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TERMINAL INVESTMENT INDUCED BY IMMUNE CHALLENGE AND FITNESS TRAITS ASSOCIATED WITH MAJOR HISTOCOMPATIBILITY COMPLEX IN THE HOUSE SPARROW

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Abstract.—The terminal investment hypothesis predicts that individuals should invest more in their present reproduction if they are less likely to survive to future reproductive events. Infections, which reduce viability, may be used by individuals as a cue of a diminishing residual reproductive value and could therefore theoretically trigger an intensification of breeding effort. We tested this hypothesis in a natural population of house sparrows (*Passer domesticus*). We manipulated the immune system of breeding females by injecting them with a vaccine against the Paramyxo virus, the agent of Newcastle disease. Females were captured and treated immediately after completion of their first clutch either with the vaccine (NDV) or with phosphate buffered saline (PBS). The entire clutch was subsequently removed. We also screened *Mhc* class I genes of females to assess possible genotype-by-immune treatment interactions on reproductive investment. Our results indicate that vaccinated females were more likely to lay replacement clutches and that the difference in number of eggs between first and replacement clutches was greater for NDV females than for controls. In addition, chick size, both in terms of tarsus length and body mass, was affected by immune activation but in interaction with nestling age and female body mass, respectively. *Mhc* genotype-by-immune treatment interactions were never significant; however, allelic diversity was positively correlated with nestling survival. These results show that immune system activation is potentially used as a cue of reduced survival prospect and appears to induce a costly terminal investment behavior, and *Mhc* diversity might be under selection in a natural population of house sparrows.

Key words.—Immune response, *Mhc* class I genotype, Newcastle disease virus, reproductive effort, terminal investment, trade-off.

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Trade-offs have a central role in the theory of life-history evolution (Roff 1992; Stearns 1992). Given that organisms cannot simultaneously maximize all life-history traits (growth, maintenance, reproduction), natural selection is expected to favor the optimal combination of such traits over the life span of individuals (Michod 1979). Optimal investment in current versus future reproduction is likely to depend on the relative life span expectancy (Williams 1966). Indeed, as the residual reproductive value decreases, individuals are expected to increase their investment in current reproduction. This rule has been named ''terminal investment'' as it likely corresponds to the last reproductive bout of a given individual (Clutton-Brock 1984).

Empirical tests of the terminal investment hypothesis are mostly based on patterns of age-specific reproductive effort (Clutton-Brock 1984; Pärt et al. 1992; Hutchings 1993; Langley and Clutton-Brock 1998; Weladji et al. 2002; Yoccoz et al. 2002). As individuals age, their residual reproductive value decreases and as a result they should enhance their reproductive effort. In agreement with this prediction, female collared flycatchers (*Ficedula albicollis*) enhanced their breeding effort as they got older and subsequently paid a high reproductive cost (Pärt et al. 1992). This cost was revealed by a reduction of their survival probability resulting from an increased allocation of resources to current reproduction.

A central question underlying the terminal investment hypothesis concerns the cue used by individuals to trigger a modification of their resource allocation pattern to current versus future reproduction. Mortality rate is not only affected by intrinsic factors such as age but also by extrinsic factors such as predation, food shortage, and parasitism. Infection may be a good indicator of a rapidly diminishing life span (Grenfell and Dobson 1995), and both theoretical and empirical work has shown that parasitized hosts can adaptively adjust their life-history traits to reduce the cost of parasitism (Minchella and LoVerde 1981; Hochberg et al. 1992; Forbes 1993; Lafferty 1993; Polak and Starmer 1998; Adamo 1999; Agnew et al. 2000; McCurdy 2000). For instance, *Biomphalaria glabrata* snails underwent a reproductive burst immediately following exposure to *Schistosoma mansoni* (Minchella and LoVerde 1981). Infections by schistosomes usually result in snail castration so that an increase in reproductive investment may counterbalance the drastic decrease in future breeding opportunities. Following the initial increase in reproductive output, snails exposed but not parasitized lay fewer eggs than nonexposed individuals, corroborating the trade-off between current and future reproduction

(Minchella and LoVerde 1981). Along the same line, Sorci et al. (1996) showed a positive correlation between parasite load and overall reproductive investment, in terms of relative clutch size and investment per young, in the common lizard (*Lacerta vivpara*). However, this study did not resolve whether this was an adaptive response of the host to parasite infection by changing resource allocation, or whether hosts displaying high reproductive efforts had a higher susceptibility to parasites.

Modification of the allocation rule of infected hosts can also be triggered by immune system activation. Recently, several studies have used immune challenges to investigate the costs of immune activation in terms of reproductive output (Williams et al. 1999; Ilmonen et al. 2000; Råberg et al. 2000; Bonneaud et al. 2003). The use of inert antigens permits immune system activation while eliminating the costs associated with parasite infection (i.e., parasite energetic requirements). Inflammatory responses to lipopolysaccharide (LPS) have been shown to be costly and to incur reductions in the reproductive success of immunologically challenged house sparrows (*Passer domesticus*) simultaneously faced with an increase of their feeding workload (Bonneaud et al. 2003). These results point toward the existence of a trade-off between immune response and reproduction (see Sheldon and Verhulst 1996; Norris and Evans 2000) and are in apparent opposition with the terminal investment hypothesis. However, it is essential to bear in mind that this study challenged breeding females during the chick-rearing period and examined the cost of immune activation on parental effort within the same reproductive event (Bonneaud et al. 2003). These results therefore are likely to reflect the inevitable physiological cost of immune function. Interestingly, female house sparrows injected with LPS produced second-brood offspring that grew faster than offspring of control females. Although this result is suggestive of a readjustment of life-history decisions, it may be simply explained by the fact that LPS females saved energy when raising the first brood (Bonneaud et al. 2003).

This last finding led us to test whether diseases may initiate a terminal investment behavior. We manipulated the immune system of breeding female house sparrows in a natural population by injecting them with a Newcastle disease virus (NDV) vaccine. NDV is responsible for a contagious infection characterized by a clinical polymorphism that may result in host death (Alexander 1997). NDV occurs worldwide and many free-ranging birds can function as carriers (Seal et al. 2000 and references therein). In particular, house sparrows have been reported to harbor the infection (Gustafson and Moses 1953; Alexander et al. 1987) and display local prevalence of antibodies to certain NDV strains as high as 69% (Maldonado et al. 1994). The major histocompatibility complex (*Mhc*) encodes for highly polymorphic molecules responsible for recognizing nonself antigenic peptides and then elicits a specific immune response (Janeway et al. 1999). Because of its key role in immune defense, we expect *Mhc* variants to alter disease susceptibilities (Hill 1998). As such, studies on chickens and turkeys show that *Mhc* genotypes are associated with variation in resistance to NDV (Dunnington et al. 1992; Nestor et al. 1996). It is therefore possible that interindividual variation in *Mhc* alleles affects the physiological and behavioral response to the vaccine. To test this possibility we screened the most variable *Mhc* class I gene family, using a polymerase chain reaction (PCR) based genotyping method (Bonneaud et al. 2004). Females were captured and treated immediately after completion of their first clutch and all the eggs were removed to stimulate relaying. This allowed us to control for previous intraseasonal breeding costs to assess reproductive investment in replacement clutches solely on the basis of the immune treatment. If immune stimulation by NDV is used as a cue of a decreasing probability of survival, vaccinated females are expected to make a higher investment in replacement clutches than controls, once the physiological cost of mounting a defense (i.e., time required for antigen clearance, Russell and Ezeifeka 1995; King 1999) is over. We also predicted that reproductive investment of vaccinated females might be modulated by their *Mhc* diversity.

Materials and Methods

This study was conducted during spring 2002 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. Prior to onset of breeding and during egg laying, all nests-boxes were checked daily to determine dates of clutch initiation, completion of laying, and clutch size.

Breeding females were captured at the nest the night after completion of their first clutch (i.e., at start of incubation) and were assigned to two groups. One group received 0.025 ml of a vaccine against NDV (Nobi-Vac-Paramyxo, Buckinghamshire, U.K.) by subcutaneous injection into the back of the neck, the other group received an identical volume of phosphate buffered saline (PBS) as control ($N_{NDV} = 15$, $N_{PBS} = 15$). The NDV vaccine is an oil emulsion with inactivated paramyxovirus serotype-1 (PMV-1). In a pilot study performed on house sparrows maintained in captivity, vaccinated individuals displayed detectable anti-NDV antibodies two weeks after the injection as opposed to controls injected with PBS ($\chi^2 = 17.17$, $P < 0.0001$, $N_{NDV} = 11$, $N_{PBS} = 7$). At capture, we measured body mass (± 0.1 g) and tarsus length (± 0.01 mm), and we sampled blood from the brachial vein (~ 150 μl). This allowed us to assess levels of serum immunoglobulin G (IgG) specifically directed against NDV to determine whether individuals had previously been exposed to the virus. Blood was immediately centrifuged and plasma stored at -20°C.

All the eggs (but not the nest) of the first brood were removed to stimulate laying of a replacement clutch, allowing us to assess the effect of immune challenge on reproductive investment independent of previous effort. Replacement clutches were monitored in the same way as first clutches to gather date of laying, clutch size, and hatching success. In this population, house sparrows are seldom found far from buildings and, during the breeding season, pairs chose to nest in nest-boxes because all crevices within houses and outbuildings were supervised and as much as possible blocked. We can therefore rightfully assume that the females that did not lay a replacement clutch did not do so in unmonitored breeding sites.

Within each replacement clutch, half of the nestlings were immunologically challenged with the NDV vaccine (0.010 ml) when they were 5 days old, while the other half was injected with the same volume of PBS. Blood was sampled from the chicks when they were 5 and 11 days old to assess their anti-NDV antibodies before and after immune challenge. However, because chicks had no measurable levels of anti-NDV antibodies when they were 5 days old and because they did not respond to the vaccine, we did not consider chick immune treatment in the statistical analyses. The lack of detectable antibodies in chicks may be attributed to an immature immune system or to the presence of maternal antibodies avoiding stimulation of the chicks' immune system (yet in too low concentration to be detected). Nestling condition was evaluated by weighing and by measuring tarsus length at days 5, 8, and 11.

To determine whether females had previously been exposed to the virus, we assessed levels of IgG specifically directed against the Paramyxo virus. We used a PMV-1 specific monoclonal antibody blocking ELISA that can detect antibodies present in serum (Czifra et al. 1996) by means of the commercially available test (SVANOVA Biotech, Uppsala, Sweden). Sample antibodies were expressed as the percent inhibition (PI) of the positive control serum. According to the kit, samples with a PI higher than 40% are considered positive for antibodies to NDV, whereas samples with a PI under 30% are negative.

Assessing Mhc Diversity

We screened the females to assess allelic diversity at the 211/214 bp *Mhc* class I gene family using the PCR-based DGGE method (Bonneaud et al. 2004). Genomic DNA was extracted using the Perfect gDNA Blood Mini kit (Eppendorf, Hamburg, Germany) and amplified by PCR using the standard procedures of the GeneAmp PCR kit (Perkin Elmer, Foster City, CA). Amplifications were run in a final volume of 20 μl including 20–50 ng of genomic DNA, 40 ng of GCA21M-fA23M primers (we added a GC-clamp to the A21M primer and a fluoresceine probe to the A23M primer), 2 μl 1× PCR buffer, 0.125 μM dNTP, 2 μM $MgCl_2$, and 0.5 unit of *Taq* polymerase. The reaction was run for 35 cycles in a thermal cycler GeneAmp PCR System 2400 (Perkin Elmer) at 94°C, 64°C, and 72°C for 30 sec each.

The DGGE gel contained 7% of 40% 37.5:1 acrylamide: bis solution, 1× TAE, formamide, and a 40–65% denaturating gradient of urea (Myers et al. 1987). The gels were run at 60°C in 1× TAE buffer for 18 h at 180 V and always included at least one copy of two individuals used as standards. Gels were visualized in a FluorImage SI (Molecular Dynamics, Inc., Sunnyvale, CA). Each DGGE band is considered to be one allele (Bonneaud et al. 2004), so individuals with the highest number of alleles are the most diverse.

Statistical Analyses

We used general linear models and generalized linear models according to the distribution of the dependent variables. The effect of immune treatment on chick growth (tarsus length and body mass) was assessed using mixed models of covariance with nestling identity nested within the nest as a random factor. The number of *Mhc* alleles was always entered in the models, as well as its *z*-transformed squared term (to disrupt colinearity between the two terms of the polynomial equation). This was based on previous work (Wegner et al. 2003; Kurtz et al. 2004), which showed that optimal *Mhc* diversity might reside at intermediate rather than maximum values. Model selection was performed by including the main factors plus the two-way 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).

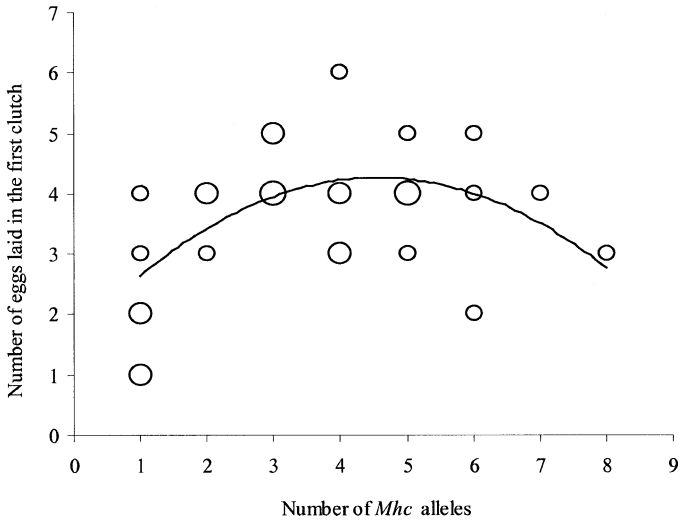

FIG. 1. Number of eggs laid in the first clutch as a function of the female number of *Mhc* alleles. Circles vary in size according to the number of observations (n = 1, 2, or 3).

RESULTS

Upon capture, no female was found to be positive for NDV antibodies (all PI < 30%), suggesting that they were unlikely to have recently been exposed to the virus. Body mass and tarsus length did not differ between NDV and PBS females (*t*-test: body mass: $t_{28} = 0.09$, $P = 0.93$, tarsus length: $t_{19.5} = 1.34$, $P = 0.19$), nor did dates of first clutch laying and numbers of eggs laid on this occasion (*t*-test; date of first clutch laying: $t_{28} = -0.24$, $P = 0.81$; number of eggs laid in the first clutch: $t_{28} = -0.15$, $P = 0.88$). Similarly, *Mhc* allelic diversity did not differ between the two treatment groups (NDV, mean ± SE = 3.64 ± 0.43, $n = 14$; PBS = 3.60 ± 0.60, $n = 15$; Wilcoxon two-sample test, $Z = 0.199$, $P = 0.842$). These results suggest that initial condition was similar across treatments.

Interestingly, *Mhc* diversity was associated with the number of eggs laid in the first clutch in a nonlinear way (Fig. 1). A quadratic regression showed that females with intermediate numbers of alleles had the largest clutch size (slope ± SE, number of alleles = 0.28 ± 0.096, $P = 0.008$; squared number of alleles = −0.14 ± 0.044, $P = 0.004$). Laying date of first clutch was neither related to allelic diversity nor to its squared terms (all *P*-values > 0.35).

About two-thirds of the females (21/30) laid a replacement

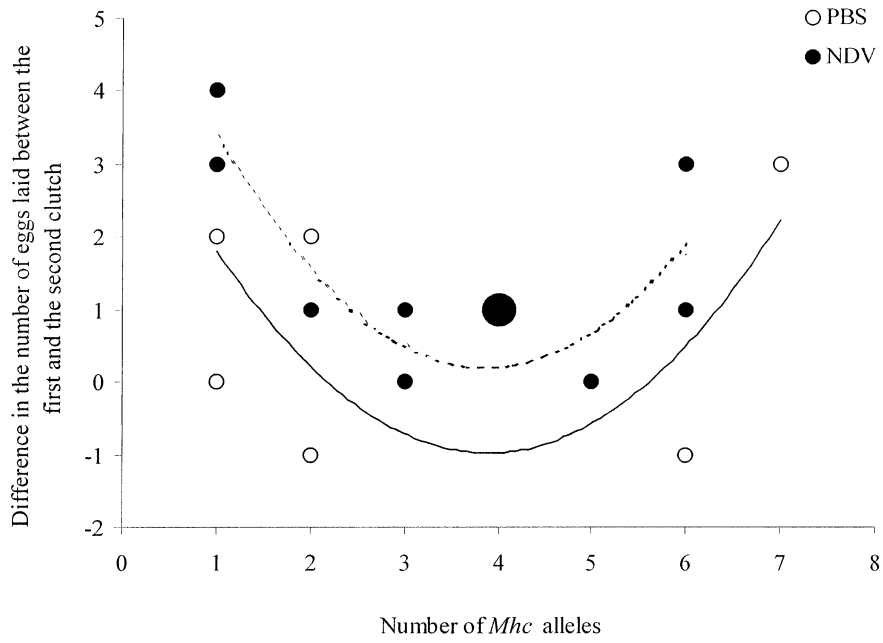

FIG. 2. Variation in the number of eggs laid between the first and replacement clutch in function of the number of *Mhc* alleles for both immune treatment groups. The Newcastle disease virus (NDV) group is symbolized by the dashed line, and the phosphate buffered saline (PBS) group by the solid line. Circles vary in size according to the number of observations (n = 1 or 4).

clutch. Vaccinated females were more likely to lay a replacement clutch than control females, regardless their numbers of *Mhc* alleles (logistic regression: immune treatment, $\chi^2 = 4.12$, $P = 0.042$; number of *Mhc* alleles, $\chi^2 = 1.47$, $P = 0.225$; squared number of *Mhc* alleles, $\chi^2 = 1.15$, $P = 0.283$).

The time interval between first and replacement clutches equaled 18 days on average (range = 8–39). This is slightly higher than the reported amount of time required for antigen clearance in chicken (Russell and Ezeifeka 1995; King 1999). The delay between first and replacement clutches was not significantly affected by any of the independent variables included in the ANCOVA model (immune treatment, $F_{1,15} = 0.98$, $P = 0.338$; number of *Mhc* alleles, $F_{1,15} = 0.89$, $P = 0.360$; squared number of *Mhc* alleles, $F_{1,15} = 0.13$, $P = 0.727$; number of eggs laid in the first clutch, $F_{1,15} = 0.58$, $P = 0.458$).

Variation in number of eggs laid between the first and replacement clutch was affected by the immune treatment as well as by the squared number of *Mhc* alleles (ANCOVA: immune treatment, $F_{1,15} = 5.71$, $P = 0.030$, number of *Mhc* alleles, $F_{1,15} = 0.85$, $P = 0.371$; squared number of *Mhc* alleles, $F_{1,15} = 13.82$, $P = 0.002$; number of eggs laid in the first clutch, $F_{1,15} = 17.20$, $P = 0.001$). For a given number of *Mhc* alleles, the difference between the number of eggs laid in the two clutches was larger for NDV than control females (Fig. 2). Females with intermediate *Mhc* allelic diversity maintained the large clutch sizes they had produced on their first attempt, whereas females with small or large number of *Mhc* alleles laid more eggs in the replacement clutch (Fig. 2).

Hatching success was neither affected by immune treatment nor by number of *Mhc* alleles or its squared term (ANCOVA: immune treatment, $F_{1,16} = 0.07$, $P = 0.795$, number of *Mhc* alleles, $F_{1,16} = 0.26$, $P = 0.615$; squared number of *Mhc* alleles, $F_{1,16} = 0.02$, $P = 0.886$).

We ran two mixed models of covariance to analyze chick mass gain and tarsus growth. Beyond the obvious effects of nestling age and squared nestling age, mass gain was also affected by the interaction between female immune treatment and female body mass (Table 1, Fig. 3). Female *Mhc* diversity did not affect nestling mass gain (Table 1). In the same way, tarsus length was affected by nestling age and squared nestling age (Table 1). In addition, tarsus growth was affected by female immune treatment in interaction with nestling age (Table 1). This implies that nestlings of NDV females were bigger than nestlings of PBS females when they were 5 days old, but this difference disappeared as they got older (Fig. 4). Female *Mhc* diversity did not affect nestling tarsus length (Table 1).

Finally, number of fledged young correlated neither with female immune treatment nor with the total number of *Mhc* alleles or its squared term (ANCOVA: immune treatment, $F_{1,16} = 0.51$, $P = 0.487$; number of *Mhc* alleles, $F_{1,16} = 1.67$, $P = 0.215$; squared number of *Mhc* alleles, $F_{1,16} = 0.74$, $P = 0.403$). Fledgling success (i.e., the proportion of hatchlings that fledged), although independent of female immune treatment, was positively and linearly correlated with *Mhc* allelic diversity (ANCOVA: immune treatment, $F_{1,15} = 0.03$, $P = 0.862$; number of *Mhc* alleles, $F_{1,15} = 11.99$, $P = 0.004$; squared number of *Mhc* alleles, $F_{1,15} = 1.74$, $P = 0.207$; Fig. 5), indicating that nestling survival during rearing was function of female *Mhc* diversity.

DISCUSSION

Challenging the immune system of breeding female house sparrows induced measurable effects on their reproductive

TABLE 1. Mixed models of covariance of nestling body mass (g) and tarsus length (mm) in chicks at 5, 8, and 11 days of age. Sources of variation are female immune treatment (Newcastle disease virus vs. phosphate buffered saline), number of *Mhc* alleles, squared number of *Mhc* alleles, chick age, squared chick age, female body mass, plus all the two-way interactions. Nonsignificant interactions were dropped from the model. Nestling identity within the nest was taken into account as a random factor.

Sources of variation	df	*F*	*P*
Chick mass gain			
Female immune treatment	1,52	4.59	0.037
Number of *Mhc* alleles	1,52	1.17	0.284
Squared number of *Mhc* alleles	1,52	0.99	0.325
Age	1,108	378.40	<0.0001
Squared age	1,108	35.24	<0.0001
Female body mass	1,52	1.58	0.215
Female immune treatment × female body mass	1,52	4.71	0.035
Random factor		*Z*	*P*
Nestling(Nest)		4.15	<0.0001
Sources of variation	df	*F*	*P*
Chick tarsus growth			
Female immune treatment	1,53	9.75	0.003
Number of *Mhc* alleles	1,53	0.40	0.530
Squared number of *Mhc* alleles	1,53	1.86	0.179
Age	1,106	761.81	<0.0001
Squared age	1,106	78.91	<0.0001
Female body mass	1,53	0.08	0.775
Female immune treatment × chick age	1,106	5.73	0.019
Random factor		*Z*	*P*
Nestling(Nest)		3.52	<0.001

investment in the subsequent brood of the same breeding season. Females injected with a NDV vaccine were more likely to lay a replacement clutch after complete experimental removal of their first clutch than control females. Similarly, they invested more in replacement clutches and produced heavier and larger nestlings, although these effects were in interaction with female body mass and nestling age, respectively. Hence, a simulated infection appears to induce an adjustment of allocation rules to current reproduction, in agreement with the terminal investment hypothesis. Interestingly, even though the life-history adjustment induced by immune challenge was not modulated by the number of *Mhc*

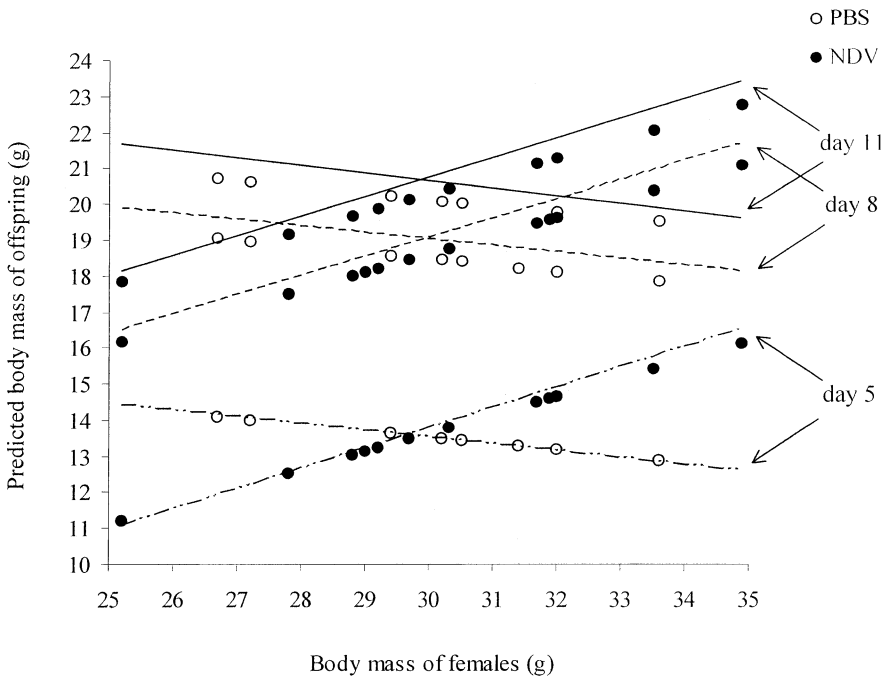

FIG. 3. Chick body mass values (predicted values from a mixed model of covariance) as a function of female body mass for both immune treatments. The Newcastle disease virus (NDV) treatment group is symbolized by black dots and the phosphate buffered saline (PBS) treatment group by white dots. Predicted values of chick body mass are shown at three different chick ages: 5, 8, and 11 days.

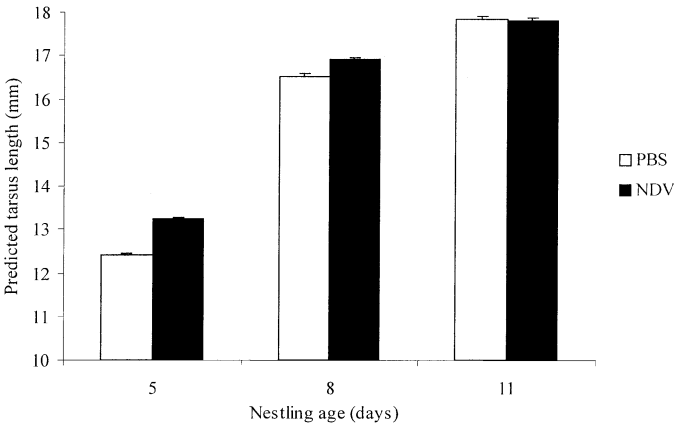

FIG. 4. Chick tarsus length (predicted values from a mixed model of covariance) as a function of chick age (in days) for both groups of female immune treatment. The Newcastle disease virus (NDV) treatment group is symbolized by the black bars and the phosphate buffered saline (PBS) treatment group by the white bars. Error bars represent standard errors.

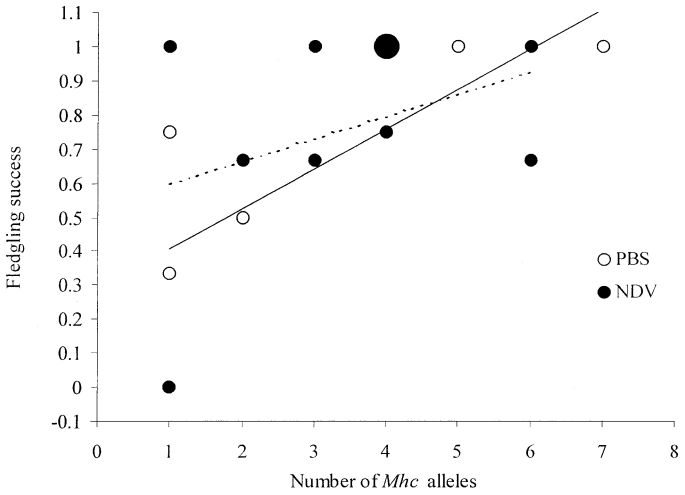

FIG. 5. Fledging success (the proportion of fledged hatchlings) as a function of the number of *Mhc* alleles. The Newcastle disease virus (NDV) group is symbolized by the dashed line and the phosphate buffered saline (PBS) group by the solid line. Circles vary in size according to the number of observations ($n = 1$ or 3).

alleles, *Mhc* diversity (or its squared term) was correlated with two breeding parameters, clutch size and fledging success. This suggests that *Mhc* diversity might be under selection in this population of house sparrows.

Natural selection is expected to shape the combination of life-history traits so as to maximize individual fitness (Roff 1992; Stearns 1992). It is generally accepted that in iteroparous species investment in current reproduction trades off with subsequent survival and/or reproduction (Ghalambor and Martin 2001). As individuals age and their survival prospect diminishes, their residual reproductive value also decreases (Williams 1966). In this case, saving energy for future reproduction might not be worthwhile and selection should hence favor individuals that increase their current reproductive effort as their number of further reproductive attempts declines (Clutton-Brock 1984; Pärt et al. 1992). Beyond age, extrinsic mortality factors can produce the same shift in allocation rule between life-history traits. These factors include physical damage (Geller 1990; Candolin 1999; Fox and McCoy 2000), food availability (Reznick and Bryga 1987), temperature (Kight et al. 2000), social structure (Harvell and Grossberg 1988), and the presence of predators (Crowl and Covich 1990; Reznick et al. 1990) and pathogens (Minchella and LoVerde 1981; Lafferty 1993; Polak and Starmer 1998; Adamo 1999). In this study we show, to the best of our knowledge for the first time, that simulated infections by means of injections with an inert antigen is a sufficient cue to produce an alteration in patterns of reproductive investment in a natural population of house sparrows. The simulated nature of the infection is also suggestive of a plastic response solely governed by the host. This finding is in agreement to those reported for a snail parasitized by a schistosome (Minchella and LoVerde 1981). In this case, exposure of the snails to water that had held the parasite produced an increase of reproductive efforts as predicted by models of evolution of life histories (Gadgil and Bossert 1970; Michod 1979).

Increased reproductive investment of female house sparrows exposed to NDV translated into a higher likelihood of laying a replacement clutch, as well as improved nestling quality. Chick growth in terms of body mass was increased for NDV mothers in good body condition. Similarly, nestlings produced by NDV females had longer tarsi at 5 days of age compared to chicks of PBS females, even though this difference vanished at fledging.

The cost of reproduction can be measured as a decline in fitness either in terms of reduced survival prospect or diminished future reproductive output (Reznick 1992). Previous correlative and experimental work has shown negative effects of current reproduction on survival and subsequent fecundity in the house sparrow (Summer-Smith 1956; McGillivray 1983; Hegner and Wingfield 1987). Because both reproduction and immune defense are costly (Bonneaud et al. 2003), the fitness cost of increased reproductive output for vaccinated females observed in this study is likely to be even more severe than the cost associated with amplified parental effort alone.

One of the major assumptions underlying the terminal investment hypothesis is that the cue used to change the patterns of resource allocation to current versus future reproduction reliably mirrors residual reproductive value. Intrinsic factors such as age, degenerative processes, or accumulation of deleterious mutations are likely to be relevant and reliable cues of declining survival (Finch 1990). Exposure to parasites and pathogens is also a likely source of mortality in most natural populations of animals and plants (Grenfell and Dobson 1995). But how do organisms appraise infection and related mortality risk? Contrary to predation for which the environmental cue triggering the life-history shift can be visual, acoustic, or even chemical (Crowl and Covich 1990), infections are likely to be detected by means of internal physiological cues, mostly provided by the activation of the immune system. Infections with NDV have repercussions on the individual's general status with respiratory, digestive, and nervous symptoms that can provoke host death (Alexander 1997). NDV is known to contaminate many species of wild birds including house sparrows (Maldonado et al. 1994), so

exposure through vaccination potentially mimics a real-life situation to which individuals have evolved. In addition, vaccination programs require multiple administrations to reach a satisfactory level of protection against the virus (Allan et al. 1978). As a result, recovery after one exposure is more likely to be associated to a potential threat of re-infection than to life-long resistance. We are therefore confident that immune activation can be interpreted as a relevant cue for a diminishing environmental quality potentially inducing a decrease in life span.

But should we expect a readjustment of breeding investments regardless of the kind of antigen used for immune system activation? Although there has not been enough experimental work to provide a clear answer to this question, we can speculate on various requirements for the induction of terminal investment behaviors. In a study by Williams et al. (1999), female European starlings injected with sheep red blood cells (SRBC) did not exhibit a higher renesting probability, nor did they rear heavier chicks or show higher fledging success than PBS-injected females. Here the lack of support for the terminal investment theory may stem from the characteristics of the antigen used and hence the type of life-threatening signal it likely produces. Indeed, SRBC is not a pathogen against which individuals naturally coevolved. Other factors that may influence the occurrence or level of behavioral modifications may be the intensity of the immune response or the dose of antigen used to induce it.

Resistance to NDV is known to be influenced by *Mhc* genotypes in chickens and turkeys (Dunnington et al. 1992; Nestor et al. 1996), so the number of *Mhc* alleles could have been a confounding factor in the life-history readjustments set out by individuals challenged with NDV. In theory, the optimal number of *Mhc* alleles is predicted to be determined by the trade-off between two opposing selective forces. The first maximizes the number of alleles to permit the recognition of the largest antigenic peptide repertoire (Arkush et al. 2002; Penn et al. 2002;). In contrast, the number of alleles is limited to minimize the loss of T-cell clones due to self-tolerance induction (thymic negative selection, Nowak et al. 1992). Heterozygocity has been found to be advantageous in multiple-strain infections (Arkush et al. 2002; Penn et al. 2002) and associated with higher fitness (Sauermann et al. 2001). Recent experimental studies have also provided evidence of an advantage associated to the existence of an optimal, rather than a maximal, number of *Mhc* alleles (Wegner et al. 2003; Kurtz et al. 2004).

We found significant relationships between the number of *Mhc* alleles and female fitness traits. The size of first clutches was maximal for individuals with intermediate numbers of *Mhc* class I alleles. In the same way, the difference between the number of eggs laid between first and replacement clutches was minimal for these individuals, indicating that they maintained a high reproductive effort throughout the two breeding attempts. Similarly, fledgling success (the proportion of fledged hatchlings) was linearly correlated with *Mhc* diversity. Sorting out whether it is more beneficial to have more *Mhc* alleles or an intermediate number requires further exploration. Regardless of the type of selection existing on the number of *Mhc* alleles (stabilizing vs. diversifying selection), this is one of the first reports of an association between numbers of *Mhc* alleles and fitness components in a natural population of birds (see also von Schantz et al. 1996).

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