

Experimental test of a trade-off between moult and immune response in house sparrows *Passer domesticus*

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Abstract

A trade-off between immune system and moulting is predicted in birds, given that both functions compete for resources. However, it is unclear whether such a trade-off exists during post-breeding moult. This study tests such a trade-off in the house sparrow (*Passer domesticus*). Males injected with an antigen (lipopolysaccharide) significantly moulted slower than sham-injected males. Moreover, males whose seventh primaries were plucked to simulate moult showed smaller immune response to phytohaemagglutinin than control males, in which seventh primaries were clipped. A trade-off between moult speed and body mass was also found. The results show a clear trade-off between moult and immune response in the house sparrow: immune response negatively affected moult and moult negatively affected immune response. These findings suggest that only individuals in good condition may have an efficient moult and simultaneously respond effectively in terms of immunity to pathogens, which could explain how plumage traits honestly indicate parasite resistance in birds.

Introduction

Life-history theory suggests that as resources are typically limited, the investment in a given fitness-related trait implies a decrease in the resources available for investing in other life-history traits (Sibly & Calow, 1986; De Jong & Van Noordwijk, 1992; Zera & Harshman, 2001). Therefore, to maximize their fitness, organisms should optimize the investment in the different fitness-related traits that compete for the same resources, making a number of trade-offs (Stearns, 1992; Roff, 2002). The study of trade-offs between fitness-related traits has focused primarily on reproduction (e.g. Stearns, 1992). Nevertheless, fitness is determined also by their capacity to survive. The strategy to invest optimally in different survival-related traits may be important for an organism's fitness, because it affects its future reproduction. However, the trade-offs between traits related to survival

have been studied relatively less than trade-offs between traits related to reproduction (some examples in Martín & López, 1999; López *et al.*, 2005; Cooper, 2009).

An important function for organisms' survival is investment in the immune system, which fights parasites and pathogens (Wakelin & Apanius, 1997; Davison *et al.*, 2008). The immune system is probably energetically expensive (reviews in Lochmiller & Deerenberg, 2000; Demas, 2004) and requires specific resources such as proteins and particular amino acids (Lochmiller *et al.*, 1993; Hoi-Leitner *et al.*, 2001; De Neve *et al.*, 2007). Its function, moreover, may increase the risk of physiological damage (auto-immune response, oxidative stress; Råberg *et al.*, 1998; Costantini & Møller, 2009; Sorci & Faivre, 2009). For these reasons, the immune response to an antigen should be traded against other life-history components, such as reproduction or self-maintenance (Sheldon & Verhulst, 1996; Norris & Evans, 2000; Zuk & Stoehr, 2002; Schmid-Hempel, 2003; Ardia & Schat, 2008). For example, the stimulation of the immune system reduces growth rate (Soler *et al.*, 2003; Brommer, 2004; Mauck *et al.*, 2005), breeding success (Råberg *et al.*, 2000; Bonneaud *et al.*, 2003; French *et al.*, 2007) and

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survival prospects (Moret & Schmid-Hempel, 2000; Hanssen *et al.*, 2004; Eraud *et al.*, 2009).

In birds, another important process for survival is moulting (Jenni & Winkler, 1994). Moulting renews worn or broken feathers, and new plumage enhances flight efficiency and thermoregulation (Nilsson & Svensson, 1996; Williams & Swaddle, 2003; Pap *et al.*, 2007). Thermoregulation may be especially important for winter survival (in temperate climates) and flight efficiency for migration (in migratory birds). In fact, in an experiment, dark-eyed junco (*Junco hyemalis*) males that did not moult had a lower probability to survive the winter (Nolan *et al.*, 1992). However, moult is costly in terms of production of new feathers and thermoregulation (Klaassen, 1995; Moreno *et al.*, 2001; Vezina *et al.*, 2009). Like the immune system, moulting also requires a large input of proteins (Murphy & King, 1992; Cheral *et al.*, 1994; Pap *et al.*, 2008). Other costs include increased predation risk, presumably because flight is impaired during the moult (Slagsvold & Dale, 1996; Swaddle & Witter, 1997; Swaddle *et al.*, 1999). Evidence that moulting involves a trade-off with other fitness-related traits comes from studies showing that moult reduces breeding success (Slagsvold & Lifjeld, 1990; Svensson & Nilsson, 1997; Hemborg & Lundberg, 1998) and a stronger breeding effort negatively affects moult (Lessells, 1986; Siikamäki *et al.*, 1994; Norris *et al.*, 2004).

According to this information, a trade-off between immune response and moult is expected in birds, considering that both processes are physiologically costly and compete for available proteins. Given the importance of the two processes in terms of survival, such a trade-off should have major implications for future reproduction. However, evidence in favour of that trade-off is inconclusive. During the breeding season, the stimulation of the immune system in pied flycatcher (*Ficedula hypoleuca*) females inhibits the growth of tail feathers experimentally plucked (Ilmonen *et al.*, 2000). Similarly, the stimulation of immune system of males delays the onset of the moult (Sanz *et al.*, 2004). These studies, nonetheless, were performed during the breeding season, when moulting is not the norm (Hemborg *et al.*, 2001) and birds face a heavy demand of resources (Moreno *et al.*, 2001). Therefore, it is unclear whether such a trade-off between moult and immune system remains outside the breeding period, when moulting usually occurs.

The trade-off between moult and immune system has been extensively studied in domestic poultry, mainly in chickens (*Gallus gallus domesticus*). In these studies, some researchers found that the heterophil-to-lymphocyte ratio (H:L) increases during induced moult (Holt, 1992; Alodan & Mashaly, 1999; Davis *et al.*, 2000). This might suggest a trade-off between moult and immune system; however, in these studies, moult was induced by processes such as food restriction (among others; see Alodan & Mashaly, 1999), and thus immunosuppression may be a consequence of the method used to force the

moult, not of moult *per se*. In fact, an increased H:L ratio may signal elevated stress (Gross & Siegel, 1983). Supporting this statement, the levels of corticosterone, another stress indicator (Buchanan, 2000), also increased during these experiments (Davis *et al.*, 2000), despite the fact that corticosterone levels are usually lower during moult, given that it inhibits protein synthesis and thus is harmful to the moult (Romero *et al.*, 2005). These studies also found a diminished number of leucocytes (Alodan & Mashaly, 1999) and a weaker cell response to the antigen dinitrofluorobenzene (Holt, 1992), but it is unknown whether such effects are a consequence of the method used to induce moult. On the other hand, these researchers failed to find a trade-off between moult and the response to other antigens [sheep red blood cells (SRBC) and the bacteria *Brucella abortus*; Holt, 1992; Alodan & Mashaly, 1999]. Therefore, studies with poultry are inconclusive.

Work with wild species has focused almost exclusively the house sparrow (*Passer domesticus*), but the conclusions are unclear, again. Moulting house sparrows have an increased number of basophils and monocytes (Nava *et al.*, 2001), and their response to phytohaemagglutinin (PHA) is higher than at other times of their annual cycle (Greenman *et al.*, 2005). However, the response to PHA is the smallest immediately after the moult, suggesting that moulting may affect the immune response in a delayed way (Martin *et al.*, 2006a). Available studies experimentally stimulating the immune response in moulting birds are limited to the house sparrow, but with contradictory results. Martin (2005) inoculated sparrows from two populations (North American and Central American) with PHA, and these sparrows moulted more slowly than did control. Moreover, the level of response to PHA was negatively correlated with moulting speed. Both results imply a trade-off between moult and the immune response. However, in a Romanian population, Pap *et al.* (2008) failed to find an effect of the antigen SRBC on moult parameters; thus, this study did not support the existence of a trade-off between moult and immune system.

All this information appears to indicate that a trade-off between moult and immune system exists during the breeding season, but it remains unclear whether such a trade-off persists outside the breeding season. The goal of this study is to experimentally examine the trade-off between moult and immune system in a Spanish population of house sparrow. Such a trade-off is expected, given that, in the house sparrow, it has been demonstrated that both the moult and immune response are energetically expensive (Blackmore, 1969; Lindström *et al.*, 1993; Martin *et al.*, 2003) and require proteins (Gonzalez *et al.*, 1999; Poston *et al.*, 2005; Pap *et al.*, 2008). In an initial experiment [similar to those performed by Martin (2005) and Pap *et al.* (2008)], I stimulated the immune system of a group of male house sparrows with an antigen (lipopolysaccharide [LPS] from

the cell wall of *Escherichia coli*) during the moult. Additionally, I performed another experiment outside the moulting period, in which I stimulated moulting by plucking two primaries, clipped in the control group, and examined the effect of induced moult on the immune response to PHA.

Materials and methods

Study animals

The study was performed with a set of male house sparrows in an outdoor aviary located in Moraleda de Zafayona (3°57'W, 37°11'N; SE Spain). Sparrows were captured in December 2007 on a farm of the nearby town of El Padul (3°38'W, 37°01'N). Food supplied to sparrows was composed of a commercial mixture of seeds for canaries, wheat and sunflower seeds. Standard vitamins for canaries were included in the seed mixture. Birds were individually marked with colour rings. Standard biometric measurements such as body mass (with a digital balance, accuracy 0.1 g) and tarsus length (with a digital calliper, accuracy 0.01 mm) were taken. House sparrows moult between mid-July and end-October (Ginn & Melville, 1983).

Experiment 1: Effect of immunity on moult

In this experiment, I stimulated the immune system of house sparrows during the moult and examined the effect of this stimulation on the moulting rate. After the end of breeding, on August 27, I scored the moult of 37 male house sparrows, using a modification of the method of Ginn & Melville (1983). Each primary (the outermost excluded) of the left wing was scored according to its status: 0 – when the old feather remained, 1 – when the old feather was missed or the new feather was in pin, 2 – from the onset of feather emergence to half of its final length, 3 – when the new feather reached more than half of its final size and 4 – when the new feather was completely developed. The moult score was the sum of scores for all the primaries, ranging from 0 (moult of primaries still not started) to 36 (moult of primaries already ended). Therefore, this score is an index of the state of the moult. Note that Ginn & Melville (1983) used six levels (0–5), whereas I used only five (0–4), for simplicity. A week after the first moult evaluation (3 September), I again scored the moult of the house sparrows. The difference between the second score and the first score was calculated to estimate the speed of the moult. The higher the difference in the moult score, the faster the moult. In this way, I estimated the moult speed during a control week.

On 3 September, I subcutaneously injected 18 male house sparrows (randomly selected) with 0.1 mg of a lipopolysaccharide (LPS) from the cell wall of *Escherichia coli* (serotype 055:B5, L-2880; Sigma Aldrich, St. Louis,

MO, USA), diluted in 0.01 mL of isotonic phosphate buffer. This substance acts as an antigen mimicking an infection, provoking an immune reaction, and may sequester resources for other functions in the house sparrow (Bonneaud *et al.*, 2003). For this reason, I expected individuals injected with this antigen to have lower moulting efficiency. Although the concentration used here was higher than that used by Bonneaud *et al.* (2003), the immune response caused should be smaller than that provoked by a real infection. Another 18 males were injected with 0.01 mL of isotonic phosphate buffer saline (PBS), as control. Preliminary experiments on 12 nonmoulting individuals showed no effect of the treatment on survival, but six birds died the next day of the experiment (probably from the stress of manipulation, although an effect of LPS was also possible), which reduced the sample size to 14 LPS- and 16 PBS-injected males. This reduction did not change the composition of the samples with respect to the measures of moult score and biometry (Tables S1–S3).

On 10 September, I again evaluated the moult and estimated the moult speed as the difference between the moult score on 10 September and that on 3 September. This second week was the experimental week. According to the hypothesis of a trade-off between moult and immune response, I predicted that the moult speed would be slower in the LPS group than in the PBS group during the experimental week (but not during the control week), and the moult speed of the LPS group would be slower during the experimental week than during the control week.

Experiment 2: Effect of moult on immunity

This experiment was performed after the end of the moult. On 2 December, I randomly assigned 27 male house sparrows from other stock to two treatments. One group of 14 males was subjected to the moulting treatment, which was designed to simulate the moult in sparrows. For this, I plucked the seventh primary of both wings, to provoke the regrowth of new feathers. It should be noted that this treatment is conservative, as during the moult, several primaries are shed simultaneously (Ginn & Melville, 1983). A second group of 13 sparrows was submitted to the control treatment, which was designed to simulate feather loss (as provoked in the moulting treatment), but avoiding the growth of new feathers. The moult affects not only the physiology of the bird but also its flight because of the lack of feathers (Swaddle & Witter, 1997; Hedenström & Sunada, 1999; Swaddle *et al.*, 1999), and, in turn, it may affect other traits such as body mass (Lind & Jakobsson, 2001). In this experiment, I was not interested in the effect of flight impairment. Next, I clipped the seventh primary of both wings in the control birds, leaving only the basis of the rachis protruding approximately 1 cm from the skin. Thus, the flight was

similarly impaired in the birds of the two groups. The differences found between groups, thus, can be explained only as a consequence of feather growth and not as a consequence of impaired flight.

Three days afterwards (5 December), to allow birds to become accustomed to the treatment, I injected 0.1 mg of phytohaemagglutinin (PHA-P, L-8754; Sigma Aldrich) diluted in 0.02 mL of isotonic phosphate buffer in the patagium of the left wing (following Smits *et al.*, 1999). PHA is an innocuous protein that provokes an immune response in birds mediated by T cells (Kennedy & Nager, 2006), although other components of the immune system are also involved in the response (Martin *et al.*, 2006b; Tella *et al.*, 2008). Previously, I measured (three times) the patagium thickness with a pressure-sensitive micrometer (Mitutoyo, Kawasaki, Japan; accuracy 0.01 mm). The repeatability of measuring the patagium thickness was 0.98 ($n = 8$; Lessells & Boag, 1987). Then, I measured the patagium again 24 h later. However, because no bird was growing new feathers at that time, I measured patagium thickness again 1 week later (13 December). At that time, most of the birds in the moulting group were already regrowing the seventh primary, and birds still showed a response to PHA (see Results). The immune response was estimated as the patagium thickness in the last measure minus the initial thickness. The degree of swelling provoked by PHA is considered a measurement of the strength of the immune response (Kennedy & Nager, 2006). The prediction for this experiment is that if moulting and the immune response undergo a trade-off, the immune response should be weaker in the moulting group than in the control group.

Statistical analyses

The effects of treatments on the dependent variables were examined by ANOVAs with type III sums-of-squares. In some analyses, I tested the effect of covariates such as 'change in body mass' on the dependent variables by means of ANCOVAs (Goldberg & Scheiner, 2001). Then, the effect of the treatment on the dependent variables was controlled for the effect of these covariates, at the same time as the effect of the covariates was measured. When analysing temporal differences in some trait repeatedly measured in sparrows (e.g. moult speed), I used a repeated measures ANOVA (RM-ANOVA; Von Ende, 2001). This test examines the change in the dependent variable with the repeated measures and the effect of the treatment or covariates on that change (interaction with the repeated measures). In each model performed in this study, I checked for homoscedasticity between the groups by using Levene's test. The immune response 1 week after the injection of PHA in the experiment 2 was transformed by the squared root to be homoscedastic. The residuals of every model were tested for normality with

the Kolmogorov–Smirnov test (Quinn & Keough, 2002). Means are given with the standard error.

Results

Experiment 1: Effect of immunity on moult

Males of PBS and LPS groups did not differ significantly in average tarsus length, initial body mass or initial moult score (Student's *t*-test, always $P > 0.15$; Table S1). During the control week, males of the two groups did not differ in average moult speed, as indicated by a nonsignificant interaction between treatment and the change in moult score (RM-ANOVA, $F_{1, 28} = 0.32$, $P = 0.57$; Fig. 1). However, during the experimental week, LPS males moulted slower than PBS males, as indicated by a significant interaction (RM-ANOVA, $F_{1, 28} = 4.65$, $P < 0.05$; Fig. 1). Males of the PBS group (control) moulted at a similar speed in both the control and experimental weeks (RM-ANOVA, $F_{1, 15} = 0.10$, $P = 0.75$; Fig. 1), whereas the moult speed of males in the LPS group in the experimental week (when their immune system was stimulated) was almost half that of the control week ($F_{1, 13} = 3.64$, $P = 0.08$; Fig. 1). These results suggest a trade-off between moult speed and immune response.

The treatment had no significant effect on the change in body mass of sparrows in the 2 weeks of the study (RM-ANOVA; control week: $F_{1, 28} = 0.40$, $P = 0.53$; experimental week: $F_{1, 28} = 0.83$, $P = 0.36$). When I included the change in body mass as a covariate in the statistical models, during the control week, the change in body mass was not significantly related to moult speed ($\beta = -0.16$; Table 1). In turn, during the experimental week, I detected a significant negative relationship between moult speed and body mass change ($\beta = -0.42$; Table 1),

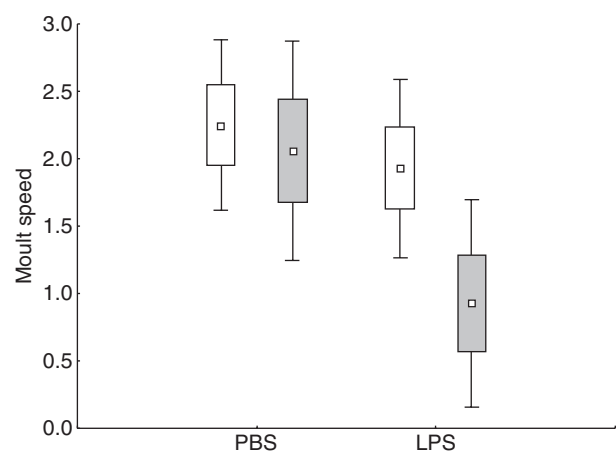


Fig. 1 Mould speed (score) of house sparrows in the phosphate buffer saline (PBS)- and lipopolysaccharide (LPS)-injected groups, during the control (white) and the experimental (grey) weeks. Boxes indicate the standard error of the mean, and bars indicate the 95% Confidence Intervals.

Table 1 Effect of treatment [phosphate buffer saline (PBS) or lipopolysaccharide (LPS) injection] and change in body mass on moult speed of house sparrows during the control week and during the experimental week.

	df	F	P
Control week			
Intercept	1	94.07	< 0.001
Change in body mass	1	0.71	0.41
Treatment	1	0.42	0.52
Error	27		
Experimental week			
Intercept	1	9.34	0.005
Change in body mass	1	6.57	0.016
Treatment	1	7.62	0.01
Error	27		

whereas the effect of the treatment remained significant. The interaction between the treatment and the change in body mass was not significant ($F_{1, 25} = 0.23$, $P = 0.64$). This result suggests a trade-off between moult speed and body mass in the house sparrow.

Experiment 2: Effect of moult on immunity

In this experiment, average tarsus length of sparrows did not differ significantly between the moulting and the control groups (moulting: 19.96 ± 0.14 mm; control: 19.29 ± 0.49 mm; $t_{25} = 1.38$; $P = 0.18$), but males in the moulting group tended to weigh more than in the control group (moulting: 28.09 ± 0.34 g; control: 26.78 ± 0.52 g; $t_{25} = 2.13$; $P = 0.04$). The immune response to PHA, measured 24 h after inoculation, did not differ between groups ($F_{1, 24} = 0.59$, $P = 0.45$; Fig. 2). However, none of the 14 sparrows in the

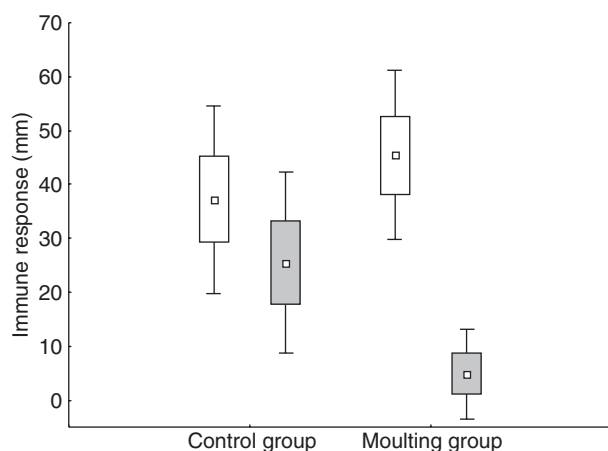


Fig. 2 Immune response (wing web swelling) to phytohaemagglutinin of house sparrows in the control and moulting groups, 24 h after the inoculation (white) and a week later (grey). Boxes indicate the standard error of the mean, and bars indicate the 95% Confidence Intervals.

moulting group were regrowing the plucked feathers at that moment. A week later, in 12 of the 14 moulting sparrows, the plucked primaries were growing, the rachis being approximately 1 cm in length, the same as the clipped feathers in the control group. The control sparrows never lost their feathers nor regrew them. I then measured the thickness of the patagium, finding that the sparrows still showed significant response to the PHA, and the response varied with the treatment (RM-ANOVA, increase in patagium thickness measured a week later in relation to initial thickness: $F_{1, 24} = 12.44$, $P = 0.001$; interaction with the treatment: $F_{1, 24} = 5.71$, $P < 0.05$; Fig. 2). The immune response had decreased since its measurement 24 h after the PHA inoculation (RM-ANOVA, $F_{1, 24} = 36.52$, $P < 0.001$), but the decrease was significantly more pronounced in the moulting group than in the control group (interaction, $F_{1, 24} = 11.07$, $P < 0.005$; Figs 2 and 3). As a result, the immune response to PHA in the sparrows a week later was significantly higher in control than in the moulting group ($F_{1, 24} = 4.47$, $P < 0.05$; Fig. 2). When initial body mass was introduced as the covariate to control for the difference in body mass between groups, the model showed a marginal effect of the treatment on immune response a week after the inoculation ($F_{1, 23} = 4.17$, $P = 0.05$; effect of body mass: $F_{1, 23} = 0.11$, $P = 0.74$), and the decrease in immune response remained significantly higher in the moulting group than in control ($F_{1, 23} = 8.60$, $P < 0.01$; effect of body mass: $F_{1, 23} = 0.03$, $P = 0.87$).

The treatment did not significantly affect the change in body mass (average change: -0.81 ± 0.11 g; $F_{1, 25} = 0.12$, $P = 0.73$). When the models were performed including the change in body mass as the

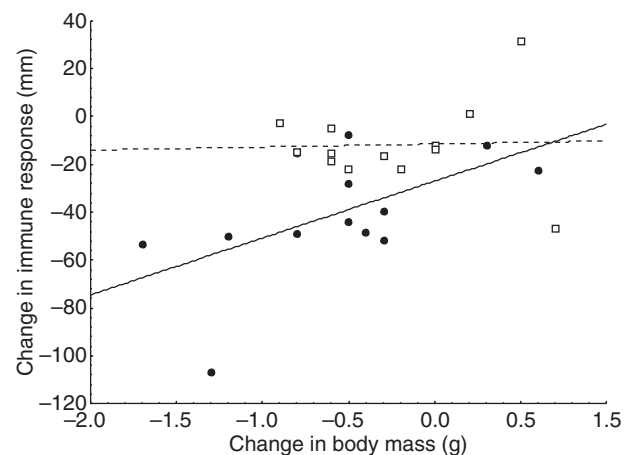


Fig. 3 Relationship between the change in body mass and the change in immune response to phytohaemagglutinin during the experiment 2. Solid dots represent data from the moulting group. Empty squares are data from the control group. Solid line is from the regression model for the moulting group, and dashed line for the control group.

covariate, I detected a nonsignificant relationship between the change in body mass and the decrease in the immune response ($\beta = 0.33$, $F_{1, 23} = 3.94$, $P = 0.06$; effect of the treatment on the decrease in immune response: $F_{1, 23} = 7.78$, $P = 0.01$). Sparrows in the moulting group showed a significant correlation between the change in body mass and the decrease in patagium thickness during the week when the plucked feathers started to grow ($r = 0.57$, $P = 0.04$, $n = 13$), whereas in the control group, this relationship was smaller and not significant ($r = 0.04$, $P = 0.91$, $n = 13$; Fig. 3), although the slopes of the two relationships did not differ significantly ($F_{1, 22} = 2.22$, $P = 0.15$).

Discussion

The results in this study show a trade-off between moulting and the immune response in the house sparrow. Stimulation of the immune system with an antigen (LPS) affected the moult by reducing its speed to half. This result supports the finding in American sparrows that inoculation with PHA slowed the moulting process (Martin, 2005). By contrast, in a Romanian population, moulting was not affected when sparrows were inoculated with the antigen SRBC (Pap *et al.*, 2008). Moreover, in the present study, the simulation of the moult by plucking two primaries depressed the immune response to PHA. This result experimentally supports the correlation found by Martin (2005), in which sparrows moulting faster showed a smaller response to PHA. The effect of this experiment was not confounded by flight impairment (Swaddle *et al.*, 1999), given that control sparrows had the same feathers clipped, the only difference between the two groups being the regrowth of feathers in the moulting group. Plucking of the feathers neither affected the results, because the immune response differed between the two groups only when the new feathers started to grow, but not before. As a whole, the findings in these studies show that the immune response to PHA and LPS is traded with moult in the house sparrow.

The house sparrow is the only species in which a trade-off between moulting and the immune system has been experimentally demonstrated during moulting (Martin, 2005; this study). In the pied flycatcher, such a trade-off has been shown, but only during the breeding season (Ilmonen *et al.*, 2000; Sanz *et al.*, 2004), a period in which birds face a heavy demand for resources, and thus to moult and to mount an immune response simultaneously may mean prohibitive costs (Moreno *et al.*, 2001). The current study shows that the trade-off between moult and immune system also occurs outside the breeding season, when it is more common, considering that birds usually avoid moulting while breeding (Ginn & Melville, 1983; Jenni & Winkler, 1994). More studies, with different species, would be necessary to examine the generality of these results.

In addition, I found evidence for a trade-off between moulting and body mass, as the sparrows that moulted faster lost more weight. Other studies have shown the importance of reserves for moult. For example, in the blue tit (*Cyanistes caeruleus*), heavier males start to moult earlier than light males (Svensson & Nilsson, 1997). Pied flycatchers supplied with extra food start to moult earlier than birds without extra food, suggesting that food availability limits the onset of the moult (Siikamäki, 1998). In the barn swallow (*Hirundo rustica*), the moult score is negatively correlated with fat reserves, suggesting a trade-off between gathering fat for migration and moult (Rubolini *et al.*, 2002). In another study with this species, the moult score was positively correlated with body condition (Møller *et al.*, 1995). Body mass is also traded with immune response, and sparrows inoculated with LPS usually lose mass (Bonneaud *et al.*, 2003; own unpublished data). However, in this study, LPS did not affect body mass during moult. Given this trade-off between moult speed and body mass, it is probable that sparrows inoculated with LPS reduced moult speed to conserve body mass. Additionally, in experiment 2, there was a trend in moulting sparrows: the greater the mass loss, the higher the decrease in the immune response. This result suggests that individuals that needed more resources for moulting took those resources from two compartments: immune system and body reserves. In the house sparrow, proteins are required for moulting (Pap *et al.*, 2008) as well as for the immune system (Gonzalez *et al.*, 1999). At the same time, both moulting and the immune response are energetically costly in the house sparrow (Blackmore, 1969; Martin *et al.*, 2003), and thus birds simultaneously facing an immune response and a moult could require more energy.

A trade-off between moult and immune response is presumably more severe in the wild, where resources are more limited, and its consequences for bird fitness should be more important. In birds, plumage condition mirrors their resistance to parasites and thus is an indicator of the immune capacity (Hamilton & Zuk, 1982). The trade-off between the immune system and moulting may mediate the relationship between plumage condition and resistance to parasites. Birds with sufficient resources may mount an immune response at the same time as they moult efficiently, resulting in plumage in good conditions (Murphy *et al.*, 1988; Borras *et al.*, 2004; De la Hera *et al.*, 2009). Birds without sufficient resources would have a limited capacity to combat pathogens, increasing their probabilities of dying (Møller & Saino, 2004), and/or they would moult plumage in bad conditions, which would affect their future fitness (Nilsson & Svensson, 1996). Therefore, results here suggest a way in which plumage quality honestly reflect individual quality (e.g. Roulin, 2007). In the case of males, this trade-off may mean that males proficient in moulting and simultaneously mounting an immune response will maintain attractive plumage (Hill & Montgomerie,

1994; Robertson *et al.*, 1998; Senar *et al.*, 2003), whereas males without sufficient resources to moult and mount an immune response will have unattractive plumage (Serra *et al.*, 2007; Ferns & Hinsley, 2008; Griggio *et al.*, 2009). Only males in good condition may simultaneously moult and mount an immune response, and thus the trade-off between the moult and the immune response may act as a handicap guaranteeing the honesty of plumage traits as indicators of immune capacity (Zahavi & Zahavi, 1997). Thus, males with attractive plumage would indicate their capacity to mount an efficient immune response and moult efficiently at the same time.

In conclusion, the current study reveals a trade-off between moult and immune response in the house sparrow. This trade-off has implications for fitness, considering that both functions are important for survival. In males, plumage condition indicates their resistance to parasites or immunocompetence (reviews in Hillgarth & Wingfield, 1997; Griffith & Pryke, 2006; Ardia & Schat, 2008), and the trade-off found here could guarantee the honesty of such a relationship. Only individuals in good condition can mount an immune response and simultaneously moult efficiently, and thus maintain attractive plumage.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Mean \pm SE of morphological traits and initial moult score of house sparrows in the phosphate buffer saline (PBS) and lipopolysaccharide (LPS) group.

Table S2 Dead and alive individuals did not significantly differ for body mass or moult score.

Table S3 Values of body mass and moult score of individuals at the start of the experiment were similar to those in the final sample.

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