Ethology

Effects of an Immune Challenge on Multiple Components of Song Display in Barn Swallows *Hirundo rustica*: Implications for Sexual Selection

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Abstract

Bird song is believed to honestly reveal male quality including the ability of singers to face parasitism. In a natural population of barn swallows *Hirundo rustica*, we experimentally imposed a cost on song production by an immune challenge. We therefore vaccinated a group of reproducing males with an antigen (Newcastle disease virus), and injected phosphate-buffered saline to a control group. Immune challenge significantly reduced one song feature, rattle duration. This decrease was related to male quality, as measured by tail length, because males with short tails reduced the duration of their rattle significantly more than males with long tails. In addition, another song feature, strophe duration, decreased in the control group, while it remained constant in the challenged group. Duration of the rattle has previously been found to be positively related to testosterone level, and it may hence reflect male competitive ability. Thus, male barn swallows may not have the potential to produce long rattles when their immune system is challenged. By maintaining their strophe duration after an immune challenge, males may compensate for the decrease in rattle duration. Our results suggest that different song features may convey different types of information, and that barn swallow song, in particular their rattle, may reliably reveal information about activation of the immune system.

Introduction

Song in male passerines is a sexual signal that is often assumed to have evolved under two main selection pressures: male competition (intra-sexual selection) and female choice (inter-sexual selection) (Darwin 1871; Andersson 1994). Sexual selection may thus have shaped this behavior to provide receivers with reliable information about phenotypic and genetic quality or attractiveness of the male signaler (Fisher 1930; Zahavi 1975; Hamilton & Zuk 1982). There is indeed growing evidence that song production displays condition dependence and hence provides reliable information about the quality of the signaler (Catchpole & Slater 1995; Buchanan et al. 1999; Nowicki et al. 2002). In fact, bird song is a complex trait and different song features may be affected by and hence reflect different factors such as maternal effects, social aggression, environmental conditions, and parasitism (Vehrencamp 2000; Gil & Gahr 2002).

An increasing number of studies have shown that males trade the expression of their sexual signals against the efficiency of their immune defense (Weatherhead et al. 1993; Birkhead et al. 1998; Kilpimaa et al. 2004; Peters et al. 2004). In particular, birds may face immune costs during song development (Spencer et al. 2005) and production (Møller 1991; Saino et al. 1997b; Buchanan et al. 1999; Møller et al. 2000; Garamszegi et al. 2004). Parasites may therefore be the cause of selection that influences the evolution of their host's song (Hamilton & Zuk 1982; Sheldon & Verhulst 1996).

The immunocompetence handicap hypothesis (Folstad & Karter 1992) suggests that a trade-off between song production and immune function is due to the antagonistic effects of testosterone or other hormones (but see Buttemer & Astheiner 2000; Hasselquist et al. 1999; Roberts et al. 2004). On the one hand, testosterone stimulates the expression of sexual signals, and song expression in particular (Catchpole & Slater 1995; Schlinger 1997; Pinxten et al. 2002), but on the other hand testosterone may lead to immunosuppression (Grossman 1990; Saino et al. 1995; Zuk et al. 1995; Casto et al. 2001; Lindström et al. 2001). An alternative mechanism to immunosuppression is based on resource allocation, if song production and immune function have competing energetic demands (Wedekind & Folstad 1994; Sheldon & Verhulst 1996). These hypotheses predict that only high-quality males can invest in song display while maintaining adequate immune defense.

Here, we analyzed the influence of immune response on the song display of male barn swallows *Hirundo rustica*, a socially monogamous passerine. For this, we injected a novel antigen vaccine [Newcastle disease virus (NDV)] to a group of reproducing males, while a control group was injected with phosphate-buffered solution. We then analyzed song production before and after treatment while comparing the two experimental groups.

As song is a complex trait, different types of information may be transmitted to different receivers by different aspects of the signal. For instance, one component might be used in individual recognition while another repels male intruders (e.g. Dabelsteen & Pedersen 1988; Lampe 1991). Catchpole (1987) suggested that sexual selection may affect the evolution of different facets of bird songs in different ways. In support of this idea, certain components but not others might influence female behavior (Marshall et al. 2005), might change during male–male competition (Guerra & Mason 2005) or might help, predict individual reproductive success (Rehsteiner et al. 1998). In the barn swallow, Galeotti et al. (1997) found that males surrounded by a large number of neighbors reduced strophe duration and complexity, but increased rattle duration, a component positively linked to circulating testosterone levels. In addition, Garamszegi et al. (2005) found that strophe duration in the barn swallow was positively related to male age and chewing lice resistance. Moreover, song rate is positively linked to gain of extra-pair paternity (Møller et al. 1998), negatively correlated with the intensity of mite load (Møller 1991) and correlated with hematological variables reflecting bird immune condition (Saino et al. 1997b). Garamszegi et al. (2006) found that different sexual signals reflect different aspects of male quality in barn swallows (the multiple signal hypothesis, Møller & Pomiankowski 1993). Our target was to assess experimentally whether song features are directly modified by immune challenge. Following the immunocompetence handicap hypothesis, we would predict in particular that rattle duration, which is positively correlated with plasma testosterone levels (Galeotti et al. 1997), would decrease after immune challenge.

We also tested whether the change in song output in response to infection could be predicted by male quality, as measured by tail length, a sexually selected character in European barn swallows (Møller 1994b). According to the immunocompetence handicap hypothesis and life-history theory, high-quality males could afford song displays without suffering high immune costs (Folstad & Karter 1992). The tail length of male barn swallows influences female choice (Møller 1988), and reliably signals viability (Møller 1994a). Moreover, as males with short tails suffer more from immunosuppression than males with long tails (Saino et al. 1997a), we predicted that song output would be reduced after an immune challenge, and that the decrease would be larger among males with short tails.

Methods

The study was carried out in Mar. to Apr. 2004, in a farmhouse near Badajoz (38°50′N, 6°59′W), Extremadura, Spain, where a population of barn swallows has been subjected to a long-term study. All adults were captured with mist nets and provided with a numbered aluminum ring and a unique combination of color plastic rings. Birds were also marked on the belly with water-resistant pens for focal identification. Sex was determined from behavioral observations and confirmed at later recaptures by inspection of the incubation patch. At first capture, we recorded tail length (mean of left and right outermost tail feathers) with a ruler, to the nearest 0.5 mm, tarsus length (mean of left and right tarsus length) with a digital caliper, to the nearest 0.01 mm, and body mass with a Pesola spring balance, to the nearest 0.5 g. Ringing data were used to assess male age; unringed birds were considered to be 1 yr old. This assumption is based on the fact that all birds from our study site (nestlings and adults) are ringed every year, and only 1-yr-old individuals are known to disperse in this species (for more detail, see Garamszegi et al. 2005).

A total of 34 males that had settled in a breeding territory had their songs recorded. Males were captured from 1 to 14 d after recording [7.5 ± 3.3 (SD)] and were randomly selected to receive either intraperitoneal placebo injection [one-third of experimental males, 20 μl of sterile phosphate-buffered saline (PBS)] or NDV vaccine (two-thirds of experimental males, 20 μl of Clona 30 strain of NDV, ≥50 PD50 units per 0.5 ml, Nobivac® Paramyxo; Intervet, Milan, Italy). We successfully recorded the song of seven controls and 21 treated individuals a second time from 6 to 14 d after injection [7.9 ± 2.1 (SD)]. These 28 experimental males were thus recorded on two recording sessions (pre-treatment and post-treatment). We recaptured these males 11 and 22 d after injection to measure their body mass. Male body condition was estimated on the day of treatment and then 11 and 22 d after treatment as residuals from a regression of body mass on tarsus length.

In addition, we evaluated the natural temporal change in song production within the first recording session for the males recorded more than 1 d (n = 31; with four non-experimental, seven control and 20 vaccinated males). The mean number of recording days for these males was 4.06 ± 1.48 (SD), and the interval (difference between last day and first day of recording for a male) was 7.87 ± 4.74 (SD).

Recordings and Song Analysis

We performed song recordings in the morning (from 07:30 to 12:30 hours.) close to the nest or on a perching place, using shotgun microphones Audio Technica (Stow, OH, USA) AT835B connected to a Marantz (Osnabrück, Germany) PMD101 cassette recorder. Male barn swallows sing successions of strophes (song units separated by a pause) of two different types (see Galeotti et al. 1997). Strophe type A is composed of varied and complex syllables arranged in various sequences (Fig. 1), while strophe type B consists of a succession of simple contact calls. For each male, we estimated the syllable repertoire size of type A strophes, as did Galeotti et al. (1997). For other song variables, we analyzed all strophes recorded (type A and B), because one of the males produced only type B strophes after treatment. The results were however very similar when considering only strophes of type A, as did Galeotti et al. (1997).

We considered only complete strophes that are characterized by the presence of a terminal rattle (a rapid succession of impulses sung at high rate, Fig. 1). Song was analyzed with the computer program AVISOFT-SASLAB PRO (Avisoft Bioacoustics, Berlin, Germany) (Specht 1998). Spectrograms were generated using a 1024-point FFT (Fast Fourier transform) and a Hamming window with 25% overlap (frequency resolution 22 Hz, time resolution 0.72 ms). Strophe and rattle duration were measured using a cursor on spectrograms. Repeatability of measurements was very high, especially for rattle duration, as impulses are very short notes assimilated to a thin line on the screen. For each male, we measured the following variables before and after treatment:

1 Strophe duration (mean duration of all complete strophes, to the nearest 0.001 s).

2 Syllable repertoire size of type A strophes. Syllable types were classified visually in 19 categories by A. N. D. A second inspection of all strophes was performed blindly with respect to the first inspection, and this second inspection produced exactly the same results. Repertoire size was estimated as the count of syllable types in more than three strophes, which is expected to give a reliable estimate of the real repertoire size (Galeotti et al. 1997).

3 Rattle duration (mean duration of the terminal rattle of all strophes, to the nearest 0.001 s).

4 Song rate (proportion of time spent singing during a 30-min sampling period). For 20 males,

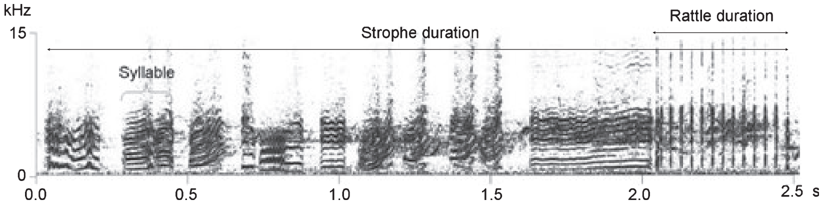

Fig. 1: Sonogram of a type A strophe of a barn swallow song.

two measurements were recorded on two different mornings. The two measurements were subsequently averaged and used in subsequent analyses.

All these recordings were made blindly with respect to treatment and tail length. The mean number of complete strophes analyzed per experimental individual was 22.7 ± 9.7 (SD) before treatment and 16.3 ± 7.4 (SD) after treatment. These strophes were recorded on different days for some of the males [mean number of recording days per experimental male before treatment: 3.6 ± 1.7 (SD); after treatment: 2.6 ± 1.1 (SD)]. The range of recording days was 5.8 ± 4.1 (SD) before treatment and 3.0 ± 2.6 (SD) after treatment. The different measurements of strophe duration and rattle duration performed on different days within the same recording session were averaged and used in the subsequent analyses.

Statistical Analysis

Variables did not differ significantly from normality using the Kolmogorov–Smirnov test (strophe duration: $p = 0.10$; syllable repertoire size: $p = 0.11$; rattle duration: $p > 0.15$; song rate: $p = 0.10$; tail length: $p > 0.15$; body condition: $p > 0.15$). Sample values are given as mean ± SE. All statistical analyses were performed with the SAS computer program (SAS 1999). Associations between male traits were assessed by simple Pearson correlations. We used the Bonferroni correction to adjust the significance level for the number of tests performed (Holm 1979).

ANOVA was used to assess the repeatability of song variables. Song rate repeatability was evaluated by comparing measurements from two observation periods on different mornings before treatment. Repeatability of strophe and rattle duration was estimated among days before treatment.

We performed Wilcoxon matched-pairs signed-ranks tests to determine whether two treatment groups differed significantly before treatment or in their response to treatment. We also performed Wilcoxon matched-pairs signed-ranks tests and t-tests to compare song output before and after treatment within control and vaccinated group, respectively. These non-parametric tests are appropriate as the sample size of the control group was smaller than 20.

To evaluate the change in song production due to temporal factors, we examined the change in song output within the first recording session for all males recorded on several days. We used a repeated-measures mixed general model with male identity as a repeated factor, day as independent variable and strophe or rattle durations as dependent variables. The first day of recording was given a value of one for each male.

We tested for the potential effect of tail length and change in body condition 11 d after treatment within the NDV-vaccinated group in a general linear model. Song after treatment was used as the dependent variable, and we used song before treatment, tail length, change in body condition and the interaction between variables as covariates.

Ethical Note

Newcastle disease virus vaccine is a non-replicative antigen that induces a humoral immune response. NDV-vaccinated male barn swallows did not suffer a decrease in reproductive success in comparison with control males (total number of fledglings produced: $Z = 1.19$, $p = 0.24$; mean fledgling body mass: $Z = 1.25$, $p = 0.21$). NDV-vaccinated males suffered a slight loss in body mass 11 d after injection in comparison with control males, but this difference was not significant 22 d after injection (see Results). We found no other adverse effects and no experimental male died during the reproductive period, although long-term effects have not been recorded.

Results

Song features were consistently repeatable within individuals among days before treatment (Strophe duration: $F_{33,81} = 2.01$, $R^2 = 0.54$, $p = 0.01$; Rattle duration: $F_{33,82} = 2.92$, $R^2 = 0.64$, $p = 0.0002$; Song rate: $F_{19,20} = 21.41$, $R^2 = 0.95$, $p < 0.0001$). Song variables were thus assumed to provide reliable estimates of male song production. Syllable repertoire size was estimated only once and thus could not be subjected to repeatability estimation.

Song features did not differ significantly between the control group and the vaccinated group before treatment (Strophe duration: $Z = 1.09$, $p = 0.28$, vaccinated: 4.142 ± 0.101, control: 4.351 ± 0.104; Syllable repertoire size: $Z = 0.77$, $p = 0.44$, vaccinated: 12.8 ± 0.4, control: 13.5 ± 0.4; Rattle duration: $Z = 0.84$, $p = 0.40$, vaccinated: 0.384 ± 0.001, control: 0.399 ± 0.012; Song rate: $Z = -0.68$, $p = 0.50$, vaccinated: 0.31 ± 0.03, control: 0.29 ± 0.07). The two treatment groups did not differ significantly in age ($Z = -0.32$, $p = 0.75$), body condition before treatment ($Z = 0.17$, $p = 0.87$), laying date ($Z = 0.02$, $p = 0.98$), recording date before ($Z < 0.0001$, $p >$

0.99) and after treatment ($Z = 1.44$, $p = 0.15$), and recording time of the day before ($Z = -0.67$, $p = 0.50$) and after treatment ($Z = -0.61$, $p = 0.54$).

Strophe duration and syllable repertoire size were the only song variables significantly correlated before treatment ($n = 34$, $r = 0.56$, $p = 0.0006$, still significant after Bonferroni adjustment for six statistical tests: critical p-value: $0.05/6 = 0.008$). Song variables were not significantly correlated with body condition and tail length (all $p > 0.1$). Body condition and tail length were not significantly correlated ($r = 0.18$, $p = 0.24$).

Effect of Treatment on Body Condition

Newcastle disease virus-vaccinated male barn swallows suffered a reduction in body condition compared with control males, as the change in body condition 11 d after treatment was significantly larger for the control group than for the vaccinated group ($Z = 2.04$, $p = 0.042$, vaccinated: -0.11 ± 0.12, control: 0.13 ± 0.11). This difference in change of body condition was no longer significant 22 d after treatment ($Z = 1.68$, $p = 0.094$, vaccinated: -0.08 ± 0.11, control: 0.23 ± 0.10).

Effect of Treatment on Song

Syllable repertoire size and song rate were not significantly affected by treatment (Syllable repertoire size: $Z = -0.60$, $p = 0.55$, vaccinated: -0.6 ± 0.3, control: -0.8 ± 0.3; Song rate: $Z = -0.08$, $p = 0.94$, vaccinated: -0.05 ± 0.06, control: -0.10 ± 0.06). However, change in strophe duration ($Z = -2.12$, $p = 0.034$, vaccinated: 0.013 ± 0.091, control: -0.477 ± 0.163) and rattle duration ($Z = 2.12$, $p = 0.034$, vaccinated: -0.017 ± 0.026, control: 0.013 ± 0.005) differed significantly between treatment groups (Fig. 2). NDV-challenged males tended to increase their strophe duration after injection and decrease their rattle duration in comparison with PBS-injected males (Fig. 2). These differences were due to a significant decrease in rattle duration for vaccinated males ($n = 21$, $t = -2.19$, $p = 0.040$) and a significant increase in strophe duration in control males ($n = 7$, Wilcoxon matched-pairs signed-ranks tests, $U = -13$, $p = 0.031$). On the other hand, change in rattle duration was not significant among control males ($n = 7$, Wilcoxon matched-pairs signed-ranks tests, $U = 6$, $p = 0.38$), and no significant change in strophe duration was observed in vaccinated males ($n = 21$, $t = 0.14$, $p = 0.89$). A *post hoc* analysis showed that the difference in rattle duration between the two treatment groups was due to a change in the number of impulses in the rattle ($Z = 2.02$, $p = 0.044$) rather than a change in the impulse rate ($Z = -1.06$, $p = 0.29$, vaccinated: -0.11 ± 0.26, control: -0.56 ± 0.19). Experimental males lost on average one impulse per rattle after vaccination (-0.9 ± 0.8, $n = 21$, $t = -2.73$, $p = 0.013$), while control males did not significantly change their number of impulses after injection (0.2 ± 0.3, $U = 6$, $p = 0.38$).

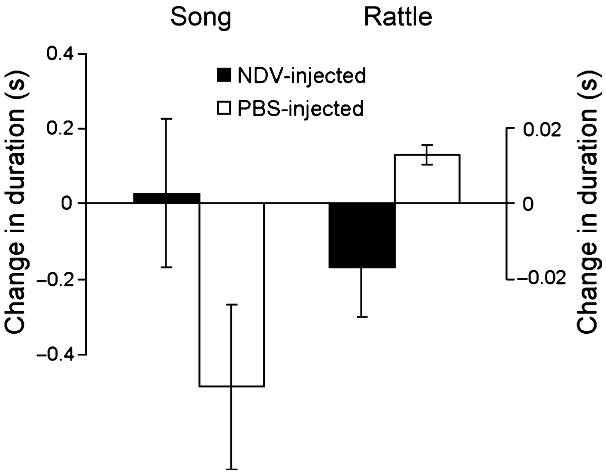

Fig. 2: Effect of immune treatment on male barn swallow song. Change in strophe duration (without the rattle) and rattle duration for Newcastle disease virus (NDV)-vaccinated males (injected with NDV vaccine, striped bars, $n = 21$) and controlled males (injected with sterile phosphate-buffered saline, grey bars, $n = 7$). Values are mean ± SE (in s).

Effect of Vaccination in Relation to Male Quality

In vaccinated males, rattle duration after treatment was significantly positively related when rattle duration before treatment ($F_{1,17} = 12.70$, $p = 0.0024$) and tail length ($F_{1,17} = 15.01$, $p = 0.0012$; Fig. 3), and was significantly related with the interaction between rattle duration before treatment and tail length ($F_{1,17} = 16.90$, $p = 0.0007$). The final model was highly significant, explaining 81% of the variance ($F_{3,17} = 9.16$, $R^2 = 0.81$, $p < 0.0001$). Other song features were not significantly related to male tail length after vaccination (Strophe duration: $F_{1,19} = 1.29$, $p = 0.\ 27$; Song rate: $F_{1,18} = 2.03$, $p = 0.\ 17$; Repertoire size: $F_{1,18} = 0.46$, $p = 0.\ 51$). No song variables after treatment were significantly related to change in body condition in vaccinated males (Rattle duration: $F_{1,19} = 1.07$, $p = 0.\ 32$; Strophe duration: $F_{1,19} = 0.02$, $p = 0.\ 90$; Song rate: $F_{1,18} = 0.01$, $p = 0.\ 98$; Repertoire size: $F_{1,18} = 3.65$, $p = 0.\ 08$).

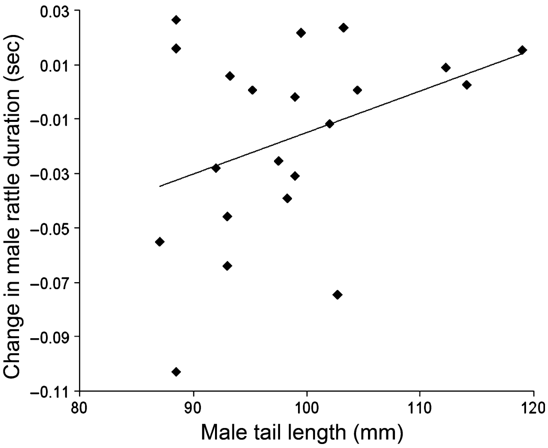

Fig. 3: Relationship between change in male rattle duration (s) and male tail length (mm), for Newcastle disease virus-vaccinated male barn swallows. The line represents the linear regression ($y = 0.002x - 0.167$, $n = 21$).

Temporal Change in Strophe and Rattle Duration Before Treatment

Rattle duration did not change significantly over days during the first recording session (mixed model with individual set as a repeated factor, day of recording as co-factor and rattle duration as the dependent variable: $F_{1,110} = 0.03$, $p = 0.86$), but strophe duration decreased significantly (similar model with strophe duration as the dependent variable: $F_{1,109} = 4.77$, $p = 0.03$).

Discussion

This study shows that two features of male song were affected by vaccination with NDV in our barn swallow population: rattle duration, which decreased, and strophe duration, which increased in vaccinated compared with control males. Hence, immune challenge affected male song production in two different ways. Finally, the effect of treatment on song features was contingent upon male quality as reflected by the size of a secondary sexual character, tail length.

Our experiment showed that rattle duration decreased after treatment among NDV-vaccinated males in comparison with PBS-injected control males. We propose two hypotheses to explain this decrease. One hypothesis is based on energy allocation (Wedekind & Folstad 1994), as both song and immune response are believed to be energetically costly activities (Brackenbury 1982; Eberhardt 1994; Moret & Schmid-Hempel 2000; Martin et al. 2002; Bonneaud et al. 2003; but see: Oberweger & Goller 2001; Ward et al. 2003). If there is a competing demand from both immune activation and song production, males could be forced to decrease the duration of their rattle when immune activity is required (Wikelski & Ricklefs 2001). Indeed, we found that injection of NDV vaccine reduced body condition in male barn swallows, suggesting that mounting an immune response is energetically costly (see also: Deerenberg et al. 1997; Klasing et al. 1987). Another possible mechanism is linked to the immunosuppressive property of testosterone (Folstad & Karter 1992). Rattle duration is positively correlated with plasma testosterone levels in barn swallow (Galeotti et al. 1997). Hence, if testosterone level decreases to allow development of an immune response, or if there is a reduction in testosterone level because of challenge to the immune system (De Vaney et al. 1977; Zhang et al. 2000; Owen-Ashleya et al. 2006), then it may have negative effects on the duration of the rattle. The change in testosterone level would have to be larger than the slight variations that naturally occur during the breeding cycle in the barn swallow (Saino & Møller 1995) as in many other bird species (Wingfield et al. 1994; Caro et al. 2005). As we performed our experimental manipulation on birds during the same breeding stage, it is possible that short-term variation in hormonal level could be biologically significant under natural conditions.

Secondly, strophe duration decreased after treatment in PBS-injected males, while it remained constant in NDV-vaccinated males. This result does not support the prediction that song output should decrease for challenged males. Although the sample size of the control group is relatively small, change in strophe duration over days during the first recording session for 31 males confirmed this tendency of decrease in strophe duration during the breeding season. This decrease in strophe duration among non-vaccinated males may be due to changes in resource availability, or changes in environmental conditions (e. g. increased day length and temperature). Song is known to fluctuate during the breeding season in several species (Merilä & Sorjonen 1994; Kloubec & Capek 2005; Kunc et al. 2005). In the barn swallow, social and competitive conditions are also changing temporally, as the number of males increased and the number of unmated females decreased during the breeding season, and this may account for the observed modification of strophe duration among control males. Contrary to that observed for control males, no change in the duration of the strophe was observed in NDV-vaccinated males although they exhibited decreased rattle duration. Previous studies of the barn swallow have shown that strophe duration and rattle duration are inversely correlated in different contexts (Galeotti et al. 1997). In a competitive context (e. g. when males have many neighbors), males emphasize the rattle, which could reflect male competitive potential, while they decrease strophe duration (Galeotti et al. 1997). Furthermore, males decrease the duration of the rattle and sing longer strophe when they have few neighbors (Galeotti et al. 1997). Therefore, vaccinated males may compensate for the decrease in rattle duration by emphasizing strophe duration. This emphasis is possible if the duration of the strophe, which is not related to testosterone level (Galeotti et al. 1997), does not trade against immune activity, or if long strophes are energetically less demanding than long rattles. The emphasis on strophe duration in vaccinated males may allow such males to compensate for the decrease in rattle duration, while reaching a threshold of stimulation, if a minimum signaling value is required for a female to mate (Holland & Rice 1998).

Syllable repertoire size and song rate were not significantly affected by immune challenge with NDV. Some studies found that repertoire size was negatively correlated with parasitism in birds (Buchanan et al. 1999; Møller et al. 2000), although this is not a general result (Read & Weary 1992; Weatherhead et al. 1993), and it has not been found in the barn swallow (Saino et al. 1997b). Song rate in barn swallows is negatively correlated with parasite load (Møller 1991), the concentration of lymphocytes in blood, and the gamma-globulin to plasma protein ratio (Saino et al. 1997b). We cannot exclude that a lack of statistical power in our experiment led to the absence of detection of changes in repertoire size and song rate with experimental treatment. However, our results suggest that these song features do not suffer from the short-term effect of immune challenge by NDV, and that they might be linked to other aspects of male quality.

The song response of NDV-vaccinated males was related to their tail length. Male rattle duration after NDV-injection was positively related to male tail length and rattle duration before injection. Rattle duration was less sensitive to immune challenge in males with long tail. Tail length in barn swallows is an important secondary sexual character involved in female choice (Møller 1988), and signals viability (Møller 1994a). In some animal species female preference has been suggested to be directed towards males that signal resistance to parasites (Taskinen & Kortet 2002; Rantala & Kortet 2004). In the barn swallow, our experiment suggests that long-tailed males may be able to sustain costs of immune response while maintaining a high level of song performance.

In contrast, variation among males in song response was not significantly related with variation in body condition, although change in body condition was smaller for vaccinated than for control males. However, other variables that we did not measure, such as testosterone levels or antibody production, may account for this variation in song change among males.

Our results show that song, and especially the rattle of male barn swallows is affected by challenge to the immune system. This suggests that male song may be used as an honest signal of male quality. The present study and previous work on the barn swallow have shown that different song features may convey different types of information, being influenced by different aspects of male quality, such as competitive ability, age and health status. Although we lack information on the reaction of receivers of the song to experimental treatment, they may obtain different kinds of information about the male signaler. Females in particular may obtain different types of benefits by using song cues to assess the current health state of males. By using rattle production, they may avoid horizontal transmission of parasites (Sheldon 1993; Hillgarth 1996), and they may benefit from increased parental care, if healthy males invest more in feeding chicks. The latter hypothesis can be rejected, because the most attractive male barn swallows feed relatively less than the average male (Møller 1994b,c). Moreover, females may use male tail length to judge male ability to deal with immune challenge and subsequently benefit from genetic transmission of parasite resistance to their offspring (Hamilton & Zuk 1982; Møller et al. 2004). Finally, by using multiple signals, females may acquire reliable information about different aspects of male quality and immune status at their disposal (Møller & Pomiankowski 1993). The change in rattle duration and in number of impulses were subtle (approx. 4% and 7%, respectively), raising the question of whether barn swallows could detect such a small difference. However, as rattle duration remained constant during the period considered (for all males before treatment and for control males before and after treatment) and was highly repeatable, it is likely that this song component is important in sexual selection, as shown for similar rapidly repeated elements in other species (Vallet & Kreutzer

1995; Illes et al. 2006; Schmidt et al. 2008). A next step would be to test whether receivers detect subtle variations in rattle performance, and how this may affect their behavior.

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