was still condition-dependent.

Conclusions/Significance: This study shows that sedentary birds might face evolutionary costs because of the molt rate–feather quality conflict. This is the first study to experimentally demonstrate that (1) molt rate affects several aspects of body feathers as well as flight feathers and (2) the costly effects of rapid molt are condition-specific. We conclude that molt rate and its association with feather quality might be a major mediator of life history trade-offs. Our findings also suggest a novel advantage of early breeding, i.e. the facilitation of slower molt and the condition-dependent regulation of feather growth.

Haste Makes Waste but Condition Matters: Molt Rate–Feather Quality Trade-Off in a Sedentary Songbird

Csongor I. Vágási[1,2]*, Péter L. Pap[1,2], Orsolya Vincze[2], Zoltán Benkő[2], Attila Marton[2], Zoltán Barta[1,3]

1 Department of Evolutionary Zoology, University of Debrecen, Debrecen, Hungary, 2 Evolutionary Ecology Group, Hungarian Department of Biology and Ecology, Babeş-Bolyai University, Cluj Napoca, Romania, 3 MTA-DE "Lendület" Behavioural Ecology Research Group, University of Debrecen, Debrecen, Hungary

Abstract

Background: The trade-off between current and residual reproductive values is central to life history theory, although the possible mechanisms underlying this trade-off are largely unknown. The 'molt constraint' hypothesis suggests that molt and plumage functionality are compromised by the preceding breeding event, yet this candidate mechanism remains insufficiently explored.

Methodology/Principal Findings: The seasonal change in photoperiod was manipulated to accelerate the molt rate. This treatment simulates the case of naturally late-breeding birds. House sparrows *Passer domesticus* experiencing accelerated molt developed shorter flight feathers with more fault bars and body feathers with supposedly lower insulation capacity (i.e. shorter, smaller, with a higher barbule density and fewer plumulaceous barbs). However, the wing, tail and primary feather lengths were shorter in fast-molting birds if they had an inferior body condition, which has been largely overlooked in previous studies. The rachis width of flight feathers was not affected by the treatment, but it was still condition-dependent.

Conclusions/Significance: This study shows that sedentary birds might face evolutionary costs because of the molt rate–feather quality conflict. This is the first study to experimentally demonstrate that (1) molt rate affects several aspects of body feathers as well as flight feathers and (2) the costly effects of rapid molt are condition-specific. We conclude that molt rate and its association with feather quality might be a major mediator of life history trade-offs. Our findings also suggest a novel advantage of early breeding, i.e. the facilitation of slower molt and the condition-dependent regulation of feather growth.

Introduction

The trade-off between current reproductive effort and future (residual) reproductive value is of outstanding concern in life history theory [1,2]. However, the mechanisms that potentially mediate such long-term effects remain an evolutionary conundrum. It was recently proposed that molt (regular replacement of worn and torn feathers) and the quality of the feathers produced in particular may couple events that precede and follow the molting period via carry-over effects ('molt constraint' hypothesis, MCH; [3,4]). The MCH suggests that the long-term costs of breeding are expressed via a compromised molt, which can lead to plumage malfunctions and hence curtailed future fitness.

The MCH integrates several lines of evidence. First, it assumes that breeding and molt are scheduled sequentially during the annual cycle due to the adaptive avoidance of overlapping two costly activities [5,6], which holds for most temperate zone bird species [7,8]. Thus, prolonged breeding due to its late onset or higher investment evokes delayed molt [9–11]. Second, molting is

a costly process [12–14], and molting birds may encounter elevated energetic constraints due to lower resource availability if the initiation of molt is postponed [3]. Consequently, any delay might manifest in the production of functionally inferior feathers. This may be exacerbated because the approaching winter shortens the time available for plumage exchange, which forces birds to accelerate their rate of molt [4,8]. However, this seems a best-of-a-bad-job strategy because the rate of molt is traded off against the quality of flight feathers [4,15–19] and presumably the structure of body feathers as well [3,20]. Third, poor-quality feathers might not serve their main functions adequately, e.g. insulation, flight and communication. These losses of function are obviously precipitated in fitness reduction by abating future performance [3,7]. Compromised molt (1) impinge fundamental adverse effects on flight [21–23] and thermoregulation [3,20] due to lower-quality feathers, (2) diminishes reproductive success because of breakage or altered pigmentation and/or microstructure of feathers involved in visual displays ([24] and refs therein, [25]), or (3) might have multiple costs through starvation–predation and

thermoregulation–predation trade-offs [26,27]. In conclusion, this cause–effect cascade implies that the molting process might link past (or current) and future life history events.

The MCH has mainly been studied by the correlative approach on the flight feathers of migratory species ([16–18]; see [15] for an experiment). Exceptions include comparisons of migratory and sedentary blackcap Sylvia atricapilla populations (e.g. [17,28]) and two experiments with European starlings Sturnus vulgaris in East England [4,19]. Although starlings are sedentary or partial migrants in Britain, their annual routine resembles that of summer-molting migrants with a short breeding period (usually only one clutch in East England) and they shed their plumage early (May–August) [29]. Temperate zone migrants commonly employ the summer-molting strategy [30] that spans a shorter period and results in the production of lighter feathers compared with sedentary birds [17,18]. In contrast, sedentary birds partition their annual cycle into fewer costly activities (lacking migration), which allows more time for plumage renewal [8,31]. An open question is whether the inter-individual variation in molt rate and duration in a less time-constrained sedentary population is sufficiently large to generate variation in feather quality. Furthermore, only one correlative study of sedentary great tits Parus major dealt with molt constraints on body feather quality [20]. To the best of our knowledge, no experimental studies have explored whether the accelerated molt rate can represent one of the proximal underpinnings of altered body feather structure, which is one of the cornerstones of the MCH [3,20]. Altogether, this topic clearly deserves further investigation. The house sparrow Passer domesticus is a 'typical' sedentary bird; it is multi-brooded (up to four clutches in Central and Eastern Europe; [29]), consequently its reproductive period is much longer and hence it molts later (August–early November in our wild-living population; Pap PL, Vágási CI, Barta Z unpublished data) than similar-sized migratory species. This makes it an ideal candidate for testing this hypothesis.

Body condition is a phenotypic trait, which refers to size-adjusted, non-skeletal mass and it was defined by Peig and Green [32] as "the energy capital accumulated in the body … assume[d] to be an indicator of an animal's health and quality." Variation in body condition has a substantial heritable component in a wide range of taxa ([33] and refs therein) including the house sparrow [34]. Thus, body condition might have broad evolutionary implications because it shapes a plethora of life history and fitness-related traits [2,33,35–37]. Molt is not an exception; a trade-off has been documented between body mass (i.e. mass unadjusted to size) and molt rate, and body mass also mediates the molt–immunity trade-off [14]. Further, body mass and body condition are also relevant to the MCH because body mass can predict molt initiation [5], while body condition can predict speed of molt [38]. However, body mass does not correlate with feather growth rate [39]. Each metabolically costly activity has recourse to finite stores, so birds in a better condition can afford higher investment due to their higher amount of resources available for supporting demanding activities and/or their more efficient replenishment of stores after allocation (higher turnover rate). Embedded in the molt constraint scenario, fitness accrual can be mediated by body condition if birds with greater energy capital can better fuel the demands of molting to grow better-quality feathers, even if molt is compromised (i.e. show higher developmental homeostasis). Yet this aspect has only been addressed indirectly [17] or investigations have been limited to feather growth rate [39,40] and molt–immunity trade-off [14].

Our previous study showed that house sparrows subjected to a photoperiod regime that simulated a late molting season experienced an accelerated molt of flight feathers, wing coverts and body feathers of the melanin-based throat patch (badge of status) [24]. These indicated that the overall plumage molting program was affected by the treatment. Fast-molting birds renewed their plumage approximately two weeks earlier than controls, although molt initiation was similar in the two groups, and grew a smaller badge and a less bright wing-bar. The current study continued this experimental investigation of the molt pattern in the same birds, but the aim was to determine whether life history trade-offs might be mediated by molt rate. The following predictions were tested. First, the speed of molt should have no effect on feather attributes if the longer molting period of sedentary birds permits cost-spreading. Second, Broggi et al. [20] found that northern great tits, which face time constraints, grew shorter, more densely structured body feathers with fewer plumulaceous barbs. It is suggested that such feather architecture may provide less efficient heat conservation ([3,20] and refs therein). If the MCH of Nilsson and Svensson [3] stands, the body feather structure of fast-molting experimental sparrows should resemble that of northern great tits. This is a missing piece of the MCH. Third, an interaction between treatment and body condition is predicted if good-quality birds can cope better with a faster molt rate.

Materials and Methods

Ethics Statement

Birds were handled in strict accordance with animal care, wellbeing and ethical prescriptions [41]. The protocol for bird care and experimentation adhered to the current Romanian laws and was approved by the Romanian Academy of Sciences (permit number: 2257).

General Procedures

We caught 50 non-molting adult male house sparrows at a cattle farm near Cluj Napoca (46°46′N, 23°33′E, Transylvania, Romania) on 25 July 2008. Birds were transferred to the Campus of Babeş-Bolyai University, Cluj Napoca and randomly assigned in equal numbers into two indoor aviaries (each 4 m L×3.5 m W×4 m H). Birds allocated to the aviaries did not differ in any measured aspect (one-way ANOVA, pre-molt wing length: $F_{1,47} = 0.09$, $P = 0.76$; pre-molt tail length: $F_{1,43} = 0.22$, $P = 0.64$; body mass: $F_{1,44} = 1.47$, $P = 0.23$; tarsus length: $F_{1,47} = 0.14$, $P = 0.71$). During confinement, birds had ad libitum access to protein-rich food (seed mixture supplemented with grated boiled eggs or mealworms on odd days; [13,24]), sand and daily exchanged drinking water. Aviaries contained bushes, perches and nest boxes. Coccidian parasites that emerge spontaneously in captive populations were purged by an anticoccidial drug (toltrazuril) administered in the drinking water [24]. Except one sparrow from the control group that died for unknown reasons, the rest of the birds were released back to the population of origin in good body condition after the termination of the experiment (on 15 November 2008).

Aggressive behavior can determine resource acquisition and has physiological bases (e.g. steroid hormones) that are recognized to influence keratin synthesis. Still, group housing was issued because free-ranging house sparrows are gregarious in the molting period [29]. To preclude the possible confounding effects caused by male–male interactions, we recorded within flock agonistic behavior of individually marked sparrows from a hide, calculated

fighting success (% fights won out of total) and included this in all subsequent statistical analyses (see [24] for details).

Photoperiod Treatment

Birds were subjected to a photoperiod treatment consisting of two different lighting regimes. At capture, the local natural photoperiod was 15L:9D (light:dark). The lighting regime of control group birds was a steadily decreasing photoperiod that conformed the natural photoperiod characteristic to study latitude (control photoperiod, hereafter 'CP' group). This meant a decrease in day-length by in average 3 min per day. Birds of the experimental group experienced an accelerated seasonal decrease in photoperiod (experimental photoperiod, hereafter 'EP' group; 8 min decrease in day-length per day) until reaching 9L:15D photoperiod at the 46th day of experimentation and then held it constantly at this level (Fig. 1). This setup simulates photoperiod conditions met by early-molting (CP) and late-molting (EP) sparrows. We concomitantly followed the molting pattern of the field population and found this to be similar to that of CP birds (personal observation), indicating that captivity did not alter their normal molting pattern.

Measurements

Wing and tail length (±0.5 mm) were measured with a ruler both pre- and post-molt. At capture and on every 10th day of the 110-day-long experimentation, body mass (±0.1 g) was measured with a Pesola spring balance and molt status was scored (see below). Hereafter, 'mass' is shorthand for "mean body mass averaged over the 11 measurement sessions". Adult house sparrows perform one post-breeding molt per year, thus feathers developed in a given year are retained until the end of breeding in the next year. Only the molting status of primaries was scored because it gives a good general picture about the complete post-breeding molt [13]. The molt status of each primary on both wings was scored on a 0–5 scale according to Ginn and Melville [8] from which the molting index was derived as the sum of 9 primaries' scores per wing (range 0–45). Note that the outermost primary is rudimentary in sparrows. Molt of flight feathers starts with the innermost primary. Molt was considered to be started on the day

Figure 1. Photoperiod regime at which male house sparrows undertook their molt. Photoperiod treatment groups: 'CP' control (broken line), 'EP' experimental (continuous line). 28 July 2008 = day 1, 25 November 2008 = day 110. Male house sparrow drawing credit: Márton Zsoldos.
doi:10.1371/journal.pone.0040651.g001

when the molt index was first >0. The reaching of a molt score of 45 marks the end of molt. The duration of molt is the number of days elapsed between molt onset and molt termination.

Once molt was completed, we plucked the 5th and 7th primaries (henceforth P5 and P7) of both wings. We use descendant primary numbering, i.e. the 1st primary, P1, is the innermost. The rationale behind choosing these feathers stems from the followings: (1) P5s were grown when groups started to diverge in molt speed, whereas P7s when the difference in molt speed was the largest between groups (see Fig. 1 in [24]), and (2) P5s grow at the highest rate, whereas P7s are the longest primaries thus necessitating the most keratin, though developing at a slower pace [13]. We further removed 4–5 contour body feathers from the mantle region. All feathers were stored dry in zip-lock plastic bags. The quality of flight feathers was quantified through 4 parameters (mass, length, the width of the rachis and the number of fault bars; [4,13,21,42,43]), whereas that of body feathers through 5 parameters (barbule density, total number of barbs, proportion of plumulaceous barbs, length and area; [20]). The rachis is the solid upper part of the feather shaft from which barbs are branching. Barbs have further branches, called barbules that connect the adjacent barbs to form together the feather vane of pennaceous body and flight contour feathers. The measures of left and right wing primaries and that of 2–3 randomly chosen body feathers were averaged to increase measurement accuracy. To reduce measurement error, each trait was assessed by one author (CIV, Z. Benkő or AM) without knowledge on group identity of samples.

Dry mass of primaries was measured with a SCALTEC (SBA 32) balance (±0.1 mg). Maximum length of primaries (from the proximal end of calamus to tip) was measured with a ruler (±0.5 mm) by stretching the feather straight. Rachis width of primaries was measured at the base of the vane with a digital caliper (±0.01 mm). The measurement of primary mass, length and rachis width proved to be highly repeatable within individuals ($R>0.8$) in a previous study with the same methodology [13]. Fault bars are abnormalities that appear in form of translucent bands quasi perpendicular to the rachis where feathers are prone to break [21]. These were counted on all 4 plucked primaries and summed up. Barbule density of body feathers was measured by taking digital photographs under a stereomicroscope (Olympus SZ61, Tokyo, Japan; camera: Cool SNAP-Pro cf) at $50\times$ magnification. Feathers were placed in a frame fixed to microscope platform aspiring to expose the same feather region. Feathers were sandwiched between two microscope slides to flatten the naturally curved feathers rendering possible planar measurements. Barbules were counted at the three longest barbs along 1.5 mm length of barb starting from the rachis. The standardized 1.5 mm lengths, calibrated from a stage micrometer scale, were traced in ImageJ [44]. When counting the number of barbs we progressed along the shaft at $30\times$ magnification and made a distinction between pennaceous and plumulaceous barbs according to Broggi et al. [20]. Pennaceous barbs are compact, closer to each other, have shorter barbules and often form vanes. Plumulaceous barbs are fluffy, with long barbules and do not create vanes, hence trap air for insulation. We recorded the total number of barbs and that of pennaceous ones and calculated the proportion of plumulaceous barbs (%) as (total barbs – pennaceous barbs)/total barbs. For length and area, mantle feathers were photographed (Nikon D80 on tripod) on a grey-card with metric template and sandwiched between two microscope slides. Feather length (±0.01 mm) was measured by tracing a line along the shaft (calamus included), whereas area (±0.01 cm^2) by encircling the feather in ImageJ.
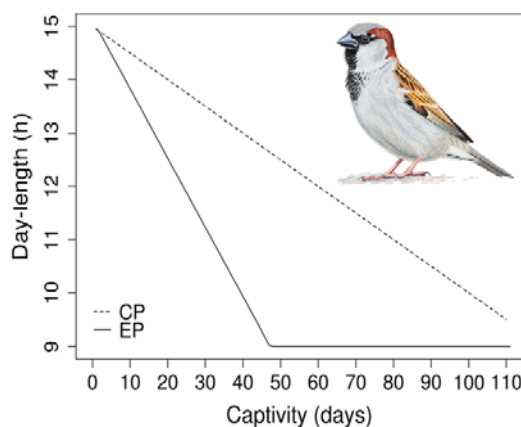
Statistical Procedures

All statistical analyses were performed in the R statistical environment version 2.14.1 [45]. We built linear models (LMs) separately for each response variable except the total number of fault bars, which was analyzed by generalized linear model (GLM) with Poisson error distribution. Treatment was entered as factor and the correlated trait, if any, of focal variables as covariate to deal with the few inter-correlations between response variables. In addition, scaled mass index (SMI; [32]) and square root-transformed fighting success were also entered as covariates. SMI ($\hat{M}_i$) is a size-corrected body condition index calculated as

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}},$$

where M_i and L_i are the mass and tarsus length of ith individual, respectively, L_0 is the mean tarsus length of the sample and exponent b_{SMA} is the slope of log-log model II standardized major axis (SMA) regression of mass on tarsus length ('lmodel2' package for R; [46]). To test whether treatment has a condition-dependent effect, the treatment × SMI interaction term was also entered. Because the widely used condition proxy computed as residuals from mass on skeletal length ordinary least squares regression violates certain statistical assumptions [37], we calculated SMI (g) as this more accurately indicates non-skeletal mass. SMI was scaled to mean = 0 in each model by subtracting the sample's mean SMI from each individual's value in order to gain meaningful parameter estimates. Entering both SMI and fighting success as continuous predictors is reasonable because they are unrelated (Pearson's product-moment correlation, $r = 0.01$, $n = 49$, $P = 0.98$). We first built saturated models that were simplified to minimum adequate models (MAMs) by backward stepwise procedure dropping the predictor with lowest improvement of model fit. For this selection we chose the $P < 0.1$ criteria to retain confounding variables with weaker influence. MAMs were not allowed to differ from the saturated model in explanatory power. The treatment main effect was always kept in the model, while SMI only when it was significant itself or non-significant itself but significantly interacted with treatment. We adopt stepwise elimination as it performs similarly as information theory approach [47]. The requirements of MAMs were checked by plot diagnosis. Each model suited the requirements of linearity, residual's variance and distribution, and no outliers (i.e. standardized residuals > |3|) were detected.

Only the MAMs are presented. Tests are two-tailed and type I error probabilities were set to 0.05. Mean ± SE of raw data and model estimate b ± SE for covariates are reported throughout. Sample sizes are $n_{CP} = 24$ and $n_{EP} = 25$ for each variable except for post-molt tail length for which $n_{CP} = 21$ and $n_{EP} = 24$. We were unable to measure post-molt tail length for some birds because of broken feathers. Repeatability calculations were performed as per Nakagawa and Schielzeth [48] using the 'rptR' package for R and restricted maximum likelihood method.

Results

Measured body feather parameters were highly repeatable ($n = 7$ randomly remeasured individuals; barbule density: $R = 0.87$, 95% CI = 0.67–0.91, $P = 0.0002$; total number of barbs: $R = 0.74$, 95% CI = 0.26–0.89, $P = 0.007$; proportion of plumulaceous barbs: $R = 0.73$, 95% CI = 0.26–0.92, $P = 0.006$; length: $R = 0.92$, 95% CI = 0.70–0.99, $P = 0.0002$; area: $R = 0.80$, 95% CI = 0.22–0.91, $P = 0.0002$). Further, the within-individual values of the 2–3 feathers were significantly correlated (Pearson's

product-moment correlation, all $n = 49$; barbule density: $r = 0.36$, $P < 0.01$; total number of barbs: $r = 0.57$, $P < 0.0001$; proportion of plumulaceous barbs: $r = 0.45$, $P < 0.001$; length: $r = 0.70$, $P < 0.0001$; area: $r = 0.63$, $P < 0.0001$).

Tarsus length (mm) in our study population was 19.38 ± 0.09 and the slope of log-log mass on tarsus length SMA regression was 1.17. Thus, SMI (g) was computed as mass$_i$ × $(19.38/$tarsus length$_i)^{1.17}$ and averaged 29.00 ± 0.16. Groups did not differ in SMI (LM, $F_{1,47} = 0.10$, $P = 0.76$), indicating that photoperiod manipulation had influenced molt speed [24] without deteriorating the mean body condition of birds.

Post-molt Wing and Tail Length, and Fault Bars

Treatment had no effect on post-molt length (mm) of wing (CP: 80.25 ± 0.29; EP: 79.52 ± 0.35) and tail (CP: 57.33 ± 0.43; EP: 56.44 ± 0.50), but interacted significantly with SMI (Table 1) indicating that CP bird's wing and tail length is unrelated to condition (wing: $b = -0.40 \pm 0.26$, $t = -1.54$, $P = 0.13$; tail: $b = 0.03 \pm 0.40$, $t = 0.08$, $P = 0.94$), whereas in the EP group only birds in good condition grew long wings and tail (wing: $b = 1.14 \pm 0.43$, $t = 3.61$, $P = 0.0008$; tail: $b = 1.31 \pm 0.65$, $t = 2.08$, $P = 0.04$) (Fig. 2). Fighting success was dropped from all models.

Total number of fault bars was significantly higher in the EP (1.52 ± 0.24, median = 1.0, range = 0–5) than in the CP group (0.38 ± 0.12, median = 0.0, range = 0–2; GLM, $\chi^2_1 = 18.08$, $P < 0.0001$). None of the other predictors were retained in the MAM.

Flight Feathers

Because feather mass and length were strongly positively correlated (P5: $b = 0.39 \pm 0.06$, $t = 6.70$, $P < 0.0001$; P7: $b = 0.36 \pm 0.08$, $t = 4.65$, $P < 0.0001$) and treatment had qualitatively similar results on these two parameters, we show only results concerning feather length. Groups did not differ in the length (mm) of P5 (CP: 65.79 ± 0.37; EP: 65.04 ± 0.28) and P7 (CP: 69.77 ± 0.27; EP: 68.78 ± 0.30), but treatment interacted with SMI (Table 1). This indicates that feather length was unrelated to condition in the CP group (P5: $b = -0.27 \pm 0.28$, $t = -0.98$, $P = 0.33$; P7: $b = -0.35 \pm 0.23$, $t = -1.50$, $P = 0.14$), whereas a positive association was found in the EP group (P5: $b = 0.94 \pm 0.46$, $t = 2.62$, $P = 0.01$; P7: $b = 0.98 \pm 0.46$, $t = 3.46$, $P = 0.001$) (Fig. 3). The rachis width (mm) of P5 was not affected by treatment (CP: 0.97 ± 0.01; EP: 0.96 ± 0.01), but was positively related to body condition ($b = 0.02 \pm 0.01$; Table 1, Fig. 3C). The rachis width of P7 was unaffected either by treatment (CP: 1.06 ± 0.01; EP: 1.04 ± 0.01) or by other predictors (Table 1, Fig. 3D). Fighting success was never retained in the MAMs.

Body Feathers

Structure of mantle feathers was determined chiefly by treatment (Table 2). Fast-molting EP birds produced feathers with higher barbule density per 1.5 mm barb length (CP: 34.44 ± 0.71; EP: 36.87 ± 0.41; Fig. 4A). The total number of barbs was marginally positively related to mantle feather length ($b = 0.50 \pm 0.27$; Table 2), hence we controlled for feather length in the model. Groups were similar in total number of barbs (CP: 63.78 ± 0.73; EP: 64.41 ± 0.64; Fig. 4B). The results remained unchanged when the control for the marginal effect of feather length was omitted (not shown). Fast-molting EP birds had lower proportion (%) of plumulaceous barbs (CP: 65.83 ± 0.40; EP: 64.53 ± 0.52; Fig. 4C). Those sparrows developed shorter mantle feathers (mm) that were in the EP group (CP: 29.58 ± 0.32; EP: 28.36 ± 0.41; Fig. 4D) and were in lower condition ($b = 0.51 \pm 0.26$; Table 2). The area (cm^2) of mantle feathers was strongly positively

Table 1. Minimum adequate models (MAMs) on the significant or marginally significant predictors of several morphological and flight feather traits.

Response/predictor	MS	F	df	P
Post-molt wing length				
SMI [a]	1.60	0.79	1,45	0.38
TREAT	6.25	3.09	1,45	0.09
SMI × TREAT	26.36	13.03	1,45	0.0008
MAM: error MS = 2.02, $F_{3,45}$ = 5.64, P = 0.002, R^2 = 0.27				
Post-molt tail length				
SMI	14.91	3.30	1,41	0.08
TREAT	7.42	1.64	1,41	0.21
SMI × TREAT	19.46	4.31	1,41	0.04
MAM: error MS = 4.51, $F_{3,41}$ = 3.09, P = 0.04, R^2 = 0.18				
Length of P5				
SMI	1.64	0.70	1,45	0.41
TREAT	6.63	2.82	1,45	0.10
SMI × TREAT	16.18	6.88	1,45	0.01
MAM: error MS = 2.35, $F_{3,45}$ = 3.46, P = 0.02, R^2 = 0.19				
Length of P7				
SMI	1.25	0.76	1,45	0.39
TREAT	11.70	7.16	1,45	0.01
SMI × TREAT	19.59	11.99	1,45	0.001
MAM: error MS = 1.63, $F_{3,45}$ = 6.64, P = 0.0008, R^2 = 0.31				
Rachis width of P5				
SMI	0.01	4.65	1,46	0.04
TREAT	0.01	0.06	1,46	0.81
MAM: error MS = 0.01, $F_{2,46}$ = 2.35, P = 0.11, R^2 = 0.09				
Rachis width of P7				
TREAT	0.01	2.06	1,47	0.16
MAM: error MS = 0.01, R^2 = 0.04				

[a]Photoperiod treatment ('TREAT') effects are shown even if not significant, whereas condition ('SMI', scaled mass index (g)) was retained only when significant but also when interacted with TREAT.
doi:10.1371/journal.pone.0040651.t001

predicted by its length ($b = 0.16 \pm 0.02$, $t = 6.49$, $P < 0.0001$). Treatment influenced mantle feather area by EP birds having smaller feathers (CP: 3.51 ± 0.09; EP: 3.12 ± 0.08; Fig. 4E) even after controlling for length (Table 2). Thus, EP birds had smaller feathers in terms of both length and width. None of the body feather parameters were related to fighting success.

Discussion

We showed that a simulated time-constraint was effective in triggering different molting patterns [24] within the bounds found in free-living birds ([29] and personal observation). The more rapid molt of experimental birds was detrimental for flight and body feather quality, which might ultimately have fitness costs (see Introduction). Moreover, the fitness costs of lower quality flight and body feathers may be exacerbated over time because the non-living tissue of poor-quality feathers deteriorates to a greater extent between molts [4,16,43]. Note that these results were obtained using a relatively mild photoperiod treatment (confer with [4]) and a sedentary model organism. We conclude that the sedentary

lifestyle does not equate to release from the molt rate–feather quality trade-off. The experimental birds harbored more fault bars (also known as stress bars), which might indicate that accelerated molting was stressful [49]. This suggests that corticosterone ('stress hormone') may be a mediator of the molt rate–feather quality trade-off, as corticosterone is known to affect a wide range of feather attributes [50]. Importantly, these results were not confounded by aggression between birds housed in flocks in accordance with our previous work [24] where groups did not differ in the frequency of fighting, and fighting success did not affect plumage ornament expression.

Birds that underwent an accelerated molt developed shorter flight feathers. These results agree with and complement previous studies on the molt rate–feather quality trade-off in migratory or partially migratory species [4,15–19]. An experimentally accelerated molt resulted in shorter and lighter primaries in starlings, and shorter primaries and wings in lesser whitethroats *Sylvia curruca* [4,15,19]. A faster molt resulted in less durable primaries and a greater loss of wing length between molts in grey plovers *Pluvialis squatarola* [16], lighter feathers in summer-molting passerines [17,18], and thinner, but surprisingly more rigid (stiffer), rachis in blackcaps [28]. The molt rate is not the only driver of feather quality because other challenges during molt alter the same feather traits in a similar direction, e.g. infestation [42]. Given that investment in molt and immune function interfere [14], suppressed immunity and consequent higher susceptibility to parasites may indirectly connect molt with feather quality. The rachis width was not influenced by molt rate *per se* or its interaction with body condition. The second moment of the cross-sectional area of the rachis is related to its material rigidity [51], so we infer that this was probably less likely to be affected by the molt rate of a sedentary species as opposed to a more time-constrained migratory species ([4]; but see [28] for how selection on migratory species that rely on assiduous long-distance flights might have led to the evolution of high flexural stiffness).

This study is the first, as far as we are aware, to test the effects of molt speed on body feather structure using an experimental approach that enables the inference of causality. Rapid molting resulted in shorter and smaller feathers with a higher barbule density and a lower proportion of plumulaceous barbs [20]. This feather architecture is proposed to have a lower capacity for heat conservation because it captures less air due to inferior coverage by small feathers and its less fluffy structure (fewer plumulaceous barbs and a higher barbule density; [20] and refs therein). Thus, this study provides experimental support for the underlying mechanism proposed by Nilsson and Svensson [3] and the supposition of Dawson et al. [4] and Broggi et al. [20] that the MCH might also apply to body feathers. Our results entirely corroborate those found by Broggi et al. [20] and highlight that merely an accelerated molt rate could be sufficient to produce the inter-population differences in contour body feather structure found in great tits. Thus, great tits living on the northern margins of their European distribution might develop feathers with an impaired insulation capacity because of a time-constrained molt. As a consequence, fast-molting birds might expend more energy during thermal challenges [3,52]. These molt rate-mediated costs could influence population processes and be responsible for the sink population attributes (e.g. lower survival rate) in the northern margins of the distribution range of the great tit [20].

An interesting aspect we found is that body condition was positively associated with feather traits either irrespective of the treatment (primaries' rachis width and mantle feather length) or only in the experimental group (wing, tail and primary lengths). Therefore, feather traits could be honest indicators of the bearer's
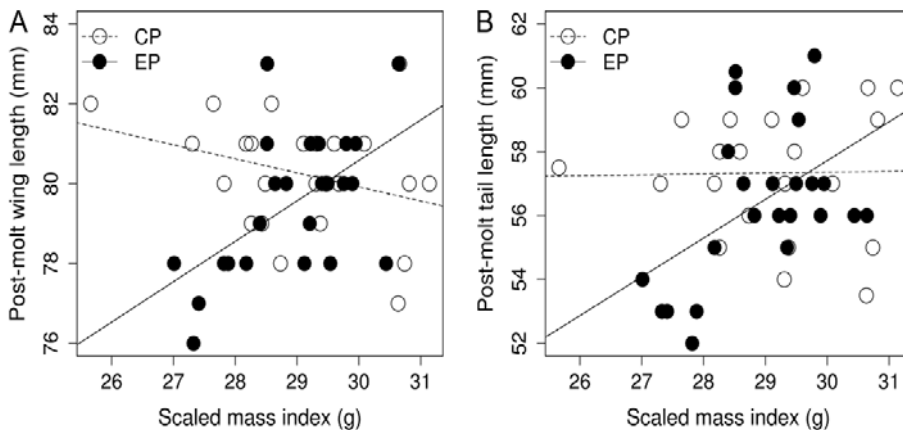
Figure 2. Photoperiod treatment effects on post-molt morphology. Panels show the treatment by condition (scaled mass index) interaction on post-molt length (mm) of (A) wing and (B) tail. Photoperiod treatment groups: 'CP' control (open circles, broken line), 'EP' experimental (filled circles, continuous line).
doi:10.1371/journal.pone.0040651.g002

condition (Figs. 2,3) in concert with Moreno-Rueda's [14] proposal. Birds can achieve functionally adequate plumage by starting to molt early in the season, but only birds with a good body condition can buffer against a compromised molt (interactions on Figs. 2,3) if they "miss the boat" of early molt. Nestling growth and body condition have been under scientific siege in
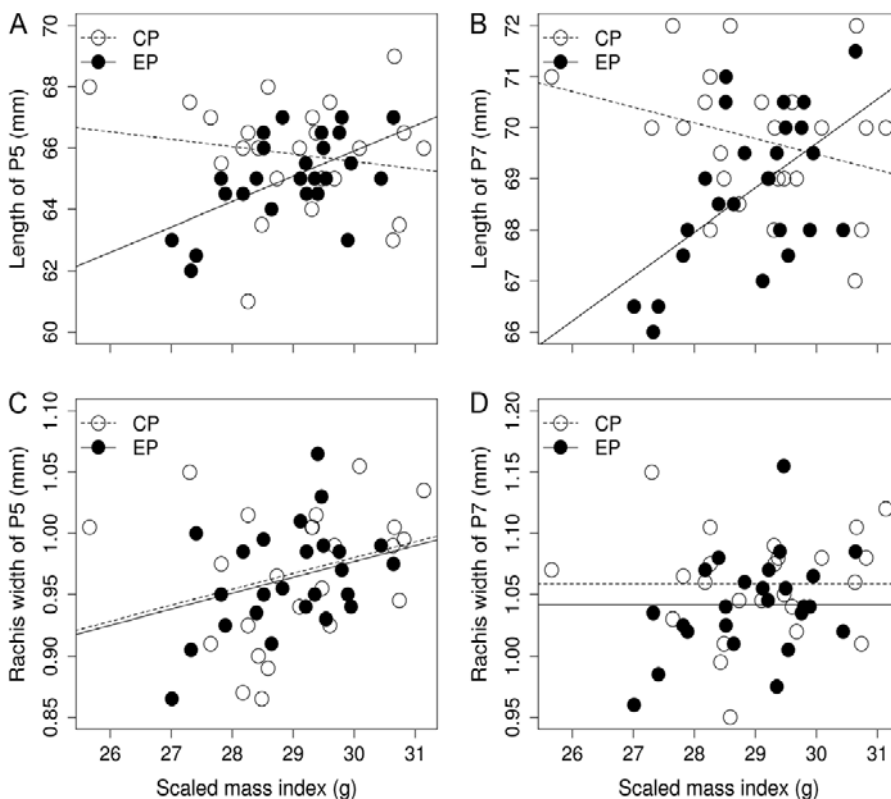

Figure 3. Photoperiod treatment effects on flight feather traits. Left side figures depict the effects of the treatment by condition (scaled mass index) interaction on length (A) and rachis width (C) of P5, whereas the right side figures (B, D) show the same attributes of P7. Photoperiod treatment groups: 'CP' control (open circles, broken line), 'EP' experimental (filled circles, continuous line).
doi:10.1371/journal.pone.0040651.g003

Table 2. Minimum adequate models (MAMs) on the significant or marginally significant predictors of several body (mantle) feather traits.

Response/predictor	MS	F	df	P
Barbule density				
TREAT [a]	72.25	9.05	1,47	0.004
MAM: error MS = 7.99, R^2 = 0.16				
Total number of barbs				
Length of mantle feather	27.15	2.46	1,46	0.07
TREAT	16.96	1.54	1,46	0.22
MAM: error MS = 11.02, $F_{2,46}$ = 2.00, P = 0.15, R^2 = 0.08				
Proportion of plumulaceous barbs				
TREAT	20.77	3.84	1,47	0.05
MAM: error MS = 5.41, R^2 = 0.08				
Length				
SMI	13.65	4.33	1,46	0.04
TREAT	16.75	5.31	1,46	0.03
MAM: error MS = 3.16, $F_{2,46}$ = 4.82, P = 0.01, R^2 = 0.17				
Area corrected to length				
Length of mantle feather	5.25	57.73	1,46	<0.0001
TREAT	0.43	4.77	1,46	0.03
MAM: error MS: 0.09, $F_{2,46}$ = 31.25, P<0.0001, R^2 = 0.58				

[a]Photoperiod treatment ('TREAT') effects are shown even if not significant, whereas condition ('SMI', scaled mass index (g)) was retained only when significant but also when interacted with TREAT.
doi:10.1371/journal.pone.0040651.t002

avian evolutionary ecology for a long while. Early-life growth costs were addressed frequently by means of brood size manipulation experiments (e.g. [53]). A general pattern emerged where growth-related costs were masked under good environmental condition (brood size reduction), whereas they were ubiquitous in the opposite situation (brood size enlargement). To the best of our knowledge, this is the first report of the body condition-dependent costs of somatic growth during adulthood using a manipulative

tool. Under good environmental condition (relaxed molt), sparrows were capable of growing good-quality feathers independent of their body condition. However, only birds with a good body condition were capable of doing so in a challenging situation (rapid molt). In other words, good condition birds had narrower reaction norms (Figs. 2,3); they could withstand challenging environmental circumstances better and by virtue of their higher quality they endure the increased demands of molt (i.e. show higher developmental homeostasis). These conclusions agree with de la Hera et al.'s [17] graphical model, as well as the molt–mass trade-off and mass-mediated molt–immunity trade-off reported by Moreno-Rueda [14]. We argue that the link between feather quality and body condition persists outside the molting period owing to a positive feedback loop across years. That is, the growth of low-quality feathers in a given year is followed by lower condition and breeding performance in the next year, which compromises the molt once again as a consequence (this is referred to as the 'running wheel' effect because of the predictable cycle of states in sequential life stages). At least two lines of empirical evidence support this reasoning. First, feather quality depended on condition-indicator traits throughout the entire annual cycle of great tits [43]. Second, feather traits were repeatable over years within individual blackcaps [54]. Low-quality birds in particular are most probably trapped in this 'running-wheel' because they are often late breeders and thus have deferred molt onset [55,56]. Understanding how individuals carry over effects from one season to the next has crucial importance, e.g. for population dynamics [57], and molt appears to be a potent modulator of such inter-season connectivity.

Our results have implications for (1) the evolution of feather trait diversity among avian taxa and (2) selective forces acting on annual routines. These results may stimulate future inquiry. First, based on current knowledge, we argue that constrained molt could be a more general mechanism at least among small-sized birds that molt their whole plumage once each year. Poor-quality flight and body feathers might have a marked imprint on the residual fitness, which provides a substrate for evolution [58] given the inheritance of feather traits, molt timing and the feather growth rate [34,39,59–62]. Consequently, species that thrive in different environmental conditions and have diverse annual routines may be exposed to species-specific selective forces, which act on molt
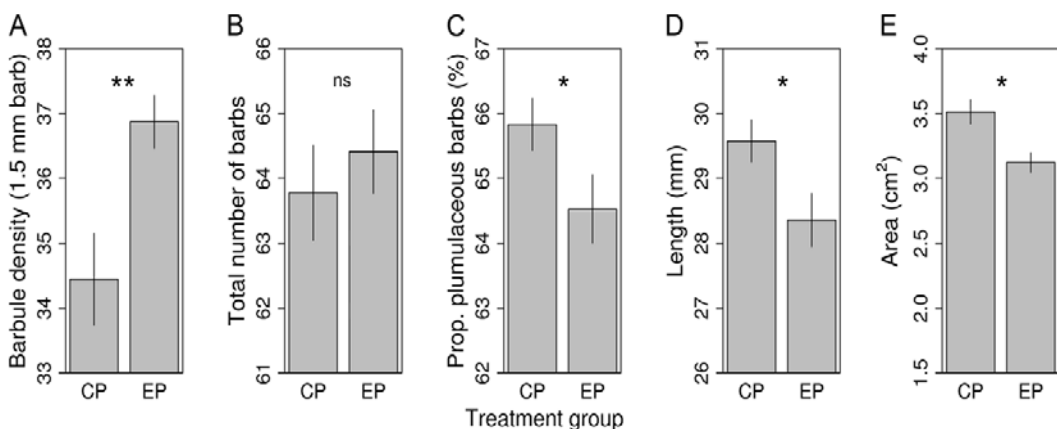

Figure 4. Photoperiod treatment effects on body feather traits. Panels show the effect of treatment on the density of barbules (A), total number of barbs (B), proportion of plumulaceous barbs (C), length (D) and area (E) of mantle feathers (mean ± SE). Photoperiod treatment groups: 'CP' control, 'EP' experimental. 'ns' non-significant, '*' $P \le 0.05$, '**' $P < 0.01$.
doi:10.1371/journal.pone.0040651.g004

rate- and condition-mediated feather quality, and this could have led to the perplexingly high diversity in feather shape, structure and their material properties (e.g. [63]). Second, the mismatch between the timing of parental labor and food peaks due to climate change could drive population declines [64,65]. Besides this climate-driven selection, our results suggest that there may be an additional selective advantage of early breeding to acquire better quality feathers via a slower molting rate. Sedentary birds may benefit in terms of molting due to the progressive advancement of spring. By contrast, this may have a highly negative impact on summer-molting migrants due to the mismatch of the timing of breeding and food abundance, which might be followed by compromised condition and molting. These aspects suggest that pluralistic approaches are needed by also looking through the prism of molting to better understand eco-evolutionary processes.

Author Contributions

Conceived and designed the experiments: CIV PLP Z. Barta. Performed the experiments: CIV PLP OV Z. Benkő AM. Analyzed the data: CIV Z. Barta. Wrote the paper: CIV PLP Z. Barta.

References

1. Lindén M, Møller AP (1989) Costs of reproduction and covariation of life history traits in birds. Trends Ecol Evol 4: 367–371.
2. Roff DA (2002) Life history evolution. Sunderland: Sinauer.
3. Nilsson J-Å, Svensson E (1996) The cost of reproduction: a new link between current reproductive effort and future reproductive success. Proc R Soc Lond B 263: 711–714.
4. 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. Proc R Soc Lond B 267: 2093–2098.
5. Svensson E, Nilsson J-Å (1997) The trade-off between molt and parental care: a sexual conflict in the blue tit? Behav Ecol 8: 92–98.
6. Hemborg C, Lundberg A (1998) Costs of overlapping reproduction and moult in passerine birds: an experiment with the pied flycatcher. Behav Ecol Sociobiol 43: 19–23.
7. Barta Z, Houston AI, McNamara JM, Welham RK, Hedenström A, et al. (2006) Annual routines of non-migratory birds: optimal moult strategies. Oikos 112: 580–593.
8. Ginn HB, Melville DS (2007) Moult in birds. BTO Guide 19. Norwich: Page Bros.
9. Morton GA, Morton ML (1990) Dynamics of postnuptial molt in free-living Mountain White-crowned Sparrows. Condor 92: 813–828.
10. Hinsley SA, Rothery P, Ferns PN, Bellamy PE, Dawson A (2003) Wood size and timing of moult in birds: potential consequences for plumage quality and bird survival. Ibis 145: 337–340.
11. Helm B, Gwinner E (2005) Carry-over effects of day length during spring migration. J Ornithol 146: 348–354.
12. Lindström Å, Visser GH, Daan S (1993) The energetic cost of feather synthesis is proportional to basal metabolic rate. Physiol Zool 66: 490–510.
13. 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? Can J Zool 86: 834–842.
14. Moreno-Rueda G (2010) Experimental test of a trade-off between moult and immune response in house sparrows Passer domesticus. J Evol Biol 23: 2229–2237.
15. Hall KSS, Fransson T (2000) Lesser Whitethroats under time-constraint moult more rapidly and grow shorter wing feathers. J Avian Biol 31: 583–587.
16. Serra L (2001) Duration of primary moult affects primary quality in Grey Plovers Pluvialis squatarola. J Avian Biol 32: 377–380.
17. 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. Biol J Linn Soc 97: 98–105.
18. de la Hera I, Pérez-Tris J, Tellería JL (2010) Relationships among timing of moult, moult duration and feather mass in long-distance migratory passerines. J Avian Biol 41: 609–614.
19. Dawson A (2004) The effects of delaying the start of moult on the duration of moult, primary feather growth rates and feather mass in Common Starlings Sturnus vulgaris. Ibis 146: 493–500.
20. Broggi J, Gamero A, Hohtola E, Orell M, Nilson J-Å (2011) Interpopulation variation in contour feather structure is environmentally determined in Great Tits. PLoS ONE 6: e24942.
21. Jovani R, Blas J (2004) Adaptive allocation of stress-induced deformities on bird feathers. J Evol Biol 17: 294–301.
22. 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. J Avian Biol 27: 103–111.
23. Swaddle JP, Lockwood R (2003) Wingtip shape and flight performance in the European Starling Sturnus vulgaris. Ibis 145: 457–464.
24. Vágási CI, Pap PL, Barta Z (2010) Haste makes waste: accelerated molt adversely affects the expression of melanin-based and depigmented plumage ornaments in house sparrows. PLoS ONE 5: e14215.
25. Smith HG, Montgomerie R (1991) Sexual selection and the tail ornaments of North American barn swallows. Behav Ecol Sociobiol 28: 195–201.

26. Macleod R, Barnett P, Clark JA, Cresswell W (2005) Body mass change strategies in blackbirds Turdus merula: the starvation–predation risk trade-off. J Anim Ecol 74: 292–302.
27. Carr JM, Lima SL (2012) Heat-conserving postures hinder escape: a thermoregulation–predation trade-off in wintering birds. Behav Ecol 23: 434–441.
28. de la Hera I, Hedenström A, Pérez-Tris J, Tellería JL (2010) Variation in the mechanical properties of flight feathers of the blackcap Sylvia atricapilla in relation to migration. J Avian Biol 41: 342–347.
29. Perrins CM (1998) The complete birds of the Western Palearctic on CD-ROM, version 1.0. Oxford: Oxford University Press.
30. Svensson E, Hedenström A (1999) A phylogenetic analysis of the evolution of moult strategies in Western Palearctic warblers (Aves: Sylviidae). Biol J Linn Soc 67: 263–276.
31. de la Hera I, Díaz JA, Pérez-Tris J, Tellería JL (2009) A comparative study of migratory behaviour and body mass as determinants of moult duration in passerines. J Avian Biol 40: 461–465.
32. Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118: 1883–1891.
33. Merilä J, Kruuk LEB, Sheldon BC (2001) Natural selection on the genetical component of variance in body condition in a wild bird population. J Evol Biol 14: 918–929.
34. Jensen H, Sæther B-E, Ringsby TH, Tufto J, Griffith SC, et al. (2003) Sexual variation in heritability and genetic correlations of morphological traits in house sparrows (Passer domesticus). J Evol Biol 16: 1296–1307.
35. van Noordwijk AJ, de Jong G (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. Am Nat 128: 137–142.
36. Andersson M (1994) Sexual selection. Princeton: Princeton University Press.
37. Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82: 1473–1483.
38. Møller AP, Magnhagen C, Ulfstrand A, Ulstrand S (1995) Phenotypic quality and molt in the barn swallow, Hirundo rustica. Behav Ecol 6: 242–249.
39. Gienapp P, Merilä J (2010) Genetic and environmental effects on a condition-dependent trait: feather growth in Siberian jays. J Evol Biol 23: 715–723.
40. Grubb TC, Jr (2006) Ptilochronology: feather time and the biology of birds. New York: Oxford University Press.
41. Animal Behavior Society (2012) Guidelines for the treatment of animals in behavioural research and teaching. Anim Behav 83: 301–309.
42. Pap PL, Vágási CI, Czirják GÁ, Titilincu A, Pintea A, et al. (2011) The effect of coccidians on the condition and immune profile of molting house sparrows (Passer domesticus). Auk 128: 330–339.
43. Vágási CI, Pap PL, Tökölyi J, Székely E, Barta Z (2011) Correlates of variation in flight feather quality in the Great Tit Parus major. Ardea 99: 53–60.
44. Rasband WS (2008) ImageJ, version 1.41. Bethesda: National Institutes of Health. URL http://rsbweb.nih.gov/ij.
45. R Development Core Team (2011) R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. URL http://www.R-project.org/.
46. Legendre P (2011) lmodel2: Model II Regression. R package version 1.7–0. URL http://CRAN.R-project.org/package = lmodel2.
47. Murtaugh PA (2009) Performance of several variable-selection methods applied to real ecological data. Ecol Lett 12: 1061–1068.
48. Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev 85: 935–956.
49. Bortolotti GR, Marchant T, Blas J, Cabezas S (2009) Tracking stress: localisation, deposition and stability of corticosterone in feathers. J Exp Biol 212: 1477–1482.
50. DesRochers DW, Reed JM, Awerman J, Kluge JA, Wilkinson J, et al. (2009) Exogenous and endogenous corticosterone alter feather quality. Comp Biochem Physiol A 152: 46–52.

51. 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. Biol Lett 1: 27–30.

52. Broggi J, Orell M, Hohtola E, Nilson J-Å (2004) Metabolic response to temperature variation in the great tit: an interpopulation comparison. J Anim Ecol 73: 967–972.

53. Alonso-Alvarez C, Bertrand S, Faivre B, Sorci G (2007) Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. Funct Ecol 21: 873–879.

54. de la Hera I, Pérez-Tris J, Tellería JL (2009) Repeatable length and mass but not growth rate of individual feathers between moults in a passerine bird. Acta Ornithol 44: 95–99.

55. Møller AP (1988) Badge size in the house sparrow *Passer domesticus*. Effects of intra- and intersexual selection. Behav Ecol Sociobiol 22: 373–378.

56. Verhulst S, van Balen JH, Tinbergen JM (1995) Seasonal decline in reproductive success of the Great Tit: variation in time or quality? Ecology 76: 2392–2403.

57. Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: unraveling migratory connectivity. Trends Ecol Evol 17: 76–83.

58. Desrochers A (2010) Morphological response of songbirds to 100 years of landscape change in North America. Ecology 91: 1577–1582.

59. Lessells CM, Ovenden GN (1989) Heritability of wing length and weight in European Bee-eaters (*Merops apiaster*). Condor 91: 210–214.

60. Grant PR, Grant BR (1995) Predicting microevolutionary responses to directional selection on heritable variation. Evolution 49: 241–251.

61. Larsson K (1996) Genetic and environmental effects on the timing of wing moult in the barnacle goose. Heredity 76: 100–107.

62. Helm B, Gwinner E (1999) Timing of postjuvenal molt in African (*Saxicola torquata axillaris*) and European (*Saxicola torquata rubicola*) stonechats: effects of genetic and environmental factors. Auk 116: 589–603.

63. Wang X, Nudds RL, Palmer C, Dyke GJ (2012) Size scaling and stiffness of avian primary feathers: implications for the flight of Mesozoic birds. J Evol Biol 25: 547–555.

64. Nussey DH, Postma E, Gienapp P, Visser ME (2005) Selection on heritable phenotypic plasticity in a wild bird population. Science 310: 304–306.

65. Both C, Bouwhuis S, Lessells CM, Visser ME (2006) Climate change and population declines in a long-distance migratory bird. Nature 441: 81–83.

Chronic coccidian infestation compromises flight feather quality

in house sparrows, *Passer domesticus*

Pap PL, <u>Vágási CI</u>, Bărbos L & Marton A (2013)

Summary

Parasites usurp indispensable resources for birds during 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 on a longer term. We conducted an experiment by medicating half of naturally infested house sparrows (*Passer domesticus*) against intestinal coccidians throughout 15 months, spanning two consecutive postnuptial moults, in aviary, while the other half was kept without medication. Coccidian infestation significantly and negatively affected the size of the uropygial gland during the second moulting period compared to the medicated group. Further, wing length was significantly shorter after the second moulting in the infested compared to the medicated female birds, which indicates that the negative effects of coccidians emerge only after a prolonged exposure of the host to parasite infestation. Infested birds grew poorer quality flight feathers detected in a large number of feather traits both after the first and second year moult. In the case of infested birds the primaries were lighter and shorter, and had smaller vane area, thinner rachis diameter and increased bending stiffness, but higher barb and barbule density, which may have various fitness consequences through reducing the flight performance. Our findings demonstrate that host–parasite interactions, besides well known immediate consequences of parasite infections on host breeding success might also have serious, long-lasting effects through influencing feather quality and ultimately fitness of the host.

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

PÉTER L. PAP[1]*, CSONGOR I. VÁGÁSI[1,2], LŐRINC BĂRBOS[1] and ATTILA MARTON[1]

[1]*Evolutionary Ecology Group, Hungarian Department of Biology and Ecology, Babeș-Bolyai University, Clinicilor St. 5–7, RO-400006 Cluj Napoca, Romania*
[2]*Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary*

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 (Hõrak *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, Strochlic & 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.

*Corresponding author. E-mail: peterlpap@gmail.com

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 & Brawner, 1998; Hõrak *et al.*, 2004; Baeta *et al.*, 2008; Mougeot *et al.*, 2009; Dolnik & Hoi, 2010) and significantly decrease an individual's condition (Hõrak *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 multiannual 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 Bălcaciu, 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
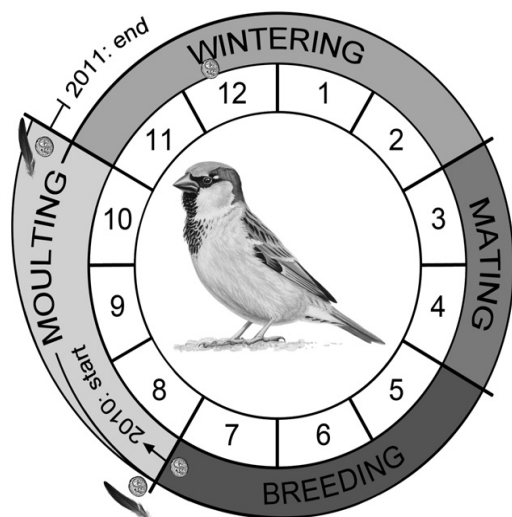
Figure 1. The annual cycle of a house sparrow and a schematic representation of the experimental protocol. The numbers within the circle show the months (i.e. 1 = January), the arrow indicates the start (30 July 2010), and the blunt-tip line marks the end of the experiment (3 November 2011). Wing length, 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
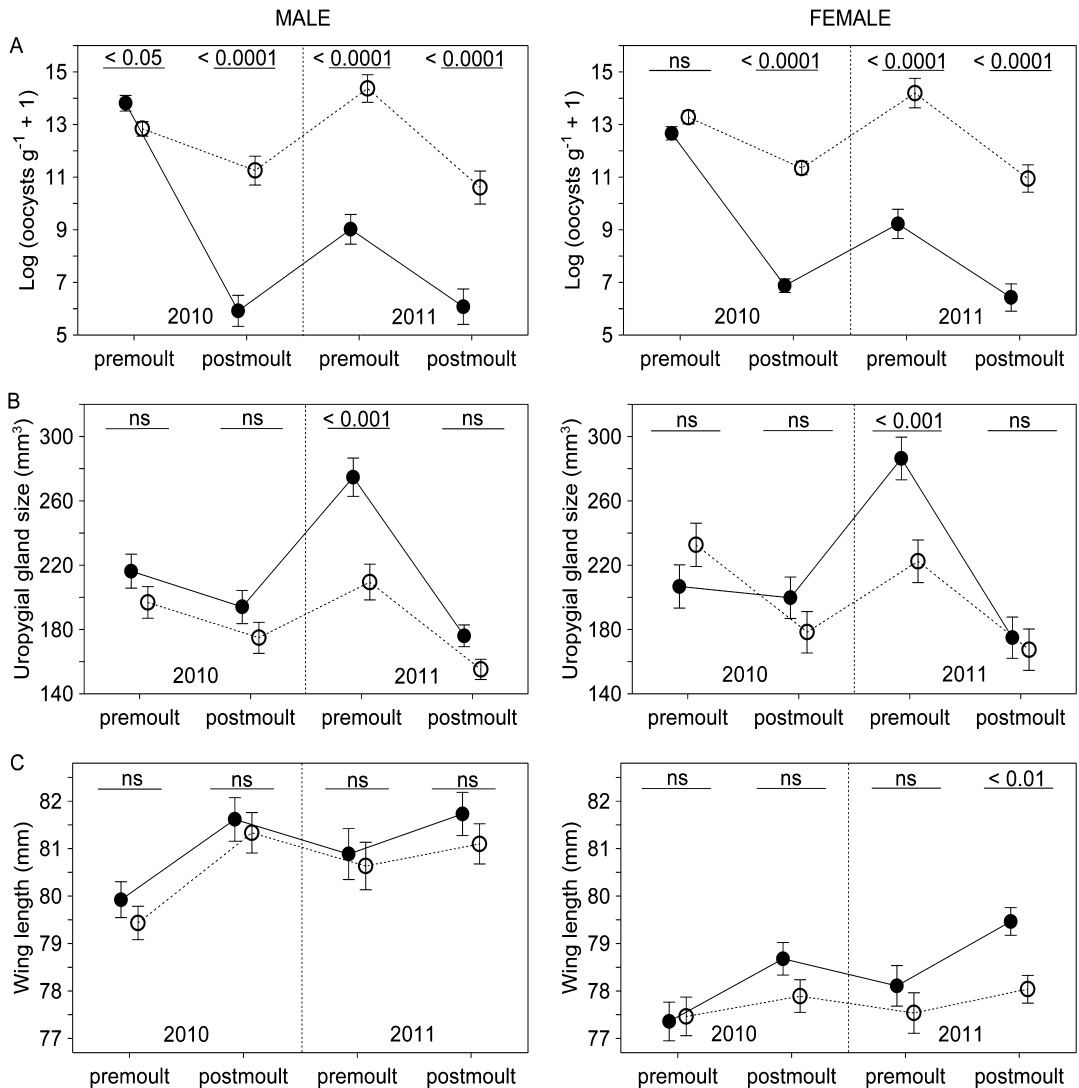
Figure 2. The effect of treatment on the coccidian infestation (A), uropygial gland size (B), and wing length (C) during the premoult and postmoult periods of the first (2010) and second study year (2011) of male and female house sparrows (Med group treated with coccidiostatic drug – black dots, continuous 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 ± 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 ± 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		
	F	d.f.	P	F	d.f.	P	F	d.f.	P	F	d.f.	P
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 × 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 × Repeated-measures	**28.77**	3	**<0.0001**	1.69	3	0.171	**18.32**	3	**<0.0001**	2.56	3	0.057
Sex × Repeated-measures	0.67	3	0.572	**6.68**	3	**0.0003**	0.43	3	0.729	**3.16**	3	**0.026**
Repeated-measures × 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 × 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 × 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 × 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 × 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 × sex interactions). The difference between experimental groups was similar between the quality measures of feathers grown after the first and second moult (nonsignificant

group × 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 × 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
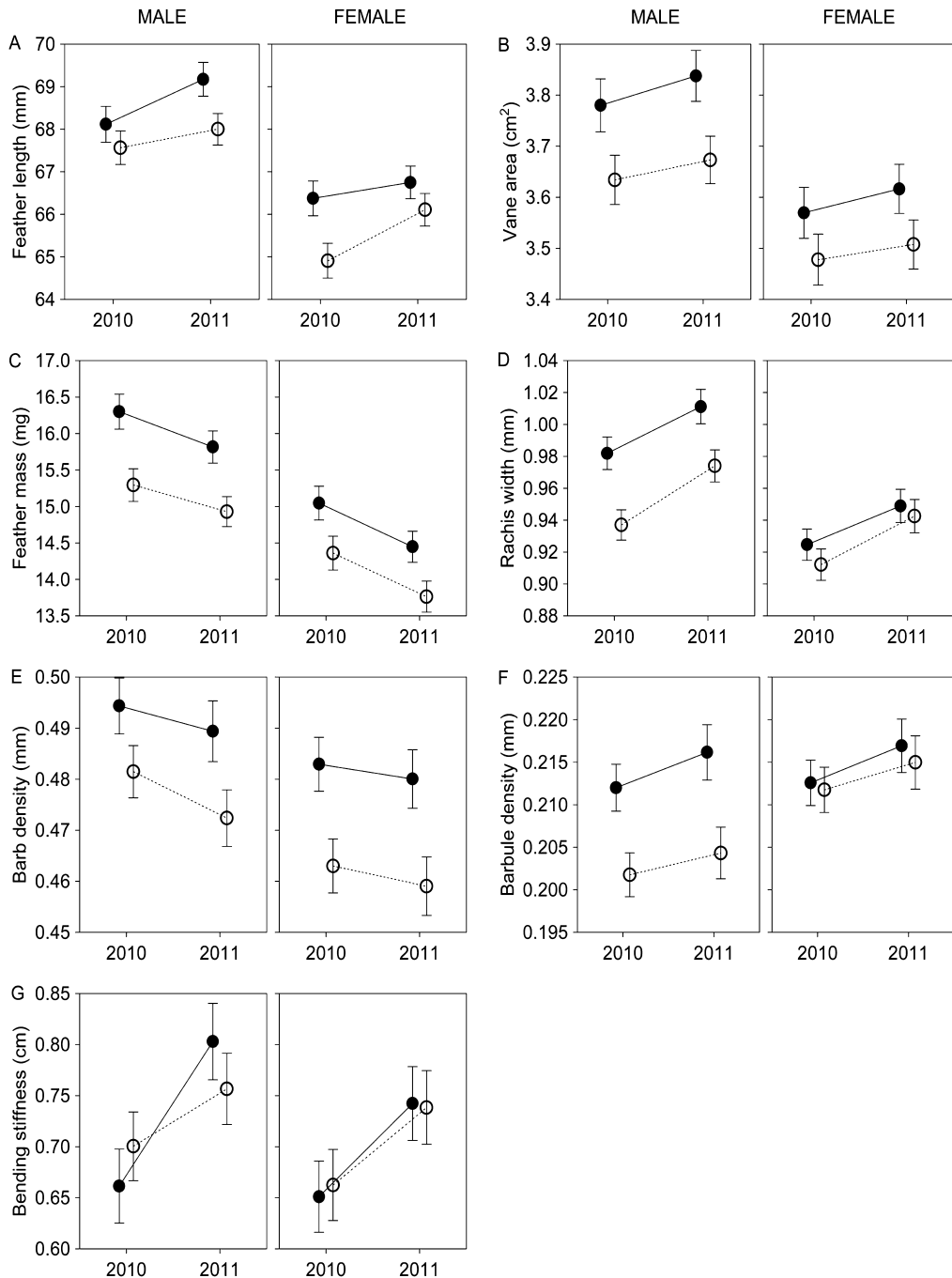
Figure 3. The effect of experimental manipulation of the coccidian infestation on the feather quality measures [feather length (A), vane area (B), feather mass (C), rachis width (D), barb density (E), barbule density (F) and bending stiffness (G)] after the first (2010) and second (2011) postnuptial 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 ± 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		
	F	d.f.	P	F	d.f.	P	F	d.f.	P	F	d.f.	P
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		
	F	d.f.	P	F	d.f.	P	F	d.f.	P
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 first postnuptial moult / After second postnuptial moult						
Feather length	—	7.45** / **0.386 (0.142)**	25.77*** / **0.547 (0.108)**	0.09 / 0.045 (0.149)	0.50 / 0.114 (0.161)	0.86 / 0.155 (0.165)	13.82** / **0.592 (0.159)**
Vane area	17.03** / **0.554 (0.134)**	—	10.60** / **0.362 (0.111)**	2.13 / 0.198 (0.135)	0.02 / −0.023 (0.149)	0.11 / −0.050 (0.153)	2.07 / 0.234 (0.163)
Feather mass	22.81*** / **0.545 (0.114)**	34.90*** / **0.561 (0.095)**	—	14.12** / **0.527 (0.140)**	0.03 / 0.030 (0.171)	0.01 / −0.019 (0.176)	0.23 / 0.091 (0.190)
Rachis width	1.33 / 0.168 (0.145)	4.15* / **0.259 (0.127)**	10.60** / **0.442 (0.136)**	—	0.00 / 0.001 (0.151)	0.16 / −0.062 (0.155)	6.40* / **−0.401 (0.158)**
Barb density	0.42 / 0.101 (0.155)	0.02 / 0.020 (0.140)	1.03 / 0.159 (0.156)	0.02 / −0.020 (0.147)	—	2.88 / −0.237 (0.140)	1.30 / 0.176 (0.154)
Barbule density	0.01 / 0.014 (0.164)	2.44 / −0.225 (0.144)	0.01 / −0.014 (0.166)	0.60 / −0.120 (0.154)	0.03 / −0.026 (0.146)	—	0.08 / 0.044 (0.151)
Bending stiffness	20.37*** / **0.682 (0.151)**	1.29 / 0.179 (0.158)	1.18 / 0.194 (0.178)	0.18 / −0.071 (0.168)	3.86 / 0.300 (0.153)	0.19 / −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 (Hõrak *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; Hõrak *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 (Hõrak *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.

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. *Naturwissenchaften* **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.

Hõrak 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, Strochlic 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, Burtt 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.

Haste Makes Waste: Accelerated Molt Adversely Affects the Expression of Melanin-Based and Depigmented Plumage Ornaments in House Sparrows

Csongor I. Vágási, Péter L. Pap & Zoltán Barta (2010) *PLoS ONE* 5: e14215 [IF$_{2010}$ 4.41]

Summary

Background: Many animals display colorful signals in their integument which convey information about the quality of their bearer. Theoretically, these ornaments incur differential production and/or maintenance costs that enforce their honesty. However, the proximate mechanisms of production costs are poorly understood and contentious in cases of noncarotenoid-based plumage ornaments like the melanin-based badge and depigmented white wing-bar in house sparrows *Passer domesticus*. Costly life-history events are adaptively separated in time, thus, when reproduction is extended, the time available for molt is curtailed and, in turn, molt rate is accelerated.

Methodology/Principal Findings: We experimentally accelerated the molt rate by shortening the photoperiod in order to test whether this environmental constraint is mirrored in the expression of plumage ornaments. Sparrows which had undergone an accelerated molt developed smaller badges and less bright wing-bars compared to conspecifics that molted at a natural rate being held at natural-like photoperiod. There was no difference in the brightness of the badge or the size of the wing-bar.

Conclusions/Significance: These results indicate that the time available for molt and thus the rate at which molt occurs may constrain the expression of melanin-based and depigmented plumage advertisements. This mechanism may lead to the evolution of honest signaling if the onset of molt is condition-dependent through the timing of and/or trade-off between breeding and molt.

Haste Makes Waste: Accelerated Molt Adversely Affects the Expression of Melanin-Based and Depigmented Plumage Ornaments in House Sparrows

Csongor I. Vágási[1,2]*, Péter L. Pap[1,2], Zoltán Barta[1]

1 Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, Debrecen, Hungary, **2** Department of Taxonomy and Ecology, Babeş-Bolyai University, Cluj Napoca, Romania

Abstract

Background: Many animals display colorful signals in their integument which convey information about the quality of their bearer. Theoretically, these ornaments incur differential production and/or maintenance costs that enforce their honesty. However, the proximate mechanisms of production costs are poorly understood and contentious in cases of non-carotenoid-based plumage ornaments like the melanin-based badge and depigmented white wing-bar in house sparrows *Passer domesticus*. Costly life-history events are adaptively separated in time, thus, when reproduction is extended, the time available for molt is curtailed and, in turn, molt rate is accelerated.

Methodology/Principal Findings: We experimentally accelerated the molt rate by shortening the photoperiod in order to test whether this environmental constraint is mirrored in the expression of plumage ornaments. Sparrows which had undergone an accelerated molt developed smaller badges and less bright wing-bars compared to conspecifics that molted at a natural rate being held at natural-like photoperiod. There was no difference in the brightness of the badge or the size of the wing-bar.

Conclusions/Significance: These results indicate that the time available for molt and thus the rate at which molt occurs may constrain the expression of melanin-based and depigmented plumage advertisements. This mechanism may lead to the evolution of honest signaling if the onset of molt is condition-dependent through the timing of and/or trade-off between breeding and molt.

Introduction

The coloration of animal integument, such as skin, scales, fur and feathers, is often determined by deposits of pigments. Melanins are the most common pigments present in almost all birds and can be categorized into two types: phaeomelanins are for various shades of yellowish and rufous brown, while eumelanins for grey, brown and black [1,2]. In addition to their ubiquitous role in naturally selected functions like crypsis, protection against solar UV-radiation and mechanical fatigue, melanin-based coloration also frequently serves as sexual or social signal [3]. Beyond pigmentary colors, several avian species possess feather patches that lack pigments, the so-called depigmented white ornaments, which also act as signals used in intra- and intersexual communication (e.g. the forehead patch in male collared flycatchers *Ficedula albicollis* [4,5]). These ornaments are built of finely structured, nanometer-scale matrix of keratin and variably-sized air vacuoles with or without spongy layer and are perceived as white because they incoherently scatter the whole spectrum of incident light [6].

A central tenet of evolutionary signaling theory posits that a communication system where information is conveyed via ornaments is susceptible to cheaters' invasion unless a significant and quality-differential production and/or maintenance cost of the signal exists [7]. A wealth of investigations have been targeting the evolution of plumage coloration in birds, but the vast majority of these studies focused on the production costs of carotenoid-based and gaudy structural ornamentation (reviews in [8]). In contrast, empirical evidences for such costs in case of melanin-based and depigmented white coloration are scarcer and open to debate, therefore the mechanisms responsible for them are poorly understood [1,9–11].

Plumage ornaments are developed when birds are molting, thus the circumstances prevailing before and during this period are of key importance. In the temperate zone, natural selection favors such an optimal scheduling of main life-history events like

breeding and molt, that they are separated [12,13], since overlapping these energetically conflicting activities induces large costs [14]. Reproduction may affect molting, because (1) low-quality birds are likely to obtain a mate and breed later in the season (e.g. [15–18]) and/or (2) a resource-based trade-off may operate between reproduction and molt [19–21]. Those birds are more likely to postpone the start of molt which are late breeders [22], which also applies for their late fledging offspring [23], and engage in high investment in current reproduction [24,25]. This delay leaves a shorter time-window for the plumage to be renewed before the onset of migration or harsh winter weather [13]. Such time constraint is often compensated for by accelerated molt, although this can be costly. For instance, in the European starling *Sturnus vulgaris*, rapid molting compromised the quality of flight feathers [26], while blue tits *Cyanistes caeruleus* with probably reduced time available for molting due to experimentally delayed breeding invested more energy in winter thermoregulation owing to less efficient insulation capacity of the plumage [27]. Molt rate might also negatively affect the elaboration of plumage ornaments, ultimately ensuring signal honesty. Accelerated molt reduced the expression of a carotenoid-based plumage trait in rock sparrows *Petronia petronia* [28], and also the UV/blue coloration in blue tits [29] ('molt speed constraint' hypothesis hereafter). However, no studies examined to date whether melanised ornaments are also affected by molt rate, and previous results on pigment-free ornaments [28,29] do not support the honesty-reinforcing effect of molt rate.

The mechanism behind the effect of molt rate on different pigmented (carotenoid- vs. melanin-based) and structural ornaments (UV/blue vs. depigmented) is probably distinct because of fundamental divergences in how these pigments are incorporated and how structural ornaments are produced, respectively. Carotenoids are transported to and incorporated in growing feathers together with other circulating proteins and lipids [1]. On the contrary, the mechanism for melanin-based coloration mediated by molt rate is probably more direct, given that melanins are manufactured *de novo* in specific cells and organelles (melanocytes and melanosomes, respectively) in the feather follicle from amino acid precursors. For this reason, we hypothesized that if feather production (i.e. keratin synthesis) is accelerated, it could outpace the rate of melanin incorporation or melanogenesis, the latter being often limited by the activity of the enzyme tyrosinase [30,31], the key enzyme in eumelanogenesis, thus less melanin granules can be embedded in the keratin matrix. As a result smaller and/or duller black signals are expected to develop. UV/blue structural ornaments are composed of large vacuoles, an underlying melanin layer and the medullary spongy layer. The precise arrangement of these components is impeded when molt is accelerated [29]. On the contrary, structural white ornaments always lack the melanin layer, and often the spongy layer too [6], so their appearance depends mostly on the arrangement of the keratin structures and air vacuoles.

The house sparrow *Passer domesticus* is a small passerine, common throughout the world that has been broadly studied with respect to males' plumage ornamentation. The male sparrow possesses a eumelanin-dominated patch of black feathers on their throat and upper breast (bib or, hereafter, 'badge') which varies greatly in size among males. Males with larger badge average higher dominance rank according to a recent meta-analysis [32]. Males also possess a depigmented white wing-bar formed by the white tip of the median secondary coverts, the conspicuousness of which also functions in status-signaling [33] or might indicate parasite resistance [34]. The factors that promote honesty to the wing-bar were, however, rarely addressed (see [35] for an

exception). We tested the 'molt speed constraint' hypothesis, namely that accelerated plumage replacement adversely affects the size and/or coloration of the melanin-based badge and structural white wing-bars of male house sparrows. To this end, we experimentally adjusted molt rate by a photoperiod treatment.

Materials and Methods

Ethics statement

Birds were handled in strict accordance with good animal welfare and ethical prescriptions. The protocol for bird care and experimentation adhered to the current Romanian laws and was approved by the Romanian Academy of Sciences (permission #2257).

Capture and housing

We caught 50 adult male house sparrows by mist nets (Ecotone, Poland) at a cattle farm near Cluj Napoca (46°46′N, 23°33′E, Transylvania, Central Romania) on 25th July 2008. These birds had finished breeding though none had started to molt. Birds were randomly assigned to one of two indoor aviaries (each 4 m L ×3.5 m W ×4 m H, 56 m³, $n = 25$ individuals per aviary; see [15] where 45 m³ were sufficient for 33 individuals) on the Campus of the Babeş-Bolyai University, Cluj Napoca. Although group housing may potentially confound the results due to inter-male aggression, we housed birds in groups to enable flocking which is characteristic for sparrows year-round and especially in the molting and wintering season [36], instead of individual caging that differs fundamentally from this natural social environment. This setup has also been applied in several previous studies about plumage ornamentation [10,37–40]. However, to exclude that aggression may alter the biological relevance of our results, we tried to moderate aggression by putting two feeding dishes per aviary (see also [40]). Moreover, we registered the aggressive encounters ($n = 1515$) between individually color-ringed birds from a hide through a one-way window using a 10×50 binocular (Zeiss, Germany) during the last month of molt (in average 1 h after the 'sunrise' and 1 h before the 'sunset' to assess fights for food and roost sites, respectively; totally 1930 min of observations). Only those fights were recorded, which had an unambiguous outcome and both the winner and the loser were identified. From these data we calculated the fighting success (expressed as the proportion of fights won) as a proxy of dominance rank [41] and statistically controlled for this variable by introducing it in models as a covariate (see below). See also the Results section for further justifications that group housing had negligible effects on our results. Although fights were registered only in the last third part of the experiment, this probably represents the social relationships throughout the experiment because dominance rank is known to be consistent at long-term (between seasons) in closed flocks [42].

Each aviary contained bushes (a pile of dead boughs) and nest boxes to provide perch and roost sites (see [43,44] for more detail on housing). Birds were provided *ad libitum* with high-quality food (protein content ranging between 50–80% [44]), sand and drinking water supplemented with vitamins. To suppress isosporan infection that emerges spontaneously in captive populations, we administered an anticoccidial drug (toltrazuril) to the drinking water [1 mL Baycox 2.5% (Bayer HealthCare, Germany) in 1 L water] for three-day periods on three occasions evenly distributed throughout the experiment. One sparrow from the control photoperiod group (see below) died on 20th August 2008 due to unknown reasons; therefore its data was omitted. Note, however, that the survival rates of wild and other aviary populations are seldom higher [45]. The remaining 49 sparrows were released at

their site of capture on 25th November 2008. Some of them were recaptured several months later during the course of other studies conducted on the same population.

Experimental procedure and data collection

On the day the birds were captured and transferred to the aviaries, the local natural photoperiod was 15L:9D. We started the experimental photoperiod treatment on 28th July 2008 (= day 1), after 3 days of acclimatization. In one aviary we simulated the natural-like light regime which is characteristic at the latitude of the population by decreasing the daylength in average by 3 min each day (control photoperiod, hereafter 'CP' group). In the other aviary we experimentally accelerated the seasonal decrease in photoperiod by decreasing the daylength by 8 min each day (experimental photoperiod, hereafter 'EP' group). The photoperiod in this aviary was decreased until it reached 9L:15D (on day 46) and was then held constantly at this level until the end of experiment (day 110). The speed of photoperiod change in EP group, the light intensity and the type of lighting is similar to Serra et al. [28].

We measured wing length (to the nearest 0.5 mm), tarsus length (to the nearest 0.01 mm) and body mass (to the nearest 0.1 g) at capture and release, and mass and molt status (see below) on every 10th day of the 110-day-long experimentation (i.e. 11 measurement sessions). We scored the molt status of each primary feather of both wings following Newton [46]: '0' = old, worn primaries, '1' = dropped primaries, '2', '3' and '4' = one-quarter-, half- and three-quarter-regrown primaries, respectively, and '5' = fully regrown, new primaries. Note that sparrows have 9 primaries, as the 10th (i.e. outermost) is vestigial. Molting index was derived by summing the scores of individual feathers and averaged the scores of the two wings. This score ranges between 0 (all primaries old, molt not started) and 45 (all primaries replaced, molt finished). The molt was considered to have started when the innermost primary was dropped (molt index >0), and to have ended when all primaries had been replaced (molt index = 45). Molt duration refers to the number of days elapsed between the onset and termination of molt. The molt status of the badge and left wing-bar was also recorded as follows: badge, '0' = no molt, '1' = 1–30%, '2' = 31–60%, '3' = 61–99% of feathers growing, respectively, and '4' = all feathers replaced (see [28]); wing-bar, '0' = no molt, '1' = few (1–3), '2' = many (>3) coverts missing, respectively, and '3' = all coverts exchanged.

Measurement of plumage ornaments

We measured ornament size and brightness from photographs taken before and after molt. We used a Nikon D80 digital camera to photograph each male's badge, holding the head perpendicular to the body, and left wing-bar, with the wing stretched out flat. Photographs were taken in a darkroom using standard lighting conditions with no flash, constant distance from subject and camera settings, and against a metric background (a grid of 1 mm^2 squares) and black and white standards. Ornament areas were measured in the ImageJ software [47] using 'set scale' (with the metric background as template) and 'freehand selection tool' by tracing the outline of the ornament. Ornament brightness was measured in the Scion Image software [48] using 'density slice' and 'wand tool' [33]. We then calculated the relative brightness expressed as a percentage where either the black or the white standard was the 100%, thus the higher values mean 'blacker' badge and 'whiter' wing-bar. The brightness of these ornaments can be appropriately estimated from photographs since neither has a reflectance peak in the UV-range [49] and there is a strong positive correlation between spectral reflectance values obtained

using a spectroradiometer and achromatic brightness measured from photographs [33]. Photographs of 15 randomly chosen sparrows were measured again several months later; repeatability between measures was highly significant for all four ornament variables (all $R_i > 0.81$, all $F > 10.17$, all $P < 0.0001$).

The feathers composing the badge can be divided into three well separated parts: depigmented white tip, melanised black middle part and plumulaceous basal part. The freshly-molted badge feathers have all three parts, thus the badge of male house sparrows is concealed owing to the white tips which partially cover the neighboring feathers' black middle vanes. These white tips gradually wear off till the next breeding season and the middle black parts, and in turn the badge, become fully exposed. It should be mentioned that the freshly molted, concealed badge area correlates with the fully-expressed, unconcealed badge area in the subsequent breeding season [50]. Moreover, in the present study the visible badge (only the black area) of freshly molted birds measured with Scion Image and the total badge (comprises the black feather parts and white tips, too) measured with ImageJ are significantly positively correlated (Pearson's correlation, $r = 0.72$, $n = 49$, $P < 0.0001$). However, since males in different treatment groups may use different tactics to pigment their badge feathers by some concealing more than others with longer white tips, or some growing larger black middle parts than others (Pap PL, Vágási CI, Barta Z unpublished data), to undoubtedly exclude the possibility of faulty badge measurements, we plucked about 5 feathers from the newly-grown badge (from a predetermined region of the breast where the badge is the widest and the badge feathers have the longer white tip). Later, we photographed two randomly chosen feathers against a metric background, measured the length (along the rachis with ImageJ) and area (with Scion Image) of the white tip, black middle part and the total feather, and averaged the values of the two feathers. Afterwards, we computed the proportion of the white tip and black middle part to the total badge feather length and area. All photographs were measured by the same person (CIV) and were performed blind with respect to the treatment.

Statistical procedures

There were no initial differences between birds placed into different aviaries in biometry, body mass and plumage ornamentation (all $F < 2.08$, all $P > 0.16$). The molt index of primaries increases following a non-linear S-shaped function, similarly to mass gain during avian ontogeny. Thus, to describe the molting pattern, we fitted a logistic growth curve used for developing nestlings [51] to each bird separately (see also [43,44]). The logistic function has the form

$$y = \frac{a}{1 + e^{-K(t-I)}}$$

where y denotes the molting score at time t, a is the maximum score or asymptote (i.e. 45 in house sparrows), K is the growth constant (indicating molt speed), I is the inflection point on the time axis at which molting switches from accelerating to decelerating, while e is the base of natural logarithm. We used Mann-Whitney U-test to analyze the effect of treatment on the relative day of molt commence. We used general linear models (GLMs) to analyze the effect of photoperiod manipulation on the following response variables: speed of molt (K and I values), duration of molt, area and brightness of badge and wing-bar. All of these response variables met the assumptions of parametric tests. Treatment was entered as factor, while wing length, tail length, tarsus length, body mass (the mean of 11 measurement sessions;

see above), fighting success and pre-molt signal values were all entered as covariates. We built repeated measures ANOVAs to test both between group (photoperiod treatment) and within individual effects. However, to render possible the control for the effects of covariates, we also tested the photoperiod treatment effect on the change in signal values during molt in a GLM by extracting pre-molt from post-molt values [hereafter differential (Δ) signal value]. Because the 4 signal variables may indicate somehow the same abilities, each time we first started with a MANOVA to see whether photoperiod treatment differentiates the two groups by the 4 signal variables. Then, we entered each signal variable separately in GLMs to closely see which variables vary more with photoperiod treatment. In all of these GLM and MANOVA analyses, the non-significant covariate terms were eliminated by a backward stepwise procedure. We present the minimal final models. Mean $\pm$ standard error (SE) is shown throughout and all tests were two-tailed with a significance level set at $\alpha = 0.05$. The sample size was reduced for wing-bar area because 7 birds (3 from CP and 4 from EP group, thus the remaining sample size, $n = 42$, is balanced with 21 individuals in both groups) lost ≥ 1 coverts before the post-molt photographing.

Results

Molt pattern and plumage ornamentation

The median date of molt commence was day 11 for primaries and the wing-bar, and day 24 for the badge. Birds started to molt their primaries and ornamental plumage patches irrespective of treatment (Mann-Whitney U-test, $n_{CP} = 24$ and $n_{EP} = 25$ in all cases; primaries: $U = 271.5$, $P = 0.57$; Fig. 1; badge: $U = 231.0$, $P = 0.17$; wing-bar: $U = 292.5$, $P = 0.88$). In contrast, molt duration

of EP birds was significantly shorter, roughly by two weeks, compared with conspecifics in the CP group: primaries (mean $\pm$ SE in days): CP 85.8 ± 1.8 vs. EP 71.8 ± 1.8 (GLM, $F_{1,40} = 33.03$, $P < 0.0001$), badge: CP 63.7 ± 2.6 vs. EP 49.0 ± 1.5 ($F_{1,38} = 27.78$, $P < 0.0001$) and wing-bar: CP 75.2 ± 3.2 vs. EP 64.6 ± 3.2 ($F_{1,38} = 11.07$, $P = 0.0019$) (Fig. 2). Analyzing the treatment effect on K and I values, we found that photoperiod treatment differentiated birds according to molt rate (MANOVA, $F_{2,39} = 38.95$, $P < 0.0001$). EP birds molted faster (GLM, K-value: $F_{1,40} = 60.63$, $P < 0.0001$) and got through the accelerate phase of molt and turned to decelerate phase more rapidly (I-value: $F_{1,40} = 34.25$, $P < 0.0001$). All the covariates turned out to be non-significant and were eliminated from the final models about molt duration and molt speed.

Photoperiod treatment groups significantly differed according to the 4 response variables (MANOVA, $F_{4,30} = 3.55$, $P = 0.017$). Table 1 shows the final minimal GLMs about the effect of treatment on each post-molt signal. Sparrows in the EP group that molted faster also developed smaller badges and less bright (i.e. less whitish) wing-bars (Table 1, Fig. 3A, D). However, after Bonferroni correction (α-level/4 response variables = 0.0125) the effect on wing-bar brightness would be non-significant ($P = 0.038$). Although badge brightness and wing-bar area were not significantly influenced by the photoperiod manipulation, EP birds tended to grow less elaborate ornaments in these respects too (Table 1, Fig. 3B, C). Sparrows with longer tarsus had larger-sized wing-bars, and pre-molt badge and wing-bar area positively predicted the post-molt badge and wing-bar area, respectively (Table1). The omission of those 7 individuals from the analyses for which the post-molt wing-bar area cannot be measured (see
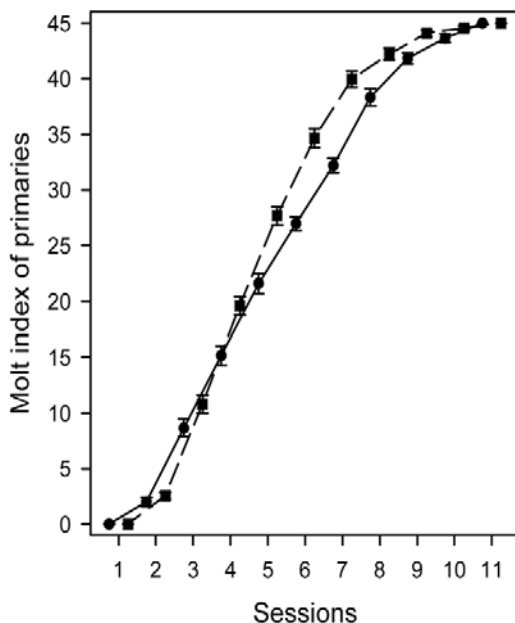

Figure 1. Change in primary molt index during the course of experiment. Male house sparrows from the accelerated 'EP' photoperiod group (rectangles and broken line) finished molting two weeks faster than birds in the natural-like 'CP' photoperiod group (circles and continuous line). First session is the date of capture (25th July 2008), then each session comes after 10-day intervals. Mean $\pm$ SE are shown. doi:10.1371/journal.pone.0014215.g001
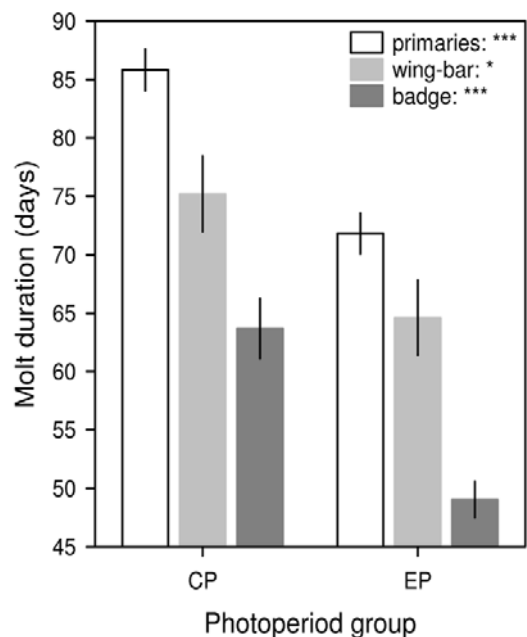

Figure 2. The effect of experimental photoperiod manipulation on molt duration. The duration of replacing the primaries (open columns), wing-bar (light grey columns) and badge feathers (dark grey columns) was significantly shorter if birds were held at experimentally accelerated 'EP' photoperiod as related to those at natural-like 'CP' photoperiod. Mean $\pm$ SE are shown. '*' $P < 0.05$, '***' $P < 0.0001$. doi:10.1371/journal.pone.0014215.g002

Table 1. Final minimal GLMs showing the photoperiod treatment effect on the area and brightness of post-molt plumage ornaments.

Response/source	Estimate	MS	F	df	P
Post-molt badge area					
Intercept	1.69	2.85	13.63	1	0.0006
Pre-molt badge area	0.47	7.39	35.28	1	<0.0001
Photoperiod treatment	0.26	3.00	14.34	1	0.0005
Error		0.21		42	
Final minimal model: $F_{2,42} = 24.53$, $P<0.0001$, $R^2 = 0.54$					
Post-molt wing-bar area					
Intercept	−0.66	0.02	1.21	1	0.28
Tarsus length	0.08	0.11	7.05	1	0.012
Pre-molt wing-bar area	0.39	0.43	27.52	1	<0.0001
Photoperiod treatment	0.04	0.05	3.16	1	0.084
Error		0.02		35	
Final minimal model: $F_{3,35} = 11.48$, $P<0.0001$, $R^2 = 0.50$					
Post-molt badge brightness					
Intercept	96.42	445494.3	373042.9	1	<0.0001
Photoperiod treatment	0.21	2.1	1.8	1	0.19
Error		1.2		46	
Final minimal model: $F_{1,46} = 1.77$, $P=0.19$, $R^2 = 0.04$					
Post-molt wing-bar brightness					
Intercept	29.28	38547.87	11074.27	1	<0.0001
Photoperiod treatment	0.60	16.04	4.61	1	0.038
Error		3.48		43	
Final minimal model: $F_{1,43} = 4.61$, $P=0.038$, $R^2 = 0.10$					

doi:10.1371/journal.pone.0014215.t001

Statistical procedures) yielded qualitatively similar results regarding the effect of photoperiod on the other signals (GLM with the same minimal models as in Table 1, badge area: $F_{1,36} = 11.08$, $P=0.002$; badge brightness: $F_{1,36} = 0.92$, $P=0.35$; wing-bar brightness: $F_{1,37} = 4.68$, $P=0.037$).

We found similar results when analyzing the within individual changes in signal values during molt by means of repeated measures ANOVAs. EP birds grew smaller badges (photoperiod treatment [pt]: $F_{1,47} = 2.43$, $P=0.13$; repeated measures [rm]: $F_{1,47} = 221.49$, $P<0.0001$; pt × rm: $F_{1,47} = 7.41$, $P=0.009$) and less bright wing-bars (pt: $F_{1,46} = 0.93$, $P=0.34$; rm: $F_{1,46} = 805.12$, $P<0.0001$; pt × rm: $F_{1,46} = 6.23$, $P=0.016$) relative to their original pre-molt values as opposed to CP birds. However, after Bonferroni correction (α-level $= 0.0125$) the effect on wing-bar brightness would turn to marginally significant ($P=0.016$). Badge brightness (pt: $F_{1,46} = 2.0$, $P=0.17$, rm: $F_{1,46} = 32.5$, $P<0.0001$, pt × rm: $F_{1,46} = 0.3$, $P=0.58$) and wing-bar area (pt: $F_{1,37} = 0.06$, $P=0.81$, rm: $F_{1,37} = 50.09$, $P<0.0001$, pt × rm: $F_{1,37} = 1.47$, $P=0.24$) were not influenced by treatment. We also found that the Δ signal values significantly differed according to photoperiod treatment (MANOVA, $F_{4,32} = 3.01$, $P=0.03$). We then analyzed each signal's Δ value separately. However, since in none of the analyses did any significant covariate term remain, these models yielded the same results as the repeated measures ANOVAs (not shown).

Condition, fights and pigmentation pattern of badge feathers

Treatment had non-significant effect on body mass measured at 10-day intervals during the whole experiment (repeated measures ANOVA, $F_{1,47} = 0.02$, $P=0.90$) and during the period when the aggressive encounters were observed ($F_{1,47} = 0.11$, $P=0.74$). Similarly, treatment did not affect the number of fights in which an individual was involved (GLM, $F_{1,27} = 2.51$, $P=0.13$) and the number of fights initiated by the individual ($F_{1,27} = 0.35$, $P=0.56$) per hour of observation. These results demonstrate that birds in the EP group were not more stressed or aggressive due to the perceived accelerated commence of winter than their CP counterparts. Interestingly, birds with larger pre- and post-molt wing-bar area were involved in fewer fights (pre-molt wing-bar area: $F_{1,27} = 4.61$, $P=0.04$; post-molt wing-bar area: $F_{1,27} = 14.21$, $P=0.0008$), and those with larger post-molt wing-bar also initiated fewer fights ($F_{1,27} = 6.84$, $P=0.015$).
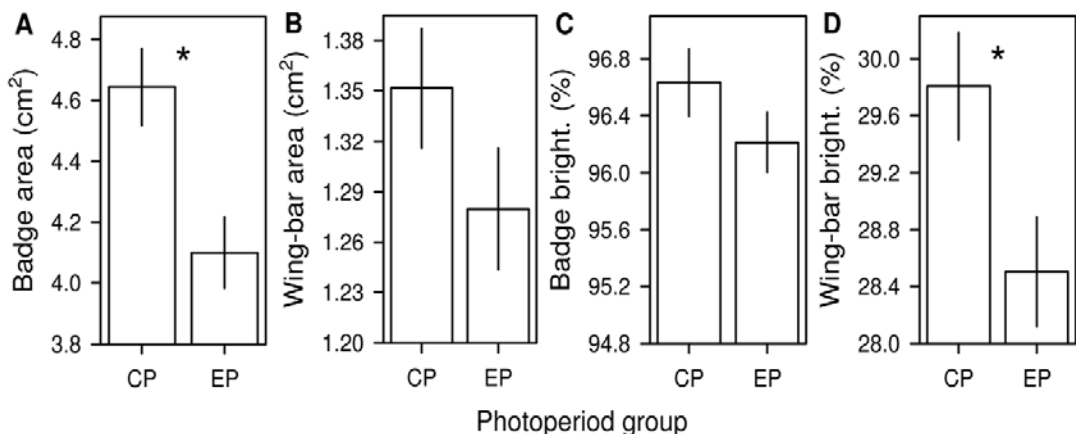

Figure 3. The effect of experimental photoperiod manipulation on plumage ornament expression. Panels show the differences in (A) badge size, (B) wing-bar size, (C) badge brightness and (D) wing-bar brightness between sparrows with experimentally accelerated molting ('EP' photoperiod group) and those molting at a natural rate ('CP' photoperiod group). Asterisks denote significant differences. Mean ± SE are shown.
doi:10.1371/journal.pone.0014215.g003

We did not find treatment effect either on absolute length or on absolute area of the white tip and black middle part of badge feathers. When the proportion-to-total badge feather length and area (i.e. relative length and area) was considered, we found similar non-significant effects for both badge feather parts (all $F < 3.05$, all $P > 0.09$).

Discussion

The 'molt speed constraint' hypothesis was largely supported for melanin-based and depigmented plumage ornaments. We have experimentally demonstrated that individuals which completed their molt faster by roughly two weeks paid a cost in terms of plumage ornament expression, because molt rate adversely affected the badge size and the wing-bar brightness. The effect on badge size might be a by-product if EP birds conceal the 'visible badge' more by the buff white feather tips, but the facultatively exposable 'hidden badge' (sensu [52]; see also Materials and Methods) did not differ between groups. However, this can be rejected as the length, area and the proportion in length and area of the feather that was comprised of the white tip were similar between treatment groups. These, together with the fact that the black middle parts' absolute and proportional length and area was also not influenced, advocate that the number of melanised badge feathers was affected similarly to the effect of a diet treatment in zebra finches *Taeniopygia guttata* [53].

Studies of melanin-based signaling have centered mostly on ultimate functions rather than proximate mechanisms that ensure reliability [53]. Empirical evidence suggests the existence of maintenance (e.g. [52,54] and production costs as well, but the mechanistic basis of the latter remained unclear. To discuss in detail the several hypotheses that have been put forward to find production costs for melanised plumage traits is beyond the scope of this study (but see thorough reviews in [1,11]). Briefly, some studies have focused on the nutritional condition during molt. Juvenile male house sparrows with higher blood protein levels grew larger badges [37] and those supplemented with an essential precursor of melanin, the phenylalanine, grew blacker badges [35], while zebra finches fed with extra calcium, which is cofactor of melanin-producing enzymes, grew larger black signals [53; but see 55]. Galván and Alonso-Alvarez [56] demonstrated the inhibitory effect of an antioxidant, the glutathione, on the melanised breast stripe of great tits *Parus major*. Others studied the effect of circulating hormone titers. Buchanan et al. [57] showed that in male house sparrows, testosterone implants enhanced both the metabolic rate and badge size. Roulin et al. [58] evidenced that corticosterone-implanted juvenile barn owls *Tyto alba* developed plumage with less phaeomelanin. All of these factors can rely on condition-dependence, as presumably prime quality birds have better access to food resources, endure more the elevated metabolic costs of testosterone-dependent ornament production, and have lower stress-induced corticosterone profiles. However, other studies could not detect nutritional influence (e.g. [38]), while the link between immunocompetence and coloration mediated by androgen hormones was partially supported and is more complicated than previously thought (e.g. [39]).

Here, we experimentally tested the 'molt speed constraint' hypothesis as a proximate mechanism that might ensure the honesty of eumelanin-based ornaments. There are several reasons to believe that variation in molt speed could explain variation in badge size and/or coloration in house sparrows. First, the two weeks difference in molt duration between our control and experimental birds was well within the range of variation that naturally occurs within most wild passerines, including house

sparrows [36]. Second, we found an association between molt duration and badge size even though our birds were provided with high-quality food *ad libitum*, therefore we expect a stronger effect under field conditions where resources are more limited. Third, it requires a rate-limiting factor in the biochemical pathway of endogenous melanin synthesis, a prerequisite which may be fulfilled by the activity of the enzyme tyrosinase [30,31] that catalyzes several biochemical reactions in the whole course of eumelanin synthesis [1]. If the process of eumelanin synthesis is of lower efficiency (e.g. reduced tyrosinase activity), a smaller and/or less intense black ornament is expected to develop [59]. However, the lower rate of melanin production may also lead to similarly sized but duller black badges (i.e. fewer pigment granules deposited per feather), but this was not the case in our experimental photoperiod group. This means that instead of a tyrosinase rate limitation there is a threshold effect in the distribution of active, pigment-producing melanosomes. Those feathers that are positioned at the bottom periphery of the badge had melanosomes activated at all in the faster molting group, thus fewer badge feathers were colored to black but with similar intensity (see also [53]). It remains for future studies to shed more light on the cellular or molecular bases of melanin granule distribution on and among feathers to elucidate why the number of melanised feathers is affected instead of the coloration [53].

Depigmented white signals are largely unexplored in terms of whether they also have production costs. Since these plumage traits do not contain any pigment and are thus emancipated from the production costs ascribed to melanins or carotenoids, they are presumed to incur mostly maintenance costs such as an increased risk of predation [60] or social control from conspecifics [61]. However, contrary to the previously established 'cheap-to-produce' concept, empirical evidence is accumulating that the expression of depigmented plumage is also costly (e.g. [10,34,62–64], Pap PL, Vágási CI, Barta Z unpublished data; but see [65]). However, the mechanism responsible for these apparent production costs is unclear. White structural signals are produced by randomly distributed and differently sized air-filled vacuoles embedded in the feather keratin that lacks the underlying melanin layer [6,66]. The magnitude of light reflected by white plumage patches depends on the interface between feather keratin and air-filled vacuoles, such that feathers that incorporate larger vacuoles into their structure appear brighter [6]. A medullary spongy layer may also be present since white plumage ornaments have evolved from structural blue feathers as a result of derived loss of the underlying melanin deposition (at least in manakins *Lepidothrix* spp. [6]; see also [66] for a case of amelanism). The precision of the arrangement of this nanometer-scale structure may also contribute to variation in the color produced [67]. Although the microscopic analysis of the nanometer-scale structure of house sparrows' white wing-bar-forming coverts are missing to date, as far as we are aware, it is possible that the birds which had undergone a rapid molt were unable to (1) either impute large vacuoles and/or (2) precisely structure the spongy layer in the ways required to produce a bright white feather patch. This scenario suggests that the appearance of white feathers is not limited by any particular resource-demand but by the ability to develop feathers with the correct microstructure, ensuring ultimately its honesty [64]. To the best of our knowledge, this study is the first to ascertain a mechanism that mediates the production cost of a depigmented, non-UV structural signal. The treatment effect on wing-bar brightness should be treated with circumspection, since after Bonferroni correction it disappeared. Albeit, we consider this result important because the treatment × repeated measures remains marginally significant and this correction procedure was

recently severely criticized (e.g. [68]). Previous studies that addressed these questions did not found any effect of molt speed on the size [28,29] or brightness [29] of white signals. Regarding coloration, the microscopic analysis of feather barbs/barbules is indispensable to disentangle whether this discrepancy is due to species (blue tits vs. house sparrows) differences in feather microstructure.

This study did not test explicitly a condition-dependent model of ornamentation, however, we can make speculative inferences on how honest signaling may be guaranteed by molt rate. The timing of molt may be connected to condition in two non-exclusive ways. Lower quality males often acquire a mate later in the season (e.g. [15–17]), thus early breeders are usually in better condition [18,29]. Late breeders may have lower survival prospects [46] further indicating their lower quality. Alternatively, birds that undertake high parental investments in current reproduction relative to their condition may run out of time and/or deplete their finite resources [69], reflecting a trade-off between breeding and molt [19–21]. For instance, Griffith [70] found that male house sparrows with experimentally increased breeding effort expressed smaller-sized badges in the subsequent molt. Moreover, juveniles males which left the nest relatively late in the season also grew smaller badges [71,72]. Thus, birds of inferior condition may initiate their molt later and then they cannot bluff their abilities because of faster molting. Only birds of superior condition that have a relaxed molt might be able to incorporate more melanins in their feathers and arrange the nanostructure precisely. Since the photoperiod treatment affected all birds in a similar way, our results are more consonant with the latter explanation. Notwithstanding, future experiments that jointly manipulate photoperiod and condition or hormonal profiles (e.g. corticosterone [58], prolactin [69]) linked to condition are encouraged to gain a clearer picture. Our findings emphasize that, through a cascade effect (delayed breeding is followed by delayed molt), molt couples the pre- and post-molt events. Molt speed may mediate the hidden costs of late breeding and/or high parental activity [69] by diminishing plumage ornamentation. Given that both the melanin-based badge and the depigmented wing-bar are subjects to intra- and intersexual selection [15,32,33,73], the coupling of successive breeding attempts by molt could explain the strong relationship between the condition-dependent ornamentation and annual [15] or lifetime reproductive success [74]. We show that the time available for molt, and thus the photoperiod it was exposed to, is an environmental factor that can generate variation in melanin-based and depigmented plumage traits (see [8,75] and references therein). This also highlights the selective advantage attained by birds which breed and fledge their nestlings earlier in the season [28,29].

Acknowledgments

Author Contributions

Conceived and designed the experiments: CIV PLP ZB. Performed the experiments: CIV PLP ZB. Analyzed the data: CIV PLP ZB. Contributed reagents/materials/analysis tools: CIV PLP ZB. Wrote the paper: CIV PLP ZB.

References

1. McGraw KJ (2006) Mechanics of melanin-based coloration. In: Hill GE, McGraw KJ, eds. Bird Coloration. Vol. I. Mechanisms and Measurements. Cambridge: Harvard University Press. pp 243–294.

2. Ducrest A-L, Keller L, Roulin A (2008) Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends Ecol Evol 23: 502–510.

3. Hill GE, McGraw KJ (2006) Bird Coloration. Vol. II. Function and Evolution. Cambridge: Harvard University Press.

4. Pärt T, Qvarnström A (1997) Badge size in collared flycatchers predicts outcome of male competition over territories. Anim Behav 54: 893–899.

5. Qvarnström A, Pärt T, Sheldon BC (2000) Adaptive plasticity in mate preference linked to differences in reproductive effort. Nature 405: 344–347.

6. Prum RO (2006) Anatomy, physics, and evolution of structural colors. In: Hill GE, McGraw KJ, eds. Bird Coloration. Vol. I. Mechanisms and Measurements. Cambridge: Harvard University Press. pp 295–353.

7. Johnstone RA (1997) The evolution of animal signals. In: Krebs JR, Davies NB, eds. Behavioural Ecology: An Evolutionary Approach. 4th ed. Oxford: Blackwell Publishing. pp 155–178.

8. Hill GE, McGraw KJ (2006) Bird coloration. Vol. I. Mechanisms and Measurements. Cambridge: Harvard University Press.

9. Jawor JM, Breitwisch R (2003) Melanin ornaments, honesty, and sexual selection. Auk 120: 249–265.

10. McGlothlin JW, Duffy DL, Henry-Freeman JL, Ketterson ED (2007) Diet quality affects an attractive white plumage pattern in dark-eyed juncos (Junco hyemalis). Behav Ecol Sociobiol 61: 1391–1399.

11. McGraw KJ (2008) An update on the honesty of melanin-based color signals in birds. Pigment Cell Melanoma Res 21: 133–138.

12. Barta Z, Houston AI, McNamara JM, Welham RK, Hedenström A, et al. (2006) Annual routines of non-migratory birds: optimal moult strategies. Oikos 112: 580–593.

13. Dawson A (2008) Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. Phil Trans R Soc Lond B 363: 1621–1633.

14. Hemborg C, Lundberg A (1998) Costs of overlapping reproduction and molt in passerine birds: an experiment with the pied flycatcher. Behav Ecol Sociobiol 43: 19–23.

15. Møller AP (1988) Badge size in the house sparrow Passer domesticus. Effects of intra- and intersexual selection. Behav Ecol Sociobiol 22: 373–378.

16. Møller AP (1990) Male tail length and female mate choice in the monogamous swallow Hirundo rustica. Anim Behav 39: 458–465.

17. Hill GE (1991) Plumage coloration is a sexually selected indicator of male quality. Nature 350: 337–339.

18. Verhulst S, van Balen JH, Tinbergen JM (1995) Seasonal decline in reproductive success of the Great Tit: variation in time or quality? Ecology 76: 2392–2403.

19. Bensch S, Gezelius L, Grahn M, Hasselquist D, Lindström Å, et al. (1985) Influence of brood size on moult in female Willow Warblers. Ornis Scand 16: 151–152.

20. Lessells CM (1986) Brood size in Canada geese: a manipulation experiment. J Anim Ecol 55: 669–689.

21. Siikamäki P, Hovi M, Rätti O (1994) A trade-off between current reproduction and moult in the Pied Flycatcher—an experiment. Funct Ecol 8: 587–593.

22. Hinsley SA, Rothery P, Ferns PN, Bellamy PE, Dawson A (2003) Wood size and timing of moult in birds: potential consequences for plumage quality and bird survival. Ibis 145: 337–340.

23. Bojarinova JG, Lehikoinen E, Eeva T (1999) Dependence of postjuvenile moult on hatching date, condition and sex in the Great Tit. J Avian Biol 30: 437–446.

24. Morton GA, Morton ML (1990) Dynamics of postnuptial molt in free-living Mountain White-crowned Sparrows. Condor 92: 813–828.

25. Svensson E, Nilsson J-Å (1997) The trade-off between molt and parental care: a sexual conflict in the blue tit? Behav Ecol 8: 92–98.

26. 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. Proc R Soc Lond B 267: 2093–2098.

27. Nilsson J-Å, Svensson E (1996) The cost of reproduction: a new link between current reproductive effort and future reproductive success. Proc R Soc Lond B 263: 711–714.

28. Serra L, Griggio M, Licheri D, Pilastro A (2007) Moult speed constrains the expression of a carotenoid-based sexual ornament. J Evol Biol 20: 2028–2034.

29. Griggio M, Serra L, Licheri D, Campomori C, Pilastro A (2009) Moult speed affects structural feather ornaments in the blue tit. J Evol Biol 22: 782–792.

30. Hearing VJ, Tsukamoto K (1991) Enzymatic control of pigmentation in mammals. FASEB J 5: 2902–2909.

31. Ito S, Wakamatsu K, Ozeki H (2000) Chemical analysis of melanins and its application to the study of the regulation of melanogenesis. Pigment Cell Res 13(Suppl 8): 103–109.

32. Nakagawa S, Ockendon N, Gillespie DOS, Hatchwell BJ, Burke T (2007) Assessing the function of house sparrows' bib size using a flexible meta-analysis method. Behav Ecol 18: 831–840.

33. Bókony V, Lendvai ÁZ, Liker A (2006) Multiple cues in status signalling: the role of wingbars in aggressive interactions of male house sparrows. Ethology 112: 947–954.

34. Moreno-Rueda G (2005) Is the white wing-stripe of male House Sparrows *Passer domesticus* an indicator of the load of Mallophaga? Ardea 93: 109–114.

35. Poston JP, Hasselquist D, Stewart IRK, Westneat DF (2005) Dietary amino acids influence plumage traits and immune responses of male house sparrows, *Passer domesticus*, but not as expected. Anim Behav 70: 1171–1181.

36. Cramp S, Perrins CM (1994) The birds of the Western Palearctic. Vol. VIII. Oxford: Oxford University Press.

37. Veiga JP, Puerta M (1996) Nutritional constraints determine the expression of a sexual trait in the house sparrow, *Passer domesticus*. Proc R Soc Lond B 263: 229–234.

38. Gonzalez G, Sorci G, Møller AP, Ninni P, Haussy C, et al. (1999) Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). J Anim Ecol 68: 1225–1234.

39. Buchanan KL, Evans MR, Goldsmith AR (2003) Testosterone, dominance signalling and immunosuppression in the house sparrow, *Passer domesticus*. Behav Ecol Sociobiol 55: 50–59.

40. McGraw KJ, Hill GE (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc R Soc Lond B 267: 1525–1531.

41. Liker A, Barta Z (2001) Male badge size predicts dominance against females in house sparrows. Condor 103: 151–157.

42. Buchanan KL, Evans MR, Roberts ML, Rowe L, Goldsmith AR (2010) Does testosterone determine dominance in the house sparrow *Passer domesticus*? An experimental test. J Avian Biol 41: 445–451.

43. 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? Can J Zool 86: 834–842.

44. Pap PL, Vágási CI, Czirják GÁ, Titilincu A, Pintea A, et al. (2009) Carotenoids modulate the effect of coccidian infection on the condition and immune response in molting house sparrows. J Exp Biol 212: 3228–3235.

45. Liker A, Bókony V (2009) Larger groups are more successful in innovative problem solving in house sparrows. Proc Natl Acad Sci USA 106: 7893–7898.

46. Newton I (1966) The moult of the Bullfinch *Pyrrhula pyrrhula*. Ibis 108: 41–67.

47. Rasband WS (2008) ImageJ, version 1.41. Maryland, Bethesda: National Institutes of Health, URL: http://rsbweb.nih.gov/ij.

48. Scion Corporation (2005) Scion Image, beta version 4.0.3. Maryland: Frederick.

49. Václav R (2006) Within-male melanin-based plumage and bill elaboration in male house sparrows. Zool Sci 23: 1073–1078.

50. Møller AP, Erritzøe J (1992) Acquisition of breeding coloration depends on badge size in male house sparrows *Passer domesticus*. Behav Ecol Sociobiol 31: 271–277.

51. Ricklefs RE (1973) Patterns of growth in birds. II. Growth rate and mode of development. Ibis 115: 177–201.

52. Veiga JP (1996) Permanent exposure versus facultative concealment of sexual traits: an experimental study in the house sparrow. Behav Ecol Sociobiol 39: 345–352.

53. McGraw KJ (2007) Dietary mineral content influences the expression of melanin-based ornamental coloration. Behav Ecol 18: 137–142.

54. Nakagawa S, Lee J-W, Woodward BK, Hatchwell BJ, Burke T (2008) Differential selection according to the degree of cheating in a status signal. Biol Lett 4: 667–669.

55. Stewart IRK, Westneat DF (2010) Dietary calcium negatively affects the size of a status signal in juvenile male house sparrows (*Passer domesticus*). Auk 127: 549–557.

56. Galván I, Alonso-Alvarez C (2008) An intracellular antioxidant determines the expression of a melanin-based signal in a bird. PLoS ONE 3: e3335.

57. Buchanan KL, Evans MR, Goldsmith AR, Bryant DM, Rowe LV (2001) Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? Proc R Soc Lond B 268: 1337–1344.

58. Roulin A, Almasi B, Rossi-Pedruzzi A, Ducrest A-L, Wakamatsu K, et al. (2008) Corticosterone mediates the condition-dependent component of melanin-based coloration. Anim Behav 75: 1351–1358.

59. Stoehr AM (2006) Costly melanin ornaments: the importance of taxon? Funct Ecol 20: 276–281.

60. Bókony V, Liker A, Lendvai ÁZ, Kulcsár A (2008) Risk-taking and survival in the House Sparrow *Passer domesticus*: are plumage ornaments costly? Ibis 150: 139–151.

61. Garamszegi LZ, Rosivall B, Hegyi G, Szöllõsi E, Török J, et al. (2006) Determinants of male territorial behavior in a Hungarian collared flycatcher population: plumage traits of residents and challengers. Behav Ecol Sociobiol 60: 663–671.

62. Gustafsson L, Qvarnström A, Sheldon BC (1995) Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. Nature 375: 311–313.

63. Török J, Hegyi G, Garamszegi LZ (2003) Depigmented wing patch size is a condition-dependent indicator of viability in male collared flycatchers. Behav Ecol 14: 382–388.

64. Hanssen SA, Folstad I, Erikstad KE (2006) White plumage reflects individual quality in female eiders. Anim Behav 71: 337–343.

65. Shawkey MD, Hill GE, McGraw KJ, Hood WR, Huggins K (2006) An experimental test of the contributions and condition dependence of microstructure and carotenoids in yellow plumage coloration. Proc R Soc Lond B 273: 2985–2991.

66. Shawkey MD, Hill GE (2006) Significance of a basal melanin layer to production of non-iridescent structural plumage color: evidence from an amelanotic Steller's jay (*Cyanocitta stelleri*). J Exp Biol 209: 1245–1250.

67. Shawkey MD, Estes AM, Siefferman LM, Hill GE (2003) Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colour. Proc R Soc Lond B 270: 1455–1460.

68. Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav Ecol 15: 1044–1045.

69. Badyaev AV, Vleck CM (2007) Context-dependent development of sexual ornamentation: implications for a trade-off between current and future breeding efforts. J Evol Biol 20: 1277–1287.

70. Griffith SC (2000) A trade-off between reproduction and a condition-dependent sexually selected ornament in the house sparrow *Passer domesticus*. Proc R Soc Lond B 267: 1115–1119.

71. Veiga JP (1993) Badge size, phenotypic quality, and reproductive success in the house sparrow: a study on honest advertisement. Evolution 47: 1161–1170.

72. Griffith SC, Owens IPF, Burke T (1999) Environmental determination of a sexually selected trait. Nature 400: 358–360.

73. Moreno-Rueda G (2010) Uropygial gland size correlates with feather holes, body condition and wingbar size in the house sparrow *Passer domesticus*. J Avian Biol 41: 229–236.

74. Jensen H, Sæther B-E, Ringsby TH, Tufto J, Griffith SC, et al. (2004) Lifetime reproductive success in relation to morphology in the house sparrow *Passer domesticus*. J Anim Ecol 73: 599–611.

75. Parejo D, Silva N (2009) Methionine supplementation influences melanin-based plumage colouration in Eurasian kestrel, *Falco tinnunculus*, nestlings. J Exp Biol 212: 3576–3582.

Diet quality affects postnuptial molting and feather quality of the house sparrow
(*Passer domesticus*): interaction with humoral immune function?

Péter L. Pap, <u>Csongor I. Vágási</u>, Gábor Á. Czirják & Zoltán Barta (2008)

Summary

We investigated the effects of nutritional limitation, humoral immune activation, and their interaction on postnuptial molting of aviary-kept house sparrows (*Passer domesticus* (L., 1758)). In a 2 × 2 experimental design, we measured the progress of molting and the quality of feathers produced during molting by house sparrows exposed to different diet qualities (high and low) and humoral immune activation with sheep red blood cells (SRBC). Food quality, but not the activation of humoral immunity, affected significantly the body mass and the process of molting. Sparrows feeding on low-quality food had decreased body mass and longer molts than the high-quality group. Low-quality food, but not the activation of humoral immunity, reduced significantly the length and mass (i.e., the quality) of primaries grown during molting. Birds responded significantly to injection with SRBC compared with the control group, but the immune response was similar between nutritional groups. The absence of a negative effect of humoral immunity on molting in house sparrows might be related to the low energy and nutritional requirements of mounting and maintaining a humoral immune response.

Diet quality affects postnuptial molting and feather quality of the house sparrow (*Passer domesticus*): interaction with humoral immune function?

Péter László Pap, Csongor István Vágási, Gábor Árpád Czirják, and Zoltán Barta

Abstract: We investigated the effects of nutritional limitation, humoral immune activation, and their interaction on post-nuptial molting of aviary-kept house sparrows (*Passer domesticus* (L., 1758)). In a 2 × 2 experimental design, we measured the progress of molting and the quality of feathers produced during molting by house sparrows exposed to different diet qualities (high and low) and humoral immune activation with sheep red blood cells (SRBC). Food quality, but not the activation of humoral immunity, affected significantly the body mass and the process of molting. Sparrows feeding on low-quality food had decreased body mass and longer molts than the high-quality group. Low-quality food, but not the activation of humoral immunity, reduced significantly the length and mass (i.e., the quality) of primaries grown during molting. Birds responded significantly to injection with SRBC compared with the control group, but the immune response was similar between nutritional groups. The absence of a negative effect of humoral immunity on molting in house sparrows might be related to the low energy and nutritional requirements of mounting and maintaining a humoral immune response.

Résumé : Nous étudions les effets des restrictions alimentaires, de l'activation immunitaire humorale et de leur interaction sur la mue post-nuptiale chez des moineaux domestiques (*Passer domesticus* (L., 1758)) gardés en volière. Dans un plan d'expérience 2 × 2, nous avons mesuré le progrès de la mue et la qualité des plumes produites par des moineaux exposés à des régimes alimentaires de qualité variable (haute et faible) et à une activation immunitaire humorale à l'aide d'érythrocytes de mouton (SRBC). La qualité de la nourriture, mais non l'activation immunitaire humorale, affecte significativement la masse corporelle et le processus de mue. Les moineaux nourris d'un régime de faible qualité ont une masse corporelle réduite et une mue prolongée par rapport aux moineaux bien nourris. La faible qualité de l'alimentation, mais non l'activation de l'immunité humorale, réduit significativement la longueur et la masse (c'est-à-dire la qualité) des pennes primaires qui poussent pendant la mue. Les oiseaux réagissent significativement à l'injection de SRBC par comparaison au groupe témoin, mais la réponse immunitaire est semblable dans les deux groupes alimentaires. L'absence d'effet négatif de l'immunité humorale sur la mue des moineaux domestiques s'explique peut-être par les exigences énergétiques et alimentaires basses requises pour l'établissement et le maintien d'une réponse immunitaire humorale.

[Traduit par la Rédaction]

Introduction

One of the most energy- and nutrition-demanding periods in a bird's life is the period of increased somatic cell growth during the regular replacement of body and flight feathers (postnuptial molting; Lindström et al. 1993; Jenni and Winkler 1994; Klaassen 1995). Given the high caloric content of feathers (Murphy and King 1982) and the very low effi-ciency of their production (compared with other animal tissues; Murphy and King 1984; Reeds 1991 cited in Klaassen 1995), the energetic cost of feather production is comparable with that of reproduction (Lindström et al. 1993; Klaassen 1995; Kuenzel 2003). Besides the increased energy requirement for molting, the need for high-quality food is also essential (Murphy and King 1982, 1986; Cherel et al. 1994). Another important energy- and nutrition-dependent

P.L. Pap[1,2] and Z. Barta. Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, H-4010 Debrecen, Egyetem square 1, Hungary.

C.I. Vágási. Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, H-4010 Debrecen, Egyetem square 1, Hungary; Department of Taxonomy and Ecology, Babeş-Bolyai University, RO-400006 Cluj Napoca, Clinicilor Street 5–7, Romania.

G.Á. Czirják. Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, RO-400372 Cluj Napoca, Mănăştur Street 3–5, Romania.

[1]Corresponding author (e-mail: peterlpap@gmail.com).
[2]Present address: Department of Taxonomy and Ecology, Babeş-Bolyai University, RO-400006 Cluj Napoca, Clinicilor Street 5–7, Romania.

doi:10.1139/Z08-060

life-history trait of a bird is the immune system. Immune activity elevates energy expenditure (Svensson et al. 1998; Ots et al. 2001; Martin et al. 2003; Eraud et al. 2005), and hence probably diverts energy from other costly functions such as growth, reproduction, and molting (e.g., Ilmonen et al. 2000; Hanssen et al. 2004; Sanz et al. 2004; Martin 2005; Mauck et al. 2005). The trade-off between the immune system and other costly activities was demonstrated by experiments where the energy expenditure has been increased. For instance, experimentally increased parental duties resulted in reduced immunocompetence (reviewed in Lochmiller and Deerenberg 2000). In addition, the effectiveness of the defense system depends on the amount and the quality of the food ingested (Lochmiller et al. 1993; Saino et al. 1997; Klasing 1998; Gonzalez et al. 1999; Alonso-Alvarez and Tella 2001; Smith et al. 2007). Birds experiencing feeding deficiency, or more specifically feeding on low-quality food, usually have a reduced immune capacity (Lochmiller et al. 1993; Saino et al. 1997; Gonzalez et al. 1999; Råberg et al. 2003; Hangalapura et al. 2005; Smith et al. 2007), which may reduce their ability to keep infections within bounds. The effects of the quality of food on the humoral immune system in wild birds are, however, still waiting to be tested. On the other hand, the energetic costs of mounting a humoral immune response is generally low (8%–13% of the basal metabolic rate; Svensson et al. 1998; Ots et al. 2001; Eraud et al. 2005), suggesting that the trade-off between the humoral immune system and the other resource-demanding functions (e.g., reproduction, molting) might be less accentuated.

Considering the energetic and nutritional costs of molting along with those related to the deployment of immune defenses, it seems reasonable to assume the existence of resource-based trade-offs between these two traits. This trade-off may be manifested as a decrease in molting speed during an immune challenge (Martin 2005), or inversely, the depression of immune response during intense feather replacement (Martin et al. 2006). In critical estates, e.g., during the molting–breeding overlap, when the energetic demand is probably at a ceiling, an induced immune response may result in delaying the onset of postnuptial molting (Sanz et al. 2004).

In the present study, we test the hypothesis that molting is traded off with food quality and humoral immunity. The hypothesis is based on the assumption that food quality and the activated humoral immunity affect condition of the birds. We addressed these questions by using a 2×2 experimental design, where within the nutritional groups half of the birds were challenged with sheep red blood cells (SRBCs), while the rest of them served as a control receiving the same dose of phosphate-buffered saline. We first explore the effects of nutritional limitation on molting and feather quality of house sparrows (*Passer domesticus* (L., 1758)) feeding under low- and high-quality foods. Second, we study the effect of humoral immune response on molting of birds from different nutritional groups.

Materials and methods

Studied species, capture, and aviary conditions

We captured 56 adult male house sparrows from a farm situated near Cluj Napoca (46°46′N, 23°33′E), Transylvania, central Romania, during two netting sessions on the 16th and 21st of July 2006. The birds were then transported into aviaries situated at the campus of the Babeş-Bolyai University, Cluj Napoca. At capture, none of the birds had started molting. Following capture, birds were randomly distributed among four aviaries (5 m long × 2 m wide × 2.5 m high).

Group feeding is a characteristic of the house sparrow social system (Anderson 2006). For this reason, birds were not housed in individual cages, but in groups to stimulate group feeding. However, the drawback of group living is the dominance interaction between individuals, which can influence the effect of manipulation, resulting in various responses of individuals with different social statuses. The food was provided in two separate dishes in each aviary to reduce dominance interactions between individuals (see McGraw and Hill 2000). Observations made during the experiment confirmed the reduced competition between birds, since the aggressive interactions between feeding individuals were almost lacking. There were no differences between the four groups in wing length ($F_{[3,52]} = 1.2$, $P = 0.33$), tarsus length ($F_{[3,52]} = 2.5$, $P = 0.07$), wing-bar area ($F_{[3,52]} = 1.13$, $P = 0.94$), and bib size ($F_{[3,52]} = 0.37$, $P = 0.77$) at capture (for measurement details see Pap 2002; P.L. Pap and C.I. Vágási, unpublished data). After 2 days of accommodation, we started the nutritional experiment. Birds in two randomly chosen aviaries (14 birds each) received low-quality food during the whole period of molting, while birds in the other two aviaries received high-quality food. In the wild, house sparrows feed on insects and seeds, with a high proportion of protein-rich insects during the molting period (Cramp 1994), but with considerable variation between populations and years. We designed the diet treatments to simulate the natural range of diet quality. The low-quality diet treatment consisted entirely of seeds, simulating the harsh conditions faced when insects are scarce and protein content of the diet is low. Groups with low-quality diet were fed ad libitum a mixture of seeds containing ground corn, sunflower, millet, oat, and barley; they also received twice weekly fresh dandelions. The composition of the dry matter of the seed mixture comprised 2.1% ash, 15.9% protein, 4.2% fat, and 77.8% carbohydrate. Every 2nd day their water was supplemented with vitamins and minerals recommended for canaries (Vita-Plus; Promedivet Ltd., Sovata, Romania). Groups with high-quality diet received the same grain mixture in addition to being supplemented with one grated boiled egg / group on every even day (comprising 3.7% ash, 51.3% protein, 40.4% fat, and 4.6% carbohydrate) and two mealworms/bird on odd days throughout molting (comprising 2.8% ash, 79.2% protein, 14.6% fat, and 3.4% carbohydrate). To suppress coccidian infection in the birds, we administered a coccidiostatic cure developed for veterinary use by adding sulfachinoxalin (Coccistops, Pasteur Ltd., Bucharest, Romania) to the drinking water of all groups during the experiment. To increase the comfort of the birds, we provided shelter (small bushes), perches, and nest boxes inside of each aviary. Sand and water were available ad libitum throughout the experiment. At capture and before release, we measured the wing and tarsus lengths, and the condition of the birds was assessed by measuring their body mass (with a Pesola spring balance

with an accuracy of 0.1 g) at weekly intervals throughout molting.

Experimental protocol

We used a 2×2 experimental design with two replicates of the nutritional groups (2 low-quality and 2 high-quality groups, respectively). Within each aviary, half of the birds were challenged with an antigen (see below), while the rest of them served as controls receiving the same dose of phosphate-buffered saline (PBS). Thus within each aviary we had 7 immunized and 7 control birds, totaling 14 house sparrows in each of the following groups: low-quality $\times$ SRBC, low-quality $\times$ PBS, high-quality $\times$ SRBC, and high-quality $\times$ PBS. One bird from the low-quality group was injured and another one from the high-quality group escaped during the second half of the molting period, hence the data for these birds on the molting process and feather quality were omitted from the analyses.

Seventy-seven percentage of the birds survived until the end of the study (the duration of the experiment was 125 days). The survival rate is comparable with those of other studies on aviary-kept house sparrows (63% during 3 months in Gonzalez et al. 1999; 71% during 4 months in Poston et al. 2005), and the death of the birds may be related to the stress associated with nutritional manipulation, repeated handling, or other factors. The survival rate of house sparrows were slightly higher than in the studies mentioned above, which is expected given the higher survival rates of adults relative to juveniles (Anderson 2006). After the birds had finished molting and feathers had been collected at the end of November, all birds were fed high-quality (protein-rich) food. After 2 weeks of intensive feeding, they were released in good condition at the farm where they had been captured. The body mass of the house sparrows at capture and before their release were similar between the nutritional groups ($F_{[1,42]} = 2.83$, $P = 0.10$); the body mass was significantly higher after 2 weeks of intensive feeding compared with the body mass at capture (Tukey's post hoc test from repeated-measures ANOVA; high-quality group: $P < 0.001$; low-quality group: $P < 0.001$).

Measurements: molting, feather quality, and biometry

Based on our observation and literature data (Jenni and Winkler 1994), the molting of primary feathers characterizes the complete postnuptial molting in birds; thus the use of the primaries gives a good general picture about the molting progress of house sparrows. Birds started molting shortly after they were introduced into the aviaries; on average, 13.5 ± 4.7 days following the start of the nutritional manipulation experiment. There was no difference between the four aviaries in the days elapsed between the start of nutritional manipulation and the start of molting (Kruskal–Wallis test: $\chi^2_{[3]} = 5.3$, $P = 0.15$). Following the settlement of birds in the aviaries, birds were captured once a week to assess the stage of molting (measure sessions hereafter). We categorized the molting stage of individual primaries following the scheme of Newton (1966): the dropped feathers were scored as 1; a quarter-, half-, or three-quarter-regrown feathers were scored as 2, 3, and 4, respectively; and fully regrown feathers were scored as 5. Old feathers received a score of 0. The molting pattern of the house sparrows is

similar to that of most passerines: primary (P) flight feathers are shed from the wing in sequential order (Jenni and Winkler 1994), from innermost P1 to outermost P9 (P10 is rudimentary in sparrows). For each measure session, the molting stage of birds was assessed by summing the scores obtained for each individual primary feather, which is called the molting score. The molting score ranges between 0 (before the beginning of molting: all primaries old) and 45 (finished molting: all primaries completely replaced). To determine the speed of growth of individual feathers, we also measured the distance between the tip and the base of the untied part of the feather with a caliper (the length of the unbounded distal part). For the analyses, we only used the measurements of those feathers that were growing at least at two consecutive measure sessions and were assigned codes between 2 and 4. We considered that molting had started if at least one primary was dropped, while molting had ended when the outermost primary was fully grown.

At the end of molting (when all primary feathers were replaced), we plucked out the P2 and P7 feathers (for the rationale of choosing these particular feathers see below) on both wings to determine the effect of experimental manipulation on the quality of feathers grown. We characterized the quality of feathers through three parameters: feather length, rachis diameter, and feather mass. The length of the feathers was measured with an accuracy of 0.5 mm by extending them on a ruler. The rachis diameter was measured with a digital caliper (with an acccuracy of 0.01 mm) at 1.5 cm from the base of the shaft. To get a more exact measure of dry mass, we dried the feathers for 24 h at 40 °C in a desiccator to evaporate the absorbed moisture, after which the feathers were weighed on an electronic balance with an accuracy of 10^{-4} g. The repeatability of all feather-quality traits estimated by remeasuring 14 feathers was significantly high ($r > 0.8$, $P < 0.0001$). To increase the accuracy of the data, we used the mean values of the feathers from the left and right wings.

Immunization protocol and assessment of humoral immunity

Humoral immune system activation against SRBC was measured using a standard haemagglutination test (e.g., Eraud et al. 2005). We assessed the primary humoral immune response of house sparrows following injection with 100 µL of 20% fresh SRBC suspension into the pectoralis muscles (Ots et al. 2001). The dose calculated for unit of body mass (71 mL $\times$ concentration of SRBC/body mass) falls within the range used in avian studies, is generally higher than the dose used in ecological immunology studies, and falls well below the maximum concentration used (P.L. Pap, C.I. Vágási, and G.Á. Czirják, unpublished data). Birds were injected when the start of their primary molt was first observed. Therefore, the effects of activating humoral immunity on the development of the next-to-be-molted primaries (P2) and their quality can be studied. At the same measuring session when SRBC or PBS was administered, 75 µL of blood was drawn from the brachial vein to determine the pre-exposure levels of antibodies. Seven days later (see Roulin et al. 2000), during the next measuring session, a second blood sample was collected to determine the post-immunization primary antibody titre in plasma. Thirty-five

days after the first injection, an identical second injection was given and 7 days later a blood sample was taken to test for the presence of antibodies of the secondary response. During the period of the second immunization, most of the birds were shortly before dropping or just started growing the P7, which permitted us to study the effect of immune activation on molting and quality of this primary. Following blood collection in heparinized capillaries, the plasma was separated by centrifugation at 10 000 rev/min (16 060g) for 10 min and preserved at –20 °C until further analysis. Antibody titres were measured using a base-2 serial dilution haemagglutination test conducted with 15 μL of heat inactivated plasma (30 min on 56 °C) on U-shaped 96-well microtitre plates. Samples were serially diluted starting with 15 μL of PBS and to each well 15 μL of a 1% suspension of SRBC in PBS was added. Plates were incubated at 37 °C for 1 h. Titres are given as the log$_2$ of the reciprocal of the highest dilution of plasma showing positive haemagglutination.

We quantified the total immunoglobulins concentration, part of the constitutive humoral immune system, in the blood samples collected before and 1 week after the two injections. The concentration of immunoglobulins in plasma was quantified using a spectrophotometrical method. Concentrations as low as 24 mg/L of heavy metal salts precipitate the immunoglobulins, since the electric charge and colloidal stability of gamma globulins are lower than those of serum albumins at pH 7.4. We mixed a volume of 3.3 μL of plasma with 197 μL of a 0.024% barbital buffer zinc sulphate solution and allowed immunoglobulins to precipitate for 30 min at room temperature (22–23 °C). Immunoglobulin concentration expressed in optical density units was read spectrophotometrically (λ = 475 nm, d = 0.5 cm; multichannel spectrophotometer SUMAL PE2; Karl Zeiss, Jena, Germany) (Khokhlova et al. 2004). The repeatability of immunoglobulins measurements was highly significant (r = 0.82, n = 14, P < 0.001).

Statistical analyses

The effect of nutritional and immunological manipulations on body mass and the growth rate of individual feathers was analyzed with general linear models using two-way repeated-measures ANOVAs, where body mass and growth rate were introduced as dependent variables. Primary molting increases in a nonlinear S-shaped function, which is very similar with the growth pattern of body mass of the developing birds. To describe the molting pattern, we fitted to each bird separately a logistic growth curve (Ricklefs 1973), which is a model used to study avian growth. The logistic growth curve has the form $y = a/\{1 + \mathrm{e}^{[-K(t - I)]}\}$, where y denotes the molting score of a bird at time t, a is the final score or asymptote (which has a fixed value of 45 in our study), K is the growth constant, I is the inflection point on the time axis in which molting changes from accelerating to decelerating, and e is the base of natural logarithm. Following the calculation of molting parameters, we tested the effect of nutritional and immunological treatments on molting speed (K) and inflection point (I) using a two-way ANOVA. The molting speed can be characterized by the number of simultaneously growing feathers and the growth rate of individual feathers. Because for most of the measuring sessions the number of growing feathers did not follow a normal

distribution and had nonhomogeneous variance, we calculated the mean number of growing feathers at each measuring session obtained during the whole molting period, which was then used in the following analyses. The effect of nutritional manipulation and immunization on the mean number of growing feathers was analyzed by two-way ANOVAs. The daily growth rate of individual feathers was calculated as the difference between the length of the unbounded distal part of feathers measured at two consecutive sessions (1 week apart) divided by the number of days elapsed between the two measures. Since in 25 of 43 birds the unbounded distal part of at least 1 of the 9 primary feathers could not be measured in one of the two consecutive measure sessions because the feather was unbounded (score 1) or reached the final length (score 5), we had a reduced sample size in measuring the effect of manipulation on the growth rate of primary feathers. The effect of nutritional manipulation on the probability of producing antibody titre against SRBC was tested with a generalized linear (binomial) model using a logit link function, while the difference in immune response of immunized birds between nutritional treatments was tested with a two-tailed t test. Values reported in the text are means ± SD unless otherwise stated.

Molting house sparrows under the same nutritional conditions but located in different aviaries did not differ significantly in the starting date of molting (Mann–Whitney U test; low-quality diet: z = 1.6, n_1 = 14, n_2 = 14, P = 0.12; high-quality diet: z = 0.6, n_1 = 14, n_2 = 14, P = 0.57), molting process (low-quality diet: $F_{[1,13]}$ = 0.9, P = 0.37; high-quality diet: $F_{[1,21]}$ = 0.4, P = 0.52), change in body mass (low-quality diet: $F_{[1,13]}$ = 2.8, P = 0.12; high-quality diet: $F_{[1,21]}$ = 0.01, P = 0.90), and the quality of feathers grown (P > 0.5 in all analyses). None of the interactions between variables and experimental groups were significant, except the change in body mass between birds fed the high-quality diet in the two aviaries during molting ($F_{[14,294]}$ = 5.6, P < 0.0001). However, the difference in change in body mass between birds fed the high-quality diet molting in different aviaries was minor compared with the change in body mass between low- and high-quality groups (see below), thus pooling the data of house sparrows from aviaries with high-quality diet had an insignificant effect on the results. We found no significant difference between aviaries of the same nutritional groups in humoral immunity and survival of birds (P > 0.05 in all cases). Therefore, in the subsequent analyses, we pooled the data of birds from the same experimental group that were kept in separate aviaries.

Results

The relationship between diet quality, humoral immunity, condition, and survival

Forty-three out of 56 house sparrows (77%) survived until the end of the study. Survival rate differed between nutrition groups (low-quality diet: 61% survival, n = 28; high-quality diet: 96% survival, n = 28; generalized (binomial) linear model with a logit link function, $\chi^2_{[1]}$ = 11.6, P = 0.001), but not among immunization treatments ($\chi^2_{[1]}$ = 0.6, P = 0.46); the interaction between nutrition type and immunization was not significant ($\chi^2_{[1]}$ = 1.1, P = 0.29).

The body mass of house sparrows in the low-quality

Table 1. The effect of nutritional and immunological manipulations on body mass and individual feather growth rate of house sparrows (*Passer domesticus*).

Source of variation	df	Sums of squares	F	P
Body mass				
Nutritional treatment	1	215.7	6.3	0.02
Immunological treatment	1	0.7	0.02	0.89
Subject within groups	38	1306.9	—	—
Repeated measures	14	374.0	60.3	<0.0001
Nutritional treatment × immunological treatment	1	20.0	0.6	0.45
Nutritional treatment × repeated measures	14	45.4	7.3	<0.0001
Immunological treatment × repeated measures	14	1.6	0.3	1.00
Repeated measures × subjects within groups	532	235.8	—	—
Growing rate of individual feathers				
Nutritional treatment	1	0.1	0.3	0.57
Immunological treatment	1	0.4	1.4	0.25
Subject within groups	14	4.2	—	—
Repeated measures	8	8.4	6.6	<0.0001
Nutritional treatment × immunological treatment	1	0.4	1.4	0.26
Nutritional treatment × repeated measures	8	1.2	1.0	0.46
Immunological treatment × repeated measures	8	1.3	1.1	0.41
Repeated measures × subjects within groups	112	17.8	—	—

Note: None of the nutritional treatment × immunological treatment × repeated-measures interaction is significant.

group was significantly lower than in the high-quality group (Table 1). None of the preinjected house sparrows had detectable SRBC antibodies, and following injection with PBS of control birds, only 1 out of 28 birds produced a reaction against SRBC. In contrast, SRBC treatment significantly elevated antibody titre compared with controls in both nutritional groups, as 93% of birds (25 of 27) produced haemagglutination against SRBC ($\chi^2_{[1]} = 56.8$, $P < 0.0001$; nutritional treatment × immune response: $\chi^2_{[1]} = 0.0$, $P = 1.00$). Nutritional manipulation had no significant effect on the primary antibody response in birds immunized with SRBCs ($F_{[1]} = 0.4$, $P = 0.56$), and including the two "negative" SRBC-treated birds (individuals that produced no antibody titre against the antigen), the mean antibody titre between low- and high-quality groups was similar (4.2 ± 2.4 vs. 4.6 ± 1.2; $t = 0.6$, $n_1 = 14$, $n_2 = 13$, $P = 0.57$). Secondary injection with SRBC produced detectable antibody titre in all but 1 bird (24/25), and the magnitude of the immune response was similar between primary and secondary immune responses (paired sample t test; low-quality group: $t = 0.66$, $n = 11$, $P = 0.53$; high-quality group: $t = 0.59$, $n = 13$, $P = 0.57$). Again, the nutritional treatment had no effect on the probability of producing antibody titre ($\chi^2_{[1]} = 1.4$, $P = 0.25$) or on the mean antibody titre (low-quality vs. high-quality groups: 4.6 ± 2.1 vs. 4.2 ± 1.9; $t = 0.5$, $n_1 = 12$, $n_2 = 13$, $P = 0.59$). The correlation between primary and secondary antibody titres of SRBC-injected birds was significant ($r = 0.44$, $n = 24$, $P = 0.03$). Preimmunization immunoglobulin concentrations were not significantly affected by nutritional condition ($t = 1.7$, $n_1 = 26$, $n_2 = 25$, $P = 0.09$), and postimmunization immunoglobulin concentrations did not differ between nutritional groups ($F_{[1,44]} = 0.9$, $P = 0.36$). Immunoglobulin concentrations at the time of secondary SRBC-injection was similar between nutritional groups ($t = 0.7$, $n_1 = 23$, $n_2 = 25$, $P = 0.52$); however,

after 1 week following the immune challenge, their concentration dropped significantly in the low-quality groups compared with the high-quality groups ($F_{[1,48]} = 6.4$, $P = 0.02$).

Immunization with SRBC produced no significant effect on body mass (Table 1). Postimmunization immunoglobulin concentrations did not differ between SRBC and PBS-injection groups ($F_{[1,44]} = 0.8$, $P = 0.37$). SRBC treatment did not affect immunoglobulin concentrations following secondary injection ($F_{[1,48]} = 0.02$, $P = 0.88$). In none of the cases, the interactions between experimental groups were significant.

Effect of nutritional manipulation and immune activation on molting and feather quality

The start of molting in house sparrows held under low- and high-quality nutritional conditions did not differ significantly ($z = 0.9$, $n_1 = 28$, $n_2 = 28$, $P = 0.39$; Fig. 1). However, the nutritional condition had a significant effect on the process of molting, as reflected in the significant increase in the K value and decrease in I value for birds feeding on high-quality food compared with birds feeding on low-quality food (K value: $F_{[1,37]} = 9.9$, $P < 0.01$; I value: $F_{[1,37]} = 8.6$, $P < 0.01$). Immunization with SRBC caused no significant change in molting parameters (K value: $F_{[1,37]} = 0.1$, $P = 0.74$; I value: $F_{[1,37]} = 0.8$, $P = 0.39$). The absence of the significant interaction between immunization and nutritional manipulation (K value: $F_{[1,37]} = 0.0$, $P = 0.92$; I value: $F_{[1,37]} = 0.0$, $P = 0.94$) indicated that the effect of nutritional manipulation was similar in groups injected with either SRBC or PBS. The duration of molting (calculated as the time elapsed between the first and the last measuring sessions, namely when a dropped innermost primary was observed first and when all primary feathers were fully grown) in birds fed a high-quality diet was 82.6 ± 9.9 days ($n = 25$) compared with 96.7 ± 10.6 days ($n = 16$) for birds

Fig. 1. Mean (SE) changes in molting score of aviary-kept house sparrows (*Passer domesticus*) held under low-quality (○) and high-quality (●) nutritional conditions during postnuptial molting between July and November 2006. The sample size for low- and high-quality groups are 17 and 25, respectively.
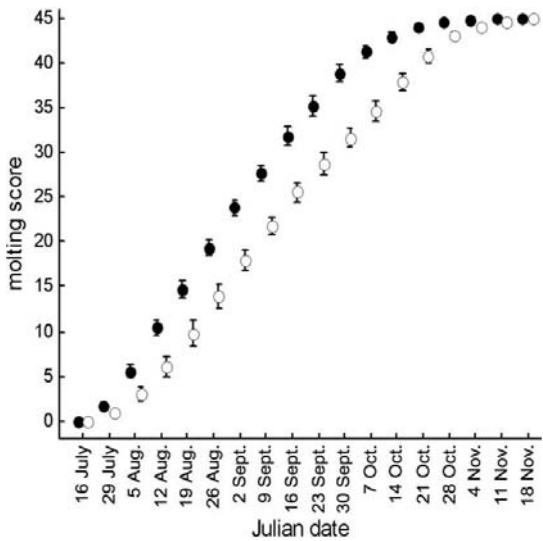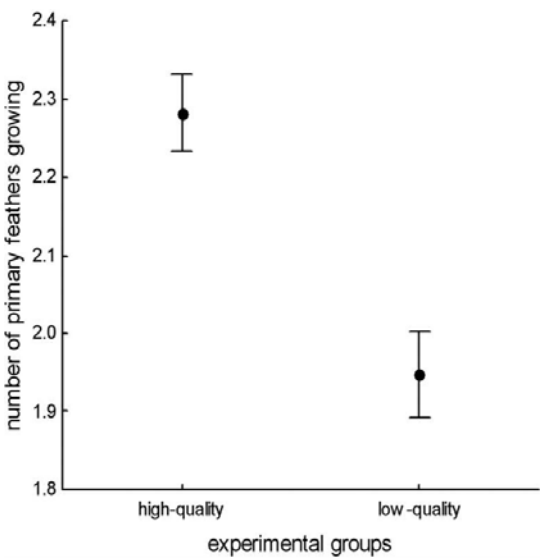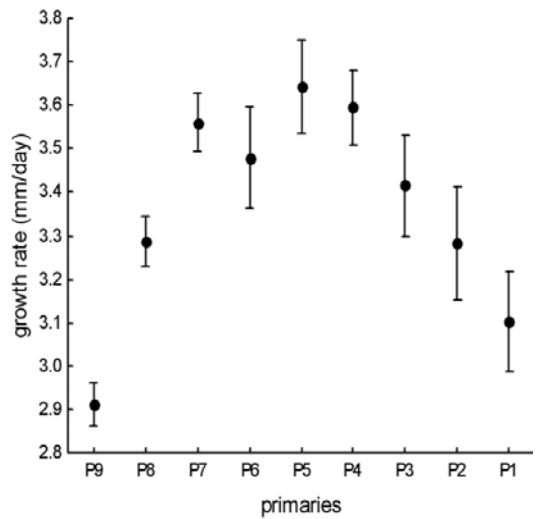

Fig. 2. Mean (SE) numbers of growing primary feathers of the left wing of aviary-kept house sparrows (*Passer domesticus*) molting under low- and high-quality nutritional conditions.

Fig. 3. Mean (SE) daily growth rates of primary feathers of the aviary-kept house sparrows (*Passer domesticus*) (*n* = 18).

namely birds feeding on low-quality diet grew less primaries simultaneously than did birds feeding on the high-quality diet ($F_{[1,37]}$ = 18.7, *P* = 0.0001; Fig. 2). In contrast, SRBC injections had no significant effect on the number of growing feathers ($F_{[1,37]}$ = 0.6, *P* = 0.44). The nonsignificant interaction between nutritional and immunization manipulations ($F_{[1,37]}$ = 0.1, *P* = 0.74) indicates that the effect of nutritional manipulation is similar in SRBC and saline-injected groups. There was no significant difference in the daily growth rate of individual feathers between nutritional and immunization groups (Table 1), which indicates that the difference between nutritional groups in molting was due to the increased number of growing primaries in the high-quality group compared with the low-quality group. The highly significant repeatability of growth rate of primary feathers shows the similarity of the growing rate of primaries within individual birds; however, the daily increase in length varied between feathers in different positions (Table 1, Fig. 3). The growth rate of primaries increased from the inner to the middle positions (Fisher's LSD post hoc test of the repeated-measures ANOVA; e.g., P1 vs. P5, *P* < 0.0001), and then decreased from the middle to the distal positions (e.g., P5 vs. P9, *P* < 0.0001).

Feather quality, measured through the feather length, rachis width, and feather mass differed significantly between birds feeding on low- and high-quality foods (Table 2). House sparrows feeding on high-quality food grew longer and heavier feathers and tended to have thicker rachis of proximal primaries compared with birds feeding on low-quality food, while none of the variables differed between immunized and control birds (*P* > 0.5 for all tests).

Discussion

Molting is recognized as one of the most energy-demanding activities of birds. The metabolic rate during molting increases by more than 100% of its premolting value (Lindström et al. 1993). In addition, the increase in metabolic

fed a low-quality diet ($F_{[1,37]}$ = 17.7, *P* = 0.0001). SRBC injections and the interaction between nutritional and immunization manipulations had no significant effect on the duration of molting (SRBC injections: $F_{[1,37]}$ = 1.3, *P* = 0.27; nutrition × SRBC injections: *F* = 0.1, *P* = 0.75).

Molting speed has two components: the number of simultaneously growing feathers and the growth rate of individual feather lengths. Nutritional condition had a significant effect on the mean number of growing feathers through molting,

Table 2. The quality of proximal (P2) and distal (P7) primary feathers grown by house sparrows (*Passer domesticus*) molting under low- and high-quality nutritional conditions.

	Group (mean ± SE)		*F*	*P*
	Low-quality	High-quality		
Feather length (mm)				
Primary 2	57.2±0.3 (24)	58.2±0.3 (25)	5.62	<0.05
Primary 7	67.5±0.4 (19)	68.6±0.3 (25)	5.01	<0.05
Rachis width (mm)				
Primary 2	0.83±0.01 (24)	0.85±0.01 (25)	3.34	0.07
Primary 7	0.99±0.01 (19)	1.00±0.01 (25)	0.23	0.64
Feather mass (1×10^{-4} g)				
Primary 2	97.3±1.2 (24)	101.6±1.2 (25)	6.01	<0.05
Primary 7	144.9±2.1 (18)	154.5±1.8 (25)	12.48	<0.01

Note: Sample sizes are in parentheses.

rate during molting is size-dependent (Lindström et al. 1993), i.e., the cost of molting is higher in species with reduced size. Therefore, it is reasonable to expect that molting can be energetically limited and as a result is negatively affected by the immune activation that consumes the same energy resources. However, contrary to expectations, our results indicated that molting and feather quality seem uncompromised by humoral immunity, as immune response to SRBC antigen had no effect on either feather growth and quality or on condition of the birds. Hence, our results do not confirm the previous findings regarding the trade-off between molting and immune response (Sanz et al. 2004; Martin 2005). Interestingly, a recent study on the annual variation of health status and immune function in the great tit (*Parus major* L., 1758) showed that immunoglobulin concentrations are highest during molting (P.L. Pap, C.I. Vágási, J. Tökölyi, G.Á. Czirják, and Z. Barta, unpublished data), questioning further the existence of a strong trade-off between humoral immune system and molting. However, we did not exclude the possibility that during critical stages of the annual cycle of the birds, such as at the molting–breeding overlap, the cost of mounting a humoral immune response may have a significant deleterious effect on fitness (Sanz et al. 2004).

Molting is a nutrition-demanding activity for birds because the feathers that are synthesized compose about 30% of the total protein mass of the birds (Murphy et al. 1988). Therefore, it is reasonable to expect that molting can be limited by nutrition. In line with this assumption, we found that house sparrows feeding on low-quality food molted slower because of the reduced number of simultaneously growing feathers and grew proximal and distal primaries of reduced quality compared with house sparrows feeding on high-quality food. The slower feather replacement of birds feeding on low-quality food resulted in a 14-day delay in the final molt (high-quality food vs. low-quality food: 82.6 vs. 96.7 days). Moreover, house sparrows feeding on low-quality food could not compensate for the nutrition deficiency by expanding the molting period as shown by the production of the low-quality feathers. These results confirm the findings of the few previous studies regarding the nutritional limitation of feather growth and quality (e.g., Murphy

et al. 1988; Murphy and King 1991). Our study also demonstrated that house sparrows could not fulfill their nutritional needs during molting with seeds alone (but for other species see Allen and Hume 2001), which is supported by the observation of house sparrows selectively feeding on insects during this period of the annual cycle (Cramp 1994).

Nutritional limitation had no effect on the individual growth rate of feathers. Contrary to a previous study involving European starlings (*Sturnus vulgaris* L., 1758) (Dawson 2003), we have shown that the growth rate of primaries varied relative to their position, namely the more proximal and distal feathers had a reduced daily growth rate compared with the primaries in the middle of the wing. In line with Dawson (2003), we found the growth rate of the outermost feather to be the lowest, which may be related to the increased mass of this primary compared with the proximals (Dawson 2005) and (or) the cost of feather replacement of secondaries that began to molt during the growth of the distal primaries.

Our experimental study showed that the humoral immunity, assessed using primary and secondary responses against SRBCs, was not affected by the food quality in molting house sparrows. Furthermore, the amount of anti-SRBC antibodies produced by house sparrows in the current study was comparable with the amount produced by other wild-living avian species (e.g., Lochmiller et al. 1993; Roulin et al. 2000; Ots et al. 2001; Hanssen et al. 2004; Eraud et al. 2005). In contrast, immunoglobulin concentrations decreased following the second immunization of birds feeding on low-quality food, which supports the nutritional limitation of constitutive humoral immunity. Following injection, the antigen-specific antibodies increase in the peripheral blood (e.g., Hanssen et al. 2004), and total immunoglobulin concentrations include both the constitutive and the acquired humoral immunities. However, the haemagglutination assay showed that the SRBC antigen-specific immune response was not affected by the nutritional manipulation, leading to the conclusion that the decreased immunoglobulin concentrations in the low-quality nutritional group compared with the high-quality nutritional group could be the result of suppression of nonspecific immunoglobulins. The absence of nutritional limitation on acquired humoral immunity and the reduction of immunoglobulin concentrations only following

prolonged malnutrition appear surprising, since both specific and nonspecific antibodies are nutrition-dependent, hence their production depends on the quality of food ingested. However, during nutritional limitations birds may use nutrition reserves deposited in muscles to supply the nutrition need for physiologically important functions, such as the immune system (Cherel et al. 1994), as is the case for migratory birds following long flights (Landys et al. 2005). In fact, birds feeding on low-quality food reduced significantly their body mass during molting. However, the duration of nutritional manipulation was probably long enough to deplete the nutritional reserves of the birds well before the termination of molting, which is supported also by the increased death rate of birds feeding on low-quality food compared with those feeding on high-quality food (see also Birkhead et al. 1999), as well as the reduced molting rate of birds feeding on low-quality food. Our results are in accordance with previous studies regarding the low energetical and nutritional costs of humoral immunity (Lochmiller et al. 1993; Gonzalez et al. 1999; Cichoń 2000; Ilmonen et al. 2002; Grindstaff et al. 2005; Poston et al. 2005), but contradicts the two recent studies that showed this branch of the immune system to be nutrition-limited (Gasparini et al. 2006; Smith et al. 2007). House sparrows on a low-quality diet mounted increased antibody responses (Buchanan et al. 2003), while sparrows on a protein-rich diet decreased antibody responses (Gonzalez et al. 1999). In addition, our study demonstrated that even during intensive protein use, such as during molting, humoral immune system is less limited by nutrition.

In conclusion, house sparrows feeding on low-quality food experienced prolonged molting and produced low-quality feathers, which may seriously reduce their future reproductive value because of increased cost of thermoregulation, higher abrasion rate of feathers, and reduced aerodynamical capacity of the flight feathers. Furthermore, we have shown that the food quality had no effect on the induced humoral immune response of the house sparrows during the energy- and nutrition-demanding periods of the molting process. Finally, the activation of humoral immunity had no effect on molting, which supports the absence of a trade-off between humoral immunity and molting in house sparrows. We propose further studies that would examine the role of energetically and nutritionally costly branches of the immune system (e.g., cellular immunity) in shaping the molting of birds.

Acknowledgements

References

Allen, L.R., and Hume, I.D. 2001. The maintenance nitrogen requirement of the zebra finch *Taeniopygia guttata*. Physiol. Biochem. Zool. **74**: 366–375. doi:10.1086/320431. PMID:11331508.

Alonso-Alvarez, C., and Tella, J.L. 2001. Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. Can. J. Zool. **79**: 101–105. doi:10.1139/cjz-79-1-101.

Anderson, T. 2006. Biology of the ubiquitous House Sparrow: from genes to populations. Oxford University Press, Oxford.

Birkhead, T.R., Fletcher, F., and Pellatt, E.J. 1999. Nestling diet, secondary sexual traits and fitness in the zebra finch. Proc. R. Soc. Lond. B Biol. Sci. **266**: 385–390. doi:10.1098/rspb.1999.0649.

Buchanan, K.L., Evans, M.R., and Goldsmith, A.R. 2003. Testosterone, dominance signalling and immunosuppression in the house sparrow, *Passer domesticus*. Behav. Ecol. Sociobiol. **55**: 50–59. doi:10.1007/s00265-003-0682-4.

Cherel, Y., Charrassin, J.B., and Challet, E. 1994. Energy and protein requirements for molt in the king penguin *Aptenodytes patagonicus*. Am. J. Physiol. **266**: R1182–R1188. PMID:8184961.

Cichoń, M. 2000. Costs of incubation and immunocompetence in the collared flycatcher. Oecologia (Berl.), **125**: 453–457. doi:10.1007/s004420000461.

Cramp, S. 1994. The birds of the Western Palearctic. Vol. 8. Oxford University Press, Oxford.

Dawson, A. 2003. A detailed analysis of primary feather moult in the Common Starling *Sturnus vulgaris* — new feather mass increases at a constant rate. Ibis, **145**: E69–E76. doi:10.1046/j.1474-919X.2003.00161.x.

Dawson, A. 2005. The scaling of primary flight feather length and mass in relation to wing shape, function and habitat. Ibis, **147**: 283–292. doi:10.1111/j.1474-919x.2005.00400.x.

Eraud, C., Duriez, O., Chastel, O., and Faivre, B. 2005. The energetic cost of humoral immunity in the Collared Dove, *Streptopelia decaocto*: is the magnitude sufficient to force energy-based trade-offs? Funct. Ecol. **19**: 110–118. doi:10.1111/j.0269-8463.2005.00934.x.

Gasparini, J.A., Roulin, V.A., Gill, S.A., and Boulinier, T. 2006. In kittiwakes food availability partially explains the seasonal decline in humoral immunocompetence. Funct. Ecol. **20**: 457–463. doi:10.1111/j.1365-2435.2006.01130.x.

Gonzalez, G., Sorci, G., Møller, A.P., Ninni, P., Haussy, C., and de Lope, F. 1999. Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). J. Anim. Ecol. **68**: 1225–1234. doi:10.1046/j.1365-2656.1999.00364.x.

Grindstaff, J.L., Demas, G.E., and Ketterson, E.D. 2005. Diet quality affects egg size and number but does not reduce maternal antibody transmission in Japanese quail *Coturnix japonica*. J. Anim. Ecol. **74**: 1051–1058. doi:10.1111/j.1365-2656.2005.01002.x.

Hangalapura, B.N., Nieuwland, M.G.B., de Vries Reilingh, G., Buyse, J., van den Brand, H., Kemp, B., and Parmentier, H.K. 2005. Severe feed restriction enhances innate immunity but suppresses cellular immunity in chicken lines divergently selected for antibody responses. Poult. Sci. **84**: 1520–1529. PMID:16335119.

Hanssen, S.A., Hasselquist, D., Folstad, I., and Erikstad, K.E. 2004. Costs of immunity: immune responsiveness reduces survival in a vertebrate. Proc. R. Soc. Lond. B Biol. Sci. **271**: 925–930. doi:10.1098/rspb.2004.2678.

Ilmonen, P., Taarna, T., and Hasselquist, D. 2000. Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. Proc. R. Soc. Lond. B Biol. Sci. **267**: 665–670. doi:10.1098/rspb.2000.1053.

Ilmonen, P., Taarna, T., and Hasselquist, D. 2002. Are incubation costs in female pied flycatchers expressed in humoral immune responsiveness or breeding success? Oecologia (Berl.), **130**: 199–204.

Jenni, L., and Winkler, R. 1994. Moult and aging of European passerines. Academic Press, London.

Khokhlova, I.S., Spinu, M., Krasnov, B.R., and Degen, A.A. 2004. Immune response to fleas in a wild desert rodent: effect of parasite species, parasite burden, sex of host and host parasitological experience. J. Exp. Biol. **207**: 2725–2733. doi:10. 1242/jeb.01090. PMID:15235001.

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 (Berl.), **104**: 424–432. doi:10.1007/BF00341339.

Klasing, K.C. 1998. Nutritional modulation of resistance to infectious diseases. Poult. Sci. **77**: 1119–1125. PMID:9706075.

Kuenzel, W.J. 2003. Neurobiology of molt in avian species. Poult. Sci. **82**: 981–991. PMID:12817454.

Landys, M.M., Piersma, T., Guglielmo, C.G., Jukema, J., Ramenofsky, M., and Wingfield, J.C. 2005. Metabolic profile of long-distance migratory flight and stopover in a shorebird. Proc. R. Soc. Lond. B Biol. Sci. **272**: 295–302. doi:10.1098/rspb.2004. 2952.

Lindström, A., Visser, G.H., and Daan, S. 1993. The energetic cost of feather synthesis is proportional to basal metabolic rate. Physiol. Zool. **66**: 490–510.

Lochmiller, R.L., and Deerenberg, C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos, **88**: 87–98. doi:10.1034/j.1600-0706.2000.880110.x.

Lochmiller, R.L., Vestey, M.R., and Boren, J.C. 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. Auk, **110**: 503–510.

Martin, L.B., II. 2005. Trade-offs between molt and immune activity in two populations of house sparrows (*Passer domesticus*). Can. J. Zool. **83**: 780–787. doi:10.1139/z05-062.

Martin, L.B., II, Scheuerlein, A., and Wikelski, M. 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? Proc. R. Soc. Lond. B Biol. Sci. **270**: 153–158. doi:10.1098/rspb.2002.2185.

Martin, L.B., II, Han, P., Kwong, J., and Hau, M. 2006. Cutaneous immune activity varies with physiological state in female house sparrows (*Passer domesticus*). Physiol. Biochem. Zool. **79**: 775–783. doi:10.1086/504608. PMID:16826503.

Mauck, R.A., Matson, K.D., Philipsborn, J., and Ricklefs, R.E. 2005. Increase in the constitutive innate humoral immune system in Leach's Storm-Petrel (*Oceanodroma leucorhoa*) chicks is negatively correlated with growth rate. Funct. Ecol. **19**: 1001–1007. doi:10.1111/j.1365-2435.2005.01060.x.

McGraw, J., and Hill, G.E. 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc. R. Soc. Lond. B Biol. Sci. **267**: 1525–1531. doi:10.1098/rspb.2000.1174.

Murphy, M.E., and King, J.R. 1982. Amino acid composition of the plumage of the white-crowned sparrow. Condor, **84**: 435–438. doi:10.2307/1367449.

Murphy, M.E., and King, J.R. 1984. Sulfur amino acid nutrition during molt in the white-crowned sparrow. 1. Does dietary sulfur amino acid concentration affect the energetics of molt as assayed by metabolized energy? Condor, **86**: 314–323. doi:10. 2307/1367002.

Murphy, M.E., and King, J.R. 1986. Composition and quantity of feather sheaths produced by white-crowned sparrows during the postnuptial molt. Auk, **103**: 822–825.

Murphy, M.E., and King, J.R. 1991. Protein intake and the dynamics of the postnuptial molt in White-crowned Sparrows, *Zonotrichia leucophrys gambelii*. Can. J. Zool. **69**: 2225–2229. doi:10.1139/z91-310.

Murphy, M.E., King, J.R., and Lu, J. 1988. Malnutrition during the postnuptial molt of White-crowned Sparrows: feather growth and quality. Can. J. Zool. **66**: 1403–1413. doi:10.1139/z88-206.

Newton, I. 1966. The moult of bullfinch *Pyrrhula pyrrhula*. Ibis, **108**: 41–67. doi:10.1111/j.1474-919X.1966.tb07251.x.

Ots, I., Kerimov, A.B., Ivankina, E.V., Ilyina, T.A., and Hõrak, P. 2001. Immune challenge affects basal metabolic activity in wintering great tits. Proc. R. Soc. Lond. B Biol. Sci. **278**: 1175–1181.

Pap, P.L. 2002. Breeding time and sex-specific health status in the barn swallow (*Hirundo rustica*). Can. J. Zool. **80**: 2090–2099. doi:10.1139/z02-200.

Poston, J.P., Hasselquist, D., Stewart, I.R.K., and Westneat, D.F. 2005. Dietary amino acids influence plumage traits and immune responses of male house sparrows, *Passer domesticus*, but not as expected. Anim. Behav. **70**: 1171–1181. doi:10.1016/j.anbehav. 2005.02.015.

Ricklefs, R.E. 1973. Patterns of growth in birds. II. Growth rate and mode of development. Ibis, **115**: 177–201.

Roulin, A., Jungi, T.W., Pfister, H., and Dijkstra, C. 2000. Female barn owls (*Tyto alba*) advertise good genes. Proc. R. Soc. Lond. B Biol. Sci. **267**: 937–941. doi:10.1098/rspb.2000.1093.

Råberg, L., Stjerman, M., and Hasselquist, D. 2003. Immune responsiveness in adult blue tits: heritability and effects of nutritional status during ontogeny. Oecologia (Berl.), **136**: 360–364. doi:10.1007/s00442-003-1287-3.

Saino, N., Calza, S., and Møller, A.P. 1997. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. J. Anim. Ecol. **66**: 827–836. doi:10.2307/5998.

Sanz, J.J., Moreno, J., Merino, S., and Tomás, G. 2004. A trade-off between two resource-demanding functions: post-nuptial moult and immunity during reproduction in male pied flycatchers. J. Anim. Ecol. **73**: 441–447. doi:10.1111/j.0021-8790.2004.00815.x.

Smith, H.G., Råberg, L., Ohlsson, T., Granbom, M., and Hasselquist, D. 2007. Carotenoid and protein supplementation have differential effects on pheasant ornamentation and immunity. J. Evol. Biol. **20**: 310–319. doi:10.1111/j.1420-9101.2006.01203.x. PMID:17210024.

Svensson, E., Råberg, L., Koch, C., and Hasselquist, D. 1998. Energetic stress, immunosuppression and the costs of an antibody response. Funct. Ecol. **12**: 912–919. doi:10.1046/j.1365-2435. 1998.00271.x.