Assessing the Cost of Mounting an Immune Response

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abstract: The evolution of parasite resistance has often been assumed to be governed by antagonistic selection pressures. Defense against pathogens, by mounting an immune response, confers evident benefits but may also incur costs, so that the optimal level of defense is expected to depend on the balance between benefits and costs. Although the benefits of immune surveillance are well known, estimates of costs are still equivocal. Here we studied the behavioral and physiological modifications associated with exposure to a nonreplicating antigen (lipopolysaccharide [LPS] of *Escherichia coli*) in a passerine species, the house sparrow (*Passer domesticus*). We further investigated whether the behavioral and physiological changes provoked by LPS induced measurable repercussions on life-history traits, such as the breeding effort and reproductive success. Finally, we tested whether the trade-off between immune activation and breeding effort was modulated by the workload required to feed the brood. Exposure to LPS reduced activity and increased body mass loss of captive individuals; similarly, LPS injection induced a dramatic drop in feeding rate and reproductive success of breeding females. However, this reduction depended on brood size, suggesting that the strength of the trade-off between immune activation and reproduction was affected by the workload required to feed the brood. Overall, this study stresses the magnitude of costs associated with mounting immune responses and the ecological and evolutionary consequences for natural populations.

Keywords: life-history traits, LPS, parasite resistance, parental effort, reproductive success, trade-off.

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It is now well established that parasites and infectious diseases exert strong selection pressures on their hosts (Grenfell and Dobson 1995). Although we expect the selective advantage of resistance to eliminate all susceptible phenotypes, hosts continue to exhibit a wide range of defense strategies (Wakelin and Apanius 1997). One explanation may be that parasites vary in their effects on hosts and thus exert variable selection pressures (Grenfell and Dobson 1995; Sorci et al. 1997). Another explanation for the maintenance of such variability may be found in the costs associated with each line of defense, since optimal resource allocation to defense will depend on allocation to other costly functions and their associated benefits (Sheldon and Verhulst 1996; Shudo and Iwasa 2001).

Although there remains some debate on the cost of maintaining immune defenses in the absence of infection (Kraaijeveld and Godfray 1997; Klasing 1998; Webster and Woolhouse 1999), mounting an immune response is undeniably energetically costly both because of the metabolic requirements of immune cells but also as a result of the indirect consequences of immune up-regulation (Lochmiller and Deerenberg 2000), such as tissue degradation or anorexia during inflammation. However, although activation of the immune machinery has been shown to induce several physiological modifications (Demas et al. 1997; Bilbo et al. 2002), we still have limited information on its impact on traits tightly related to fitness (Moret and Schmid-Hempel 2000). In addition to the energetic costs associated with mounting an immune response per se (reviewed in Lochmiller and Deerenberg 2000; Ots et al. 2001), immune activation diverts resources otherwise allocated to other costly functions, such as reproduction (Sheldon and Verhulst 1996; Norris and Evans 2000).

In several species, investment in reproduction has been

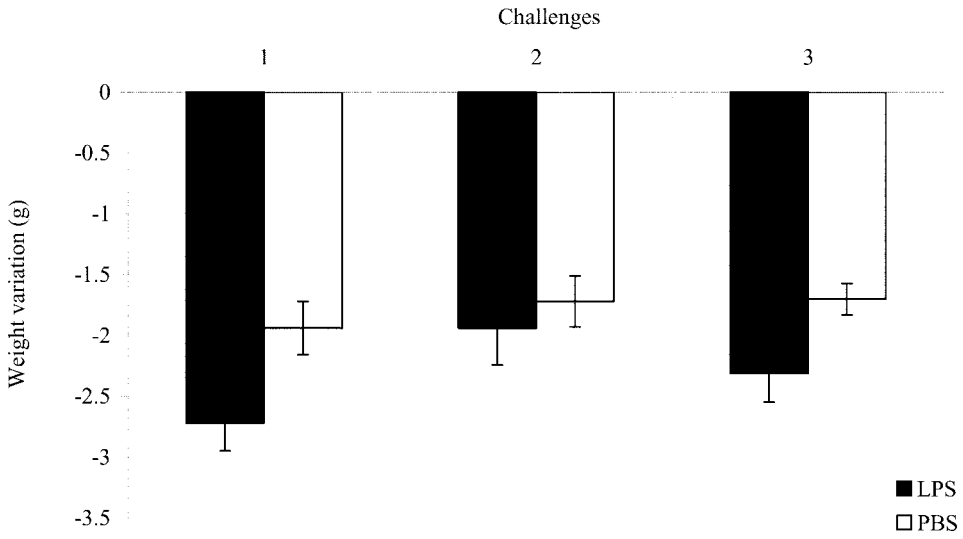

Figure 1: Overnight body weight loss (g) of male house sparrows challenged three times with either LPS or PBS; bars represent standard errors

shown to increase host susceptibility to parasites (Festa-Bianchet 1989; Gustafsson et al. 1994; Norris et al. 1994; Richner et al. 1995; Allander et al. 1995; Nordling et al. 1998), although it still remains uncertain whether this results in decreased host survival probability and future reproductive success. Immunosuppression may also partly account for the trade-off between an organism's current and future reproduction (Roff 1992; Stearns 1992). In agreement with this view, an increase in parental effort has been shown to weaken the production of antibodies specifically directed against experimentally injected antigens (Deerenberg et al. 1997; Nordling et al. 1998). Conversely, the injection of killed pathogens has been shown to induce immune responses while decreasing parental feeding rates (Ilmonen et al. 2000; Råberg et al. 2000).

The objectives of this study were threefold: first, we wished to assess the physiological and behavioral modifications associated with mounting an immune response; second, we tested whether immune system activation traded against investment in reproduction; third, we explored whether the trade-off between immune activation and reproduction was modulated by the workload required to feed the brood.

The first experiment was designed to explore the consequence of an inert antigen injection (lipopolysaccharide [LPS] from the cell wall of *Escherichia coli*) on body mass and activity of captive house sparrows. The choice of the antigen (LPS), which mimics a bacterial infection by increasing the release of cytokines (Dunn and Wang 1995), is linked to our desire to evaluate solely the cost of immune function activation while eliminating the direct negative effects of pathogens. Lipopolysaccharide induces an inflammatory response by nonspecifically activating a wide array of cells, including heterophils and B and T lymphocytes, only a few hours after exposure to the antigen (Janeway and Travers 1999). The inflammatory response is then followed by the production of specific anti-LPS antibodies (Poxton 1995).

The cost of an immune response in relation to life-history traits depends on host access to limited resources and on the investment required by other costly functions. We therefore performed a second experiment in which we activated the immune system of breeding female house sparrows in a natural population. We injected the same antigen that was used in the previous experiment (LPS), and we examined the effects on parental effort and reproductive success. In addition to immune function stimulation, we simultaneously manipulated brood size to create groups in which brood demands were increased, decreased, or kept constant.

The experiments carried out on captive and free-ranging individuals allowed us to formulate a number of predictions: first, we expect that immune activation with LPS should induce a reduction of activity and a loss of body mass; second, if these behavioral and physiological changes have ecological repercussions, we expect free-ranging breeding females injected with LPS to reduce nestling feeding provisioning and thus possibly their reproductive success compared with control females; and finally, we expect the intensity of the trade-off to be strongest for females with enlarged broods.

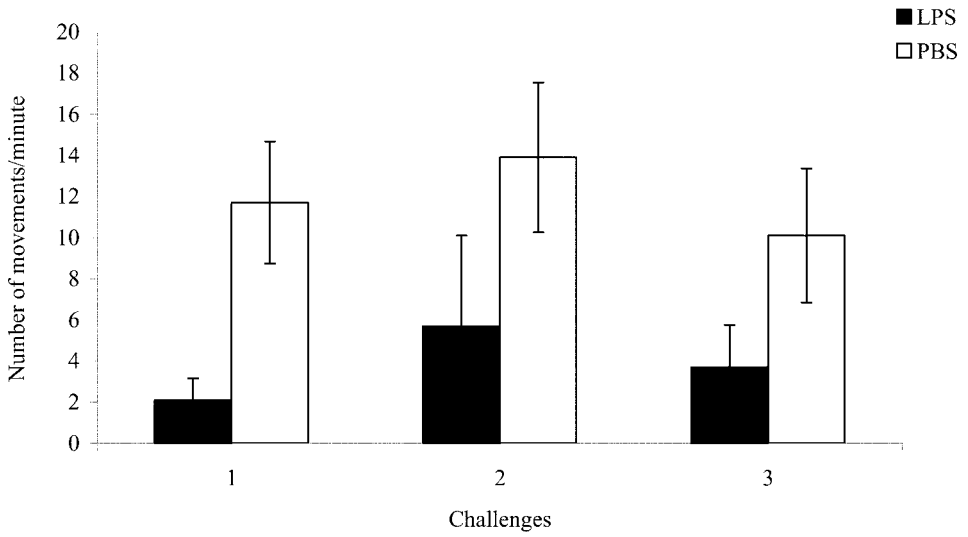

Figure 2: Number of movements per minute of male house sparrows challenged three times with either LPS or PBS; bars represent standard errors

Methods

Physiological and Behavioral Changes Induced by LPS

Male house sparrows were caught with mist nets in early spring 1999 in Badajoz (southwestern Spain) and were banded with a metal and a unique combination of color bands. We measured body mass with an accuracy of 0.1 g. Sparrows were subsequently randomly released in three outdoor aviaries (3.5 m × 1.0 m × 2.5 m) located on the campus of the University of Extremadura (Badajoz). Food (a commercial mixture of seeds for canaries) and vitamin-supplemented water were provided ad lib.

Nine male house sparrows were injected intraperitoneally with 0.01 mg of LPS (serotype O55 : B5; Sigma, St. Louis) diluted in 0.1 mL of PBS (phosphate buffered saline) so that the concentration was 0.1 mg/mL. This concentration is similar or lower than those previously used in poultry and other domesticated animals to activate the immune system (van Heugten et al. 1996; Parmentier et al. 1998*a*, 1998*b*). Nine control males were injected with the same volume (0.1 mL) of PBS. Birds were injected twice in May and once in June with an interval of 3 wk between injections (van Heugten et al. 1996). Each aviary contained an equal number of birds per treatment.

Birds were always injected at the same time in the afternoon (6:00 P.M. ± 15 min). They were weighed before injection and transferred to individual indoor cages (40 cm × 50 cm × 50 cm) where they spent the night. We recorded bird activity the following morning using a video camera attached to the ceiling. Activity was assessed as the number of perch changes per minute and was recorded for 1 h between 9:00 A.M. (±30 min) and 10:00 A.M. (±30 min). Birds were then weighed again before being released in the outdoor aviary.

Trade-off between Immune Activation and Reproduction in a Free-Ranging Population

This study was conducted during spring 2001 at the Centre d'Etude Biologique de Chizé (46°09′N, 0°24′W) in France on a nest box house sparrow population established in 1992. A large proportion of the birds are color banded. Before onset of breeding and during egg laying, all nest boxes were checked daily to determine dates of clutch initiation, completion of laying, and clutch size.

Breeding females were captured at the nest when chicks were 7 d old (females captured when nestlings are younger have a high brood-desertion probability) and assigned to two groups. One group received 0.015 mg of LPS in 0.03 mL of PBS by intraperitoneal injection (concentration = 0.5 mg/mL), while the other group received an identical volume of PBS as control. At capture, we measured body mass (±0.1 g), and we sampled blood from the brachial vein (~300 μL). This allowed us to assess levels of serum total IgG and antibodies specifically directed against LPS, which might suggest current bacterial infections (Poxton 1995). Overall, females were handled for <3 min to minimize stress. Blood was immediately centrifuged and plasma stored at −20°C.

Brood size was also manipulated when nestlings were 7 d old. Two nestlings were randomly transferred between two synchronous broods, while the brood of a third nest

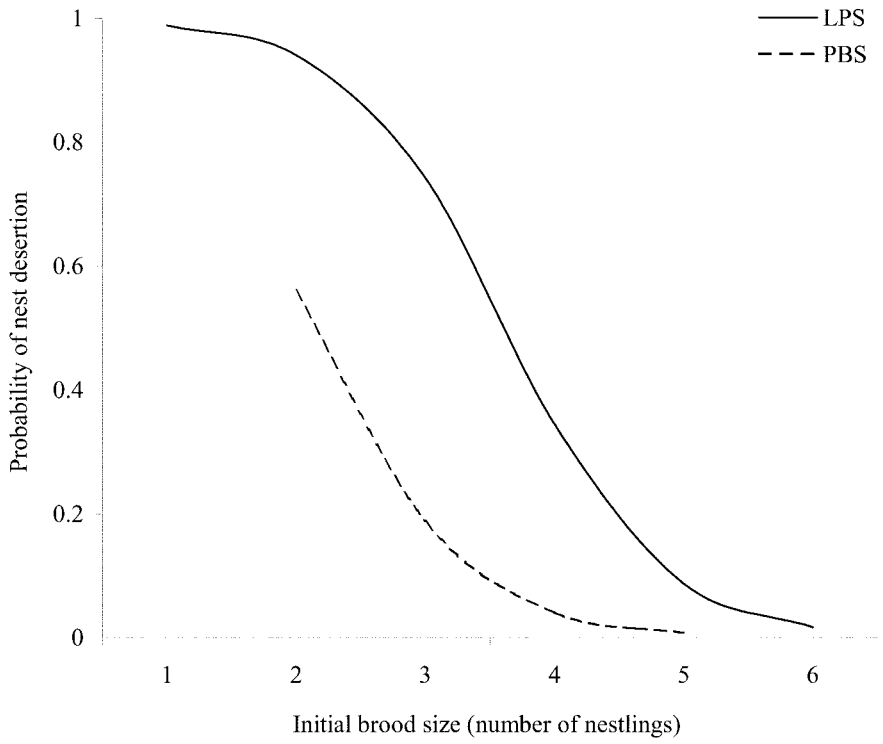

Figure 3: Probability of brood desertion for LPS (*solid line*) and PBS (*dashed line*) females as a function of initial brood size, that is, brood size before manipulation. The probability of brood desertion has been modeled with a logistic regression model.

was kept unmanipulated. This created three experimental groups with enlarged, reduced, and control broods.

Nestling condition was evaluated by regularly weighing the chicks (at days 6, 7, 8, 10, and 12), by measuring tarsus length (at day 12), and by measuring their ability to mount an immune response. Cell-mediated immune response was estimated when nestlings were 10 d old. A mitogen (phytohemagglutinin [PHA]; 0.025 mg in 0.04 mL PBS) was injected intradermically in the center of the right wing patagium, while the left wing was kept as a control and injected with an identical volume of PBS. The thickness of each patagium was measured with a thickness gauge (± 0.01 mm) at the injection site before and 24 h after injection.

Parental feeding rates of both males and females were measured between 8:00 A.M. and 6:00 P.M. using binoculars, and they were estimated as the number of visits to the nest recordable in 1-h sessions when nestlings were 6 (i.e., before the manipulation), 8, and 10 d old. Time of observation did not differ between experimental categories for any of the three nestling ages (all P's $> .3$).

Adults present at the breeding site were regularly resighted throughout the entire breeding season (all females used in this study were color banded). Replacement (after failure of the first breeding attempt) and second clutches were also recorded to assess patterns of parental investment once the physiological and behavioral effects of LPS were supposedly over. Nestlings of replacement/second clutches were weighed and their tarsus length measured when they were 5, 7, 9, and 11 d old.

Enzyme-Linked Immunosorbent Assay for IgG

Serum concentration of IgG was determined using an enzyme-linked immunosorbent assay. This method provides sensitive measures of amounts of antibodies, which specifically bind to a certain antigen (Janeway and Travers 1999).

To assess levels of total IgG, a standard curve was done using purified sparrow IgG diluted from 500 ng/mL to 15 ng/mL (Agrobio PO688). Microtiters plates were coated with antigen (250 ng/well; rabbit IgG antisparrow IgG; Agrobio L406681) by overnight incubation, washed, and incubated with phosphate buffer saline (PBS) containing 1.5% serum albumin bovine fraction V. Thawed serum samples from females were diluted 1 : 500,000 with PBS, and 100 μL of the dilution was added to the wells. The plates were incubated for 2 h at room temperature and

Table 1: Effect of immune treatment (IT) and brood-size manipulation (BS) on nestling feeding rates (number of visits/nestling/h) of female house sparrows when chicks were 6, 8, and 10 d old

Source	df	Type III SS	*F*	*P*
Tests of hypotheses for within-subject effects:				
Age	2	34.098	3.76	.0303
Age × IT	2	51.913	5.73	.0059
Age × BS	2	30.576	3.38	.0425
Age × IT × BS	2	12.832	1.42	.2525
Error	48	217.379		
Tests of hypotheses for between-subject effects:				
IM	1	38.012	3.79	.0633
BS	1	5.246	.52	.4765
IM × BS	1	63.714	6.35	.0188
Error	24	240.644		

Note: Here we report a repeated measurements ANOVA for tests of hypotheses for within- and between-subject effects.

then washed. Peroxydase-conjugated secondary antibodies (100 μL of rabbit IgG antisparrow IgG diluted $1:10^{-3}$) were added to the wells and incubated at room temperature for 2 h. Plates were again washed with PBS, and 100 μL of the chromogenous substrate o-phenylenediamine dihydrochloride (Sigma) was added to each well. Plates were protected from light during the enzyme-substrate reaction, which was terminated by adding 50 μL chlorydric acid 1 N, and the optical density of each well was determined using a plate reader equipped with 450-nm wavelength filter.

The same protocol was applied to measure levels of serum IgG specifically directed against LPS. Microtiters plates were coated with 100 μL of LPS (1,000 ng/mL; Sigma), and plasma was diluted 1 : 500. The standard curve was made using purified sparrow IgG diluted from 1.5 μg/mL to 50 μg/mL. For this reason, amounts of IgG specifically directed against LPS are expressed in equivalent mg/mL of sparrow IgG (Agrobio PO688).

Statistical Analyses

We used general linear models and generalized linear models according to the distribution of the dependent variables. Repeated measurements models were used when variables were measured several times for the same individual. Model selection was performed by including the main factors plus the interactions and then excluding all nonsignificant interactions. Differences in the number of individuals between different analyses are due to missing values. Statistical analyses were done using SAS statistical software (SAS Institute 1999).

Results

Physiological and Behavioral Changes Induced by LPS

Given the relatively small sample size, we first checked whether body mass at the beginning of the experiment differed between the two experimental groups (LPS vs. PBS). A one-way ANOVA confirmed that birds were distributed randomly in the two groups, at least with respect to body mass ($F = 2.29$, df $= 1, 16$, $P = .150$).

As expected, birds lost on average about 7.5% of their body weight during the night. Across the three immune challenges, individuals injected with LPS tended to lose more weight than did PBS controls (repeated measurements ANOVA: immune treatment, $F = 3.96$, df $= 1, 16$, $P = .064$; fig. 1). Local tests showed that LPS birds lost significantly more mass compared with PBS individuals during the first and third challenges, whereas the difference was not significant for the second challenge (first challenge: $F = 6.20$, df $= 1, 16$, $P = .024$; second challenge: $F = 0.38$, df $= 1, 16$, $P = .547$; third challenge: $F = 5.05$, df $= 1, 16$, $P = .039$). The difference between treatments was consistent across repeated exposures, as shown by the nonsignificant interaction between time and immune treatment (repeated measurements ANOVA: $F = 1.94$, df $= 2, 32$, $P = .159$). Finally, weight loss was repeatable across time within treatment (PBS: $F = 4.92$, df $= 8, 18$, $P = .0024$; LPS: $F = 3.51$, df $= 8, 18$, $P = .0128$).

The effect of LPS on body mass was not restricted to overnight weight loss. Initial body mass of LPS birds tended to decrease across the three challenges, whereas initial body mass of PBS birds remained constant (repeated measurements ANOVA: time, $F = 1.11$, df $= 2, 32$, $P = .341$; time × immune treatment, $F = 4.24$, df $= 2, 32$, $P = .023$).

Activity level was also significantly affected by immune challenge. Throughout the three challenges, birds injected with LPS were less active than controls injected with PBS (repeated measurements ANOVA on $\log_{10}$-transformed data: immune treatment, $F = 17.29$, df $= 1, 16$, $P < .0001$; fig. 2). Local tests showed that LPS birds were significantly less active compared with PBS individuals during the three challenges (first challenge: $F = 14.06$, df $= 1, 16$, $P = .002$; second challenge: $F = 9.98$, df $= 1, 16$, $P = .006$; third challenge: $F = 6.59$, df $= 1, 16$, $P = .021$). The effect of LPS on activity was constant across repeated exposures, as shown by a nonsignificant time by immune treatment interaction (repeated measurements

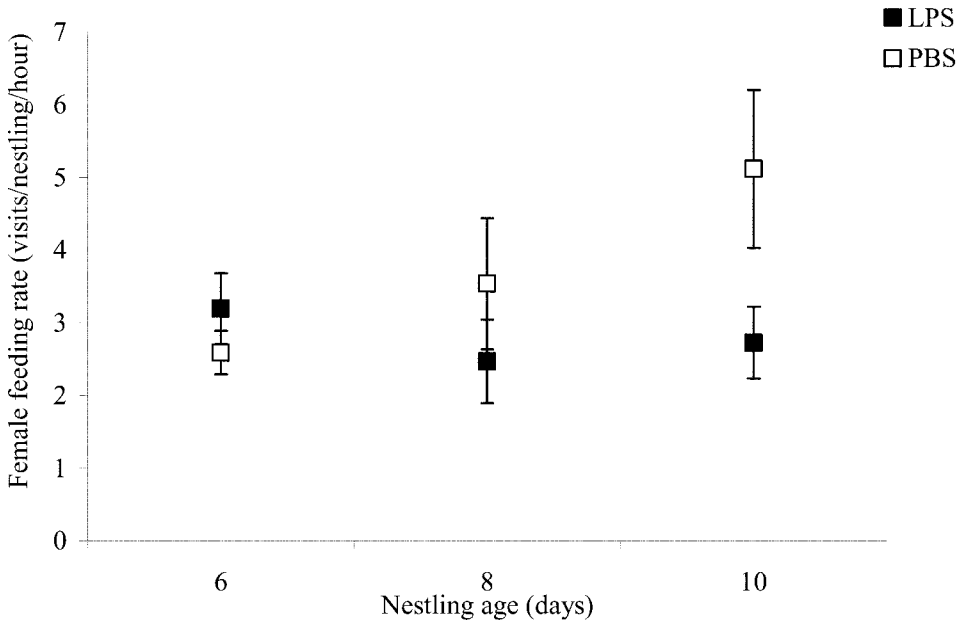

Figure 4: Effect of immune system activation on female feeding rate (number of visits/nestling/h) across the nestling rearing period (immune activation occurred at day 7). Solid squares correspond to LPS females, whereas open squares correspond to PBS females. Bars represent standard errors.

ANOVA on $\log_{10}$-transformed data: time × immune treatment, $F = 0.12$, df $= 2, 32$, $P = .890$). Finally, activity rate was repeatable within treatment (PBS: $F = 4.96$, df $= 8, 18$, $P = .0023$; LPS: $F = 2.63$, df $= 8, 18$, $P = .0421$).

Trade-off between Immune Activation and Reproduction

Initial levels of antibodies against LPS did not differ between females in the LPS and PBS groups, nor did they differ among brood-size groups (two-way ANOVA: immune treatment, $F = 0.04$, df $= 1, 39$, $P = .844$; brood-size manipulation, $F = 2.20$, df $= 1, 39$, $P = .146$). Similarly, total immunoglobulin levels were initially similar for the two experimental treatments (two-way ANOVA: immune treatment, $F = 0.61$, df $= 1, 39$, $P = .441$; brood-size manipulation, $F = 1.89$, df $= 1, 39$, $P = .177$). Finally, body mass did not differ between groups (two-way ANOVA: immune treatment, $F = 0.20$, df $= 1, 39$, $P = .658$; brood-size manipulation, $F = 0.61$, df $= 1, 39$, $P = .439$). These results suggest that initial health status was similar across treatments.

Effects of Immune Challenge on Nest Desertion. After capture and immune challenge, 30% (12/40) of the females deserted the nest (five females were removed from this analysis because nestlings died or disappeared before we could ascertain whether the female had deserted the brood or not). The likelihood of deserting the brood was significantly and negatively correlated with brood size before manipulation and immune treatment (logistic regression: brood size before manipulation, $\chi^2 = 7.55$, $P = .006$; immune treatment, $\chi^2 = 5.41$, $P = .020$; brood size before manipulation × immune treatment, $\chi^2 = 0.037$, $P = .847$). Females with small brood sizes had higher chances of deserting their nest and, for a given brood size, LPS-injected females were more likely to desert their nests than control individuals (fig. 3).

Effects of Immune Challenge and Brood-Size Manipulation on Parental Feeding Rate, Reproductive Success, and Nestling Quality. Among the females that did not desert the brood, those injected with LPS fed their young less than did PBS-injected females, and this difference increased with nestling age (table 1; fig. 4). Brood-size manipulation also affected nestling provisioning. Chicks in the brood-reduced group received more food than those in control or brood-increased groups, and this difference increased with nestling age (table 1). However, the effect of LPS on nestling provisioning was modulated by brood-size manipulation, as shown by a significant interaction (table 1). Females in the brood-reduced group were more likely to maintain a sustained feeding effort compared with control and brood-increased groups (fig. 5). Males mated to LPS-injected

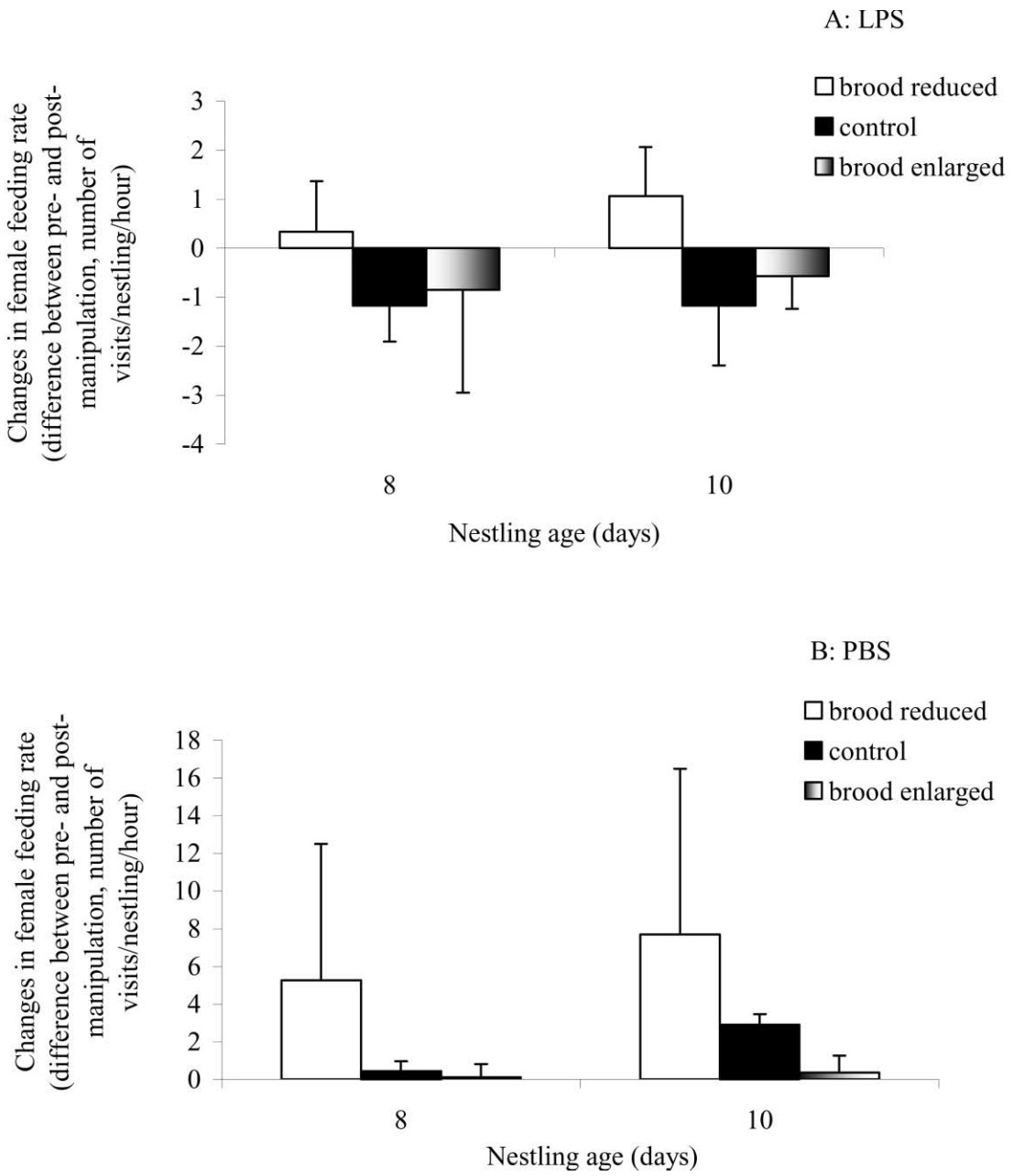

Figure 5: Changes in feeding effort of LPS females (*A*) and PBS females (*B*) as a function of brood-size manipulation. Changes in feeding effort have been computed as the difference in number of visits/nestling/h before the manipulation (day 6) and postmanipulation (days 8 and 10). Bars represent standard errors. Please note that the statistical analyses were performed on absolute feeding rates instead of rates of change.

females tended to increase their feeding rate to compensate for the reduction in female feeding effort (repeated measurements ANOVA: nestling age × immune challenge, $F = 3.3$, df $= 2, 50$, $P = .045$; nestling age × brood-size manipulation, $F = 0.33$, df $= 2, 50$, $P = .718$).

Reproductive success, assessed as the number of fledged chicks, was affected by the interaction between immune treatment and brood-size manipulation (ANCOVA: immune treatment, $F = 0.90$, df $= 1, 41$, $P = .347$; brood-size manipulation, $F = 9.29$, df $= 1, 41$, $P = .04$; immune challenge × brood-size manipulation, $F = 15.07$, df $= 1, 41$, $P = .018$). Even when the females that had abandoned after capture were excluded from the analysis, this result remained significant (ANCOVA: immune treat-

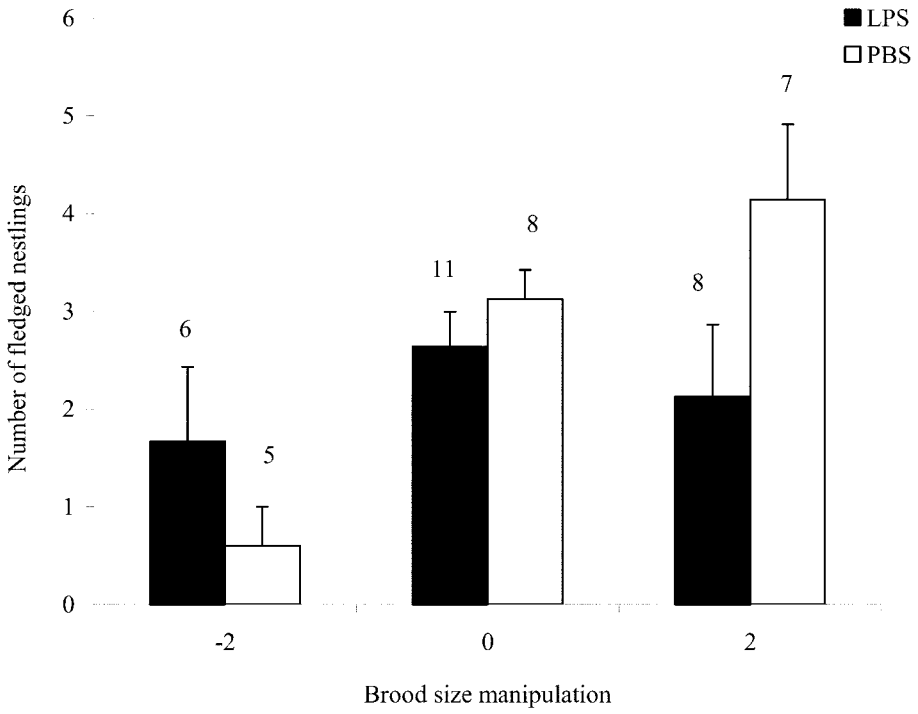

Figure 6: Reproductive success (number of fledglings) of LPS and PBS females as a function of brood-size manipulation. Bars represent standard errors.

ment, $F = 0.35$, df $= 1, 24$, $P = .560$; brood-size manipulation, $F = 9.01$, df $= 1, 24$, $P = .006$; immune challenge × brood-size manipulation, $F = 5.02$, df $= 1, 24$, $P = .035$). The PBS females produced more fledglings when their brood was experimentally increased compared with the brood-reduced and brood control groups, whereas the number of fledglings was relatively constant across the three brood-size groups for LPS females (fig. 6).

Chick quality was assessed as body mass growth, tarsus length, and cell-mediated immune response to PHA injection. Nestling mass growth was not affected by the immune treatment (repeated measurements ANOVA: nestling age × immune challenge, $F = 1.57$, df $= 3, 60$, $P = .207$) but appeared to be related to brood-size manipulation (repeated measurements ANOVA: nestling age × brood-size manipulation, $F = 6.4$, df $= 3, 60$, $P = .01$), with nestlings of reduced broods being heavier than nestlings of control and enlarged broods. Tarsus length was not statistically significantly affected by immune treatment or by brood-size manipulation (two-way ANOVA: immune treatment, $F = 0.67$, df $= 1, 15$, $P = .427$; brood-size manipulation, $F = 1.95$, df $= 1, 15$, $P = .182$). Finally, nestling cell-mediated immune response was not affected by the immune treatment or the brood-size manipulation (two-way ANOVA: immune treatment, $F = 0.09$, df $= 1, 19$, $P = .763$; brood-size manipulation, $F = 0.44$, df $= 1, 19$, $P = .517$). However, it was positively correlated with mass when chicks were 12 d old ($r = 0.503$, $P = .015$, $n = 23$).

Effects of Immune Challenge and Brood-Size Manipulation on Future Within-Season Reproductive Attempts. Although exposure to LPS induced a significantly higher nest desertion rate, LPS females were resighted at the same rate as PBS females ($\chi^2 = 1.04$, $P = .308$), suggesting that nest desertion was not due to female mortality. Similarly, the likelihood of making a replacement or a second clutch did not differ between LPS and PBS females, nor did it differ between brood-reduced, brood-increased, and control groups (immune treatment: $\chi^2 = 0.27$, $P = .604$; brood-size manipulation: $\chi^2 = 1.65$, $P = .198$). However, moderate confidence should be given to these results given the low statistical power of the analyses (to obtain a desirable power of 0.8, >100 observations per case are required to reject the null hypothesis at $\alpha = 0.05$ when frequencies differ by 20%).

The number of fledglings produced during the first reproductive attempt was positively correlated with the likelihood of making a second clutch ($\chi^2 = 5.72$, $P = .017$).

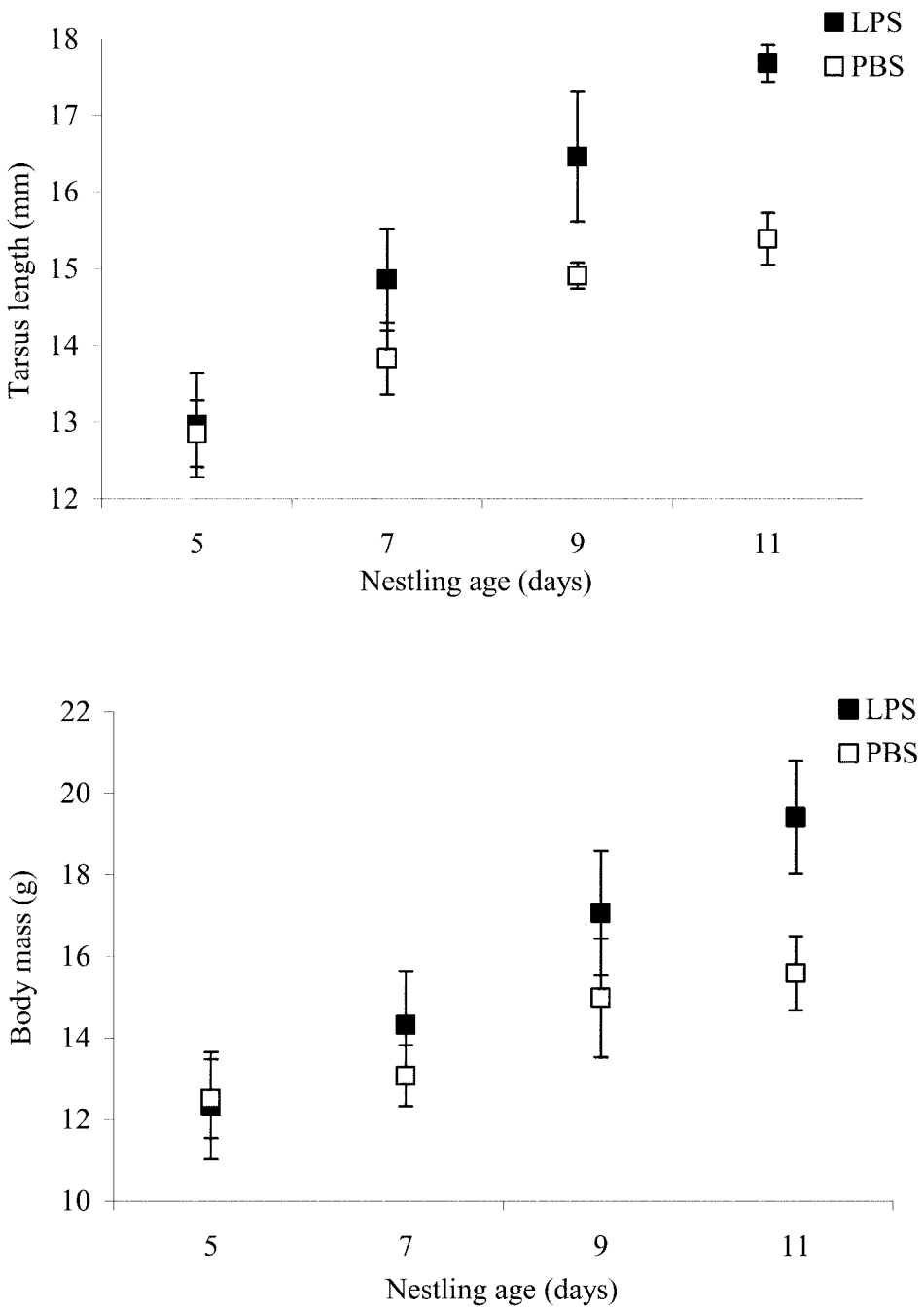

Figure 7: Effect of maternal treatment during the first breeding attempt on investment in replacement/second clutches assessed as nestling growth (*top*, tarsus length; *bottom*, body mass). Solid squares correspond to LPS females, whereas open squares correspond to PBS females. Bars represent standard errors.

Time elapsed between the first and the second clutch was not affected by treatments (two-way ANOVA: immune treatment, $F = 0.28$, df $= 1, 15$, $P = .604$; brood-size manipulation, $F = 1.89$, df $= 1, 15$, $P = .194$), but it was significantly longer for females that had produced more fledglings ($r = 0.611$, $P = .012$, $n = 16$). Neither the size of replacement clutches nor their reproductive success differed between the immune challenge or the brood-size manipulation treatments (two-way ANOVA: clutch size: immune treatment, $F = 0.20$, df $= 1, 12$, $P = .663$;

brood-size manipulation, $F = 0.05$, df $= 1, 12$, $P = .954$; number of fledglings: immune treatment, $F = 0.20$, df $= 1, 7$, $P = .668$; brood-size manipulation, $F = 0.26$, df $= 1, 7$, $P = .781$).

However, nestlings of second/replacement clutches had a significantly higher growth rate if their mother was injected with LPS compared with PBS mothers (repeated measurements ANOVA: tarsus length, nestling age × maternal treatment, $F = 7.34$, df $= 3, 15$, $P = .003$; body mass, nestling age × maternal treatment, $F = 2.92$, df $= 3, 15$, $P = .069$; fig. 7).

Discussion

We found that mimicking a bacterial infection induced a number of behavioral and physiological modifications that are likely to account for the reduction in feeding rate and reproductive success of immune-challenged birds. These costs are solely due to the process of mounting an immune response, since we injected a nonreplicating antigen. We also showed that the trade-off between immune activation and reproductive success depended on the intensity of the workload required to feed the brood. The fitness cost paid by females who mounted an immune response to LPS was also condition dependent, since it was a function of brood size both before and after manipulation (i.e., likelihood of deserting the brood and variation in the effort required to feed the young).

There are two aspects to an immune response that work together to provide organisms with a remarkably effective defense system (Janeway and Travers 1999). The specific immune response, also known as adaptive, occurs during the lifetime of an individual as an adaptation to infection with a specific pathogen and may even confer lifelong protective immunity. But most microorganisms are immediately and nonspecifically detected and destroyed by the innate immune system. Part of this early induced but nonadaptive response to infection is the induction of an inflammatory reaction, which allows inflammatory cells, such as heterophils and macrophages, to migrate to infection sites in an attempt to control the invasion. The innate system may alone succeed in repelling the pathogen while also allowing time for the specific response to be mounted (e.g., production of specific antibodies; Janeway and Travers 1999).

The injection of lipopolysaccharide nonspecifically activates a wide range of cells and induces the secretion of cytokines responsible for the inflammatory response (Janeway and Travers 1999). Exposure to LPS also has profound effects on an individual's energy metabolism, such as increased utilization of glucose by peripheral tissues (Feingold and Grunfeld 1992). The cachectin activity of cytokines provokes a decrease in food intake and an increase in resting expenditure, and previous studies in poultry and mammals have shown that stimulation with LPS results in an acute reduction of body weight gain and feed intake (Cook et al. 1993; van Heugten et al. 1996; Parmentier et al. 1998*a*). Our findings of enhanced body weight loss in LPS-injected sparrows, despite their reduced activity and when compared with control birds, are consistent with these previous results.

Behavioral responses to infection, also called "sickness behavior," as observed in this study, might have an adaptive function if they allow individuals to overcome infection and to increase the individual's survival prospect. For instance, reduced activity might conserve energy and permit tissue repair during infection (Hart 1988). However, although sickness behavior may be critical for survival during infection, it can interfere with other fitness components such as breeding effort. In agreement with the view that activation of the immune machinery induces measurable costs, we found that females challenged with LPS significantly decreased their contribution to parental care and experienced a decreased reproductive success.

As stressed in the above section, LPS induces a rapid inflammatory response a few hours after injection. A fraction of females suddenly facing a deterioration of their health deserted their brood and consequently paid a large fitness cost. Interestingly, however, females with larger brood sizes before injection and brood manipulation were more willing to sustain the feeding effort. There are several explanations for this result: first, high-quality females with large broods were more likely to stand the physiological costs of LPS injection; second, because of a negative correlation between current and future reproduction, females having already made a large breeding effort were more reluctant to desert the brood; and third, the energetic cost of brooding for small brood sizes may favor desertion by females (Chastel and Kersten 2002). A central issue in life-history theory is the trade-off between current and future reproduction (Trivers 1972; Clutton-Brock 1991; Stearns 1992). Females may consequently be more reluctant to end reproduction after investing highly in it, not because they adjust their current effort to past costs (Dawkins and Carlisle 1976) but because past investment usually decreases parental capacity for future expenditure and can therefore provide parents with a reliable index of the prospective benefits of future reproduction (Maynard Smith 1977; Coleman and Gross 1991). Whatever the reason for a lower desertion rate of females with large initial broods, this finding suggests a condition-dependent nature of the costs paid by LPS females.

When the reproductive effort was sustained, females injected with the antigen fed their young less than did control females, with the difference increasing as the chicks became older. However, reduction of food provision rate

was modulated by brood size as females in the brood-reduced group managed to maintain their feeding effort. Decreased female investment in nestling feeding effort had consequences on chick survival probability: nestling mortality was higher for LPS-injected females than for controls, but here again this effect was a function of brood-size manipulation. This advocates the existence of a cost to injections with LPS. Yet as predicted if immune function and breeding effort have common energy/resource demands, reduction in reproductive success after immune challenge was visible only when individuals experienced a concomitant workload increase while feeding the young. The trade-off between immune function and reproduction may thus only be visible when an individual must simultaneously enhance his investments in both activities.

Overall, these findings stress the magnitude of costs associated with mounting an immune response. During the course of an infection, physiological and behavioral responses might have an adaptive value if they increase the individual's likelihood of overcoming the disease. If the infection occurs when individuals are engaged in other costly functions, such as reproduction, birds might trade survival against current reproduction, as suggested by our results. For iteroparous organisms, this could be the optimal strategy as long as they have the possibility to breed again. However, if the deterioration of health during the infection is perceived as a risk for future survival prospects, then we expect females to increase their investment in future reproductive attempts once the effects of the exposure to the antigen are over. We found some evidence for this terminal investment hypothesis (Williams 1966; Pärt et al. 1992), since nestlings of replacement/second clutches of LPS-injected females grew faster and attained larger sizes at fledging than chicks of PBS-injected females. This divergence in growth rate was due to neither differences in the initial size nor differences in the clutch size. If fledgling size correlates with survival and recruitment probability (a result commonly observed in passerines; Gustafsson and Sutherland 1988; Tinbergen and Boerlijst 1990), higher investment of LPS females in replacement/second broods could compensate for the reduction of their reproductive success experienced during the course of the inflammatory response. Nevertheless, it is likely that high breeding effort during replacement/second clutches will ultimately be paid in terms of survival prospect or future reproduction (Williams 1966; Trivers 1974). However, since the experiment was not particularly designed to test the terminal investment hypothesis, we cannot rule out other alternative explanations to the difference observed between LPS- and PBS-injected females in terms of their investment in the second breeding attempt. For instance, LPS-injected females may have simply been able to invest more in their second reproduction, having made less effort raising their first clutch. Further work is definitely needed to test the terminal investment hypothesis.

In conclusion, we have shown that exposure to LPS provoked physiological and behavioral responses that, although likely to increase the probability of overcoming infection, can equally induce measurable costs in terms of reduced reproductive success. However, in this study, the cost of mounting an immune response depended on brood size. First, females were more willing to maintain their breeding effort, despite a deterioration of their health, when the potential fitness payback (as measured by the number of offspring) was high. But it also depended on the individual's physiological capacity to suffer a simultaneous increase of its investment in both immune function and reproduction, since the trade-off between those two costly activities depended on the variation in brood size after manipulation.

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