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Age-dependent health status and song characteristics in the barn swallow

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Bird song has been hypothesized to evolve, partly, to signal health status of males, and song features should therefore correlate with parasite load. Immune function, parasitism, and secondary sexual characters can, however, differ between age classes, and any apparent relationship between song and parasite loads can be the result of systematic age effects. We tested for an age-dependent relationship between sexually selected characters and measures of parasitism in a Spanish population of the barn swallow *Hirundo rustica*. A comparison across age classes revealed that chewing lice load, song duration, mean peak amplitude frequency of songs, and tail length differed significantly between yearlings and adults. In a longitudinal analysis, we found significant evidence for mean peak amplitude frequency of songs, tail length and chewing louse parasitism, and a nonsignificant tendency for song duration to change with age of an individual. We found a significant association between song duration and chewing louse load and between hematocrit and peak amplitude frequency of the rattle, the typical harsh terminal syllable. In tests for associations between song traits and health status, while controlling for age, age and chewing louse load were independently related to song duration. We found a significant relationship between pairing success and song duration, implying that females may use this song trait in their choice of parasite-free males. Although the song of the barn swallow may provide information about both male age and parasite resistance, signaling of health status appears to be independent of age effects, in accordance with the theory of parasite-mediated sexual selection, suggesting that male signals can be used as reliable indicators of parasitism. *Key words:* age, barn swallow, bird song, chewing louse, hematocrit, sedimentation rate. *[Behav Ecol 16:580–591 (2005)]*

Parasite-mediated sexual selection theory suggests an important role for parasites in the evolution of sexually selected characters based on female choice (review in Møller et al., 1999). Because bird song is usually considered to have evolved in the context of sexual selection (Searcy and Andersson, 1986; Searcy and Nowicki, 2000), females may prefer mates with elaborate songs because such males by singing complex songs at high performance reveal their relative resistance to and therefore freedom from debilitating parasites (Hamilton and Zuk, 1982). Females that select males with high-quality songs may thus obtain resistance genes for their offspring (Hamilton and Zuk, 1982), avoid the direct transmission of parasites (Able, 1996), or enjoy paternal help of superior quality (Hamilton, 1990; Price et al., 1993). A fundamental prediction of these hypotheses is that there should be a correlation between song characteristics and parasitism across individuals. This predicted relationship has been studied intensively in recent years, and available evidence provided mixed support as some tests were confirmatory (e.g., Buchanan et al., 1999; Garamszegi et al., 2004; Redpath et al., 2000; Saino et al., 1997a) but others were not (Birkhead et al., 1998; Weatherhead et al., 1993). Overall, the association between parasitism and expression of secondary sexual characters across studies is weakly negative but significant, suggesting an overall weak effect (Møller et al., 1999).

Several factors have been raised to explain such deviations between empirical and predicted relationships between parasites and sexual traits. These include the immunosuppressive side effects of testosterone that enhances trait elaboration (Folstad and Karter, 1992), the narrow focus in terms of both secondary sexual traits and parasites considered (Clayton, 1991), the investigation of relatively harmless parasites and the effects of low sampling effort (Møller et al., 1999), and the possibility that the relationship between parasites and health may vary across signalers differing in quality (Getty, 2002). However, age may also be an important, overlooked factor that can potentially mediate the relationship between song and parasitism, although such age effects mediating health status signaling via songs have usually been neglected (Thomas et al., 1995).

The abundance of parasites may vary between older and younger individuals. Parasitism can negatively covary with host age when host mortality is induced by parasite accumulation (Thomas et al., 1995). For example, species exposed to a greater abundance of parasites that attack juveniles probably exhibit greater juvenile mortality compared with species exposed to fewer parasites. Alternatively, the efficiency of the immune system may change with ageing, and different parasite loads of different age classes can be a consequence of systematic age-dependent changes in immune function. Many examples in a wide variety of animals of different taxa, including humans, have revealed significant age-class differences in parasite load, suggesting that the most severe effects of parasites often occur in nonreproductive age classes (Armstrong et al., 1999; Borgia and Collis, 1989; Buchholz, 1995; Hecking-Veltman et al., 2001; Müller-Graf et al., 1997; Poulin, 1992; Proudman et al., 1997; Rehbein et al., 2002).

Age has been thought to play a role in sexual selection, as male ornaments are often more developed in old than in young males (Andersson, 1994; Manning, 1985; Trivers, 1972). Age-dependent elaboration of sexual signals in males may be explained in two ways. First, the ornament may be designed to signal male age per se, as male quality may reflect ability to survive. Second, the ornament may reflect male

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condition that improves with age through experience. Therefore, if trait expression reliably signals male age, choosy females by preferring extravagant sexual characters may gain from pairing with older individuals. Females mating with such males may obtain either direct fitness benefits if older males have territories of high quality (Alatalo et al., 1986a) or provide superior parental care through improved foraging or defense against predators (Alatalo et al., 1986b; Marchetti and Price, 1989; Yasukawa, 1981) or absence of pathogens (Saino et al., 2003), or they may obtain genetic benefits for the offspring if viability is heritable (Trivers, 1972).

Bird song is a typical trait that is known to vary consistently between age classes. This may occur because (1) song can change during life or (2) differential survival may be related to song. In many oscine species, song or syllable repertoire size increases from young to older birds (Birkhead et al., 1997; Cosens and Sealy, 1986; Eens et al., 1992a,b; Espmark and Lampe, 1993; Galeotti et al., 2001; Gil et al., 2001; Hasselquist et al., 1996; Mountjoy and Lemon, 1995; Nottebohm F and Nottebohm ME, 1978), although not in all studies (Catchpole, 1986; McGregor et al., 1981; Searcy et al., 1985). Song learning is ubiquitous in oscine passerines, and the continuous expansion of repertoires may depend on learning processes (Kroodsma, 1982). Therefore, the link between age and repertoire size may be largely due to processes associated with individual experience (Eens et al., 1992a,b; Galeotti et al., 2001; Gil et al., 2001). Studies that tested for similar relationships between other song attributes (e.g., song performance or song structure) and male age showed inconsistent results (Galeotti et al., 2001; Garamszegi et al., 2004; Gil et al., 2001). Neural structures in the brain that are associated with song production also show age-dependent changes (Absil et al., 2003; Adret and Margoliash, 2002; Bernard et al., 1996; Wang et al., 2002).

According to the theory of parasite-mediated sexual selection, male song should allow females to assess male ability to resist parasites, predicting a negative correlation between parasite load and song elaboration. However, given that parasitism can covary with host age and that both the expression of songs and female preferences may be age dependent, the negative correlation between sexually selected traits may incorrectly suggest a causal relationship between parasitism, signal elaboration, and mating success. If immune function or parasite load show consistent changes with age, and similarly, if older individuals have more extravagant secondary sexual traits, then the relationship between the expression of the secondary sexual trait and parasite prevalence may be a systematic age effect. By preferring older males for genetic and/or material benefits, females may also be choosing less heavily parasitized individuals. Such evidence may appear to support the hypotheses of parasite-mediated sexual selection, when in fact the relationship between female preference, expression of secondary sexual characters, and parasitism is simply the result of age (Thomas et al., 1995). Therefore, tests of the hypotheses of parasite-mediated sexual selection should consider age-specific patterns of infection or immune function and test for correlations between measures of parasitism or immunity, trait expression, and mating success after controlling for age effects. This approach has so far not been adopted in studies of parasite load and song. Although a similar control is automatically made in experimental studies where individuals are randomly assigned to treatments irrespective of their age, such experiments are rare (e.g., Garamszegi et al., 2004).

Here, we studied the association between male age, sexually selected characters such as features of song and tail length, and variables reflecting health in a Spanish population of the barn swallow *Hirundo rustica*, relying on cross-sectional and longitudinal analyses of individuals. First, we compared different sexual traits, chewing louse loads, and simple hematological variables between yearling and adult males (cross-sectional analysis). Second, applying a cohort analysis based on repeated measures of individuals in consecutive breeding seasons (longitudinal analysis), we checked whether the differences in the expression of sexual traits and health status between different age classes of males are due to effects of aging or to phenotype-dependent selection. Finally, we assessed the correlation between sexual characters and measures of parasitism, and we tested whether the association between features of song, tail length, and health status was modified when the effect of age was controlled statistically. If trait elaboration and parasitism depend on age, we predicted that older males display sexual traits of superior quality, harbor fewer parasites, and have superior immune condition. In accordance with the hypotheses of parasite-mediated sexual selection, if females use song cues to assess the parasite loads, we predicted that (1) there is a negative relationship between the expression of sexual signals and variables reflecting health and (2) measures reflecting mating success are associated with signals that convey information about parasite load. However, if variables fail to relate to measures of parasitism after controlling for age, this would indicate that the apparent correlations between sexual traits and parasites do not necessarily reflect causal associations as they are the result of systematic age effects. Such a result would indicate that females primarily select elaborate traits as indicators of age and experience. On the other hand, if the correlations between parasitism and signal expression remain when age effects are held constant, the correlative results are consistent with the predictions of the hypotheses of parasite-mediated sexual selection driven by the direct and indirect benefits to females for preferring parasite-free males.

METHODS

Study population and standard field procedures

Barn swallows are small, insectivorous, socially monogamous semicolonial passerines (Cramp, 1988). Sexual dimorphism is slight, with the exception of the external tail feathers, which are on average 5–20% longer in males than in females (Møller, 1994). Males arrive earlier than females at the breeding areas and establish territories each of a few square meters where they attract females by singing and displaying their tails. Males sing throughout the breeding season with a peak during the pre-laying period. Females visit several males before making their choice and prefer long-tailed males as social and extrapair mates (Møller, 1994; Møller and Tegelström, 1997; Saino et al., 1997b). Territory quality (i.e., quality of nest sites and perches) appears unimportant for mate choice (Møller, 1994). So it seems likely that female choice directly focuses on male qualities, which may be reflected in features of the song. Features of song were related to plasma testosterone levels and male condition, and they varied according to social environment (Galeotti et al., 1997). Females prefer males that sing at high rates as extrapair mates (Møller et al., 1998).

The fieldwork was carried out near Badajoz (38° 50′ N, 6° 59′ W), Spain, where barn swallows are locally very abundant, during the reproductive seasons 2000–2001, as part of a long-term project (Cadée, 2000; Cuervo and Møller, 2001; Cuervo et al., 1996, 2003; de Lope and Møller, 1993; Perrier et al., 2002). We captured adults once a week by using mist nets. At the first capture in the season, all birds were measured and provided with an individually numbered aluminum ring and a combination of color plastic rings. For identification, all birds received individual color markings from water-resistant pens on their belly plumage. Birds recaptured in successive capture sessions and already marked and measured were immediately released. The arrival date of an individual was assessed as the first capture date, and it was standardized across years by expressing date relatively to 1 February. Each unmarked individual was measured in a standardized way to record morphological variables. For this study, we used the length of the right and left outermost tail feathers, measured using a ruler to the nearest millimeter. We calculated tail length as the mean of the length of left and right outermost tail feathers. Birds were assumed to be yearlings when first captured without a ring, while older birds already had a numbered aluminum ring from a previous year. The reliability of this assignment of age to birds was demonstrated by our finding that all local recruits (more than 150) that had been ringed in a nest were captured for the first time as adults when yearlings. Because adults always return to their previous breeding site in subsequent years (as shown by none of more than 3000 birds ever moving to another farm between years), birds that were not recaptured in a year were considered to be dead. This assumption was supported by the fact that no birds were absent in 1 year but recaptured in a subsequent year (see also Perrier et al., 2002; Saino et al., 2003). The age of birds captured with a ring was determined based on the age assessment on its first capture in previous years. Age was a categorical variable and was set to one for males caught in their first breeding year and to two for older males. We sexed all birds from the presence of a brood patch (only females) and the shape of the cloacal protuberance (males have larger protuberances). Only data for males were used in this study. We took a blood sample (about 200 μl from the ulnar vein) for hematological analyses in standard, heparinized capillary tubes. Capillaries were subsequently stored horizontally in a cooling box with frozen cooling blocks until transport to our laboratory.

We captured 102 and 69 males in the two field seasons, respectively. We recaptured 46 individuals in the second field season that were already present in the first year as yearlings or adults. When data from both years were pooled in the cross-sectional analysis of age and health status, we chose data randomly from 1 year of the study to ensure that each individual only appeared once in the analyses, reducing the maximum number of birds to 125 in the pooled analyses. The longitudinal analysis relied on repeated measures of the same males over consecutive breeding seasons.

We determined the pairing status of males from the presence of a female next to the male and the obvious behavior of unmated males (intense singing and sexual displays and chases of approaching females, including neighboring females). We estimated the pairing success of males by estimating the relative pairing date, which is the time elapsed between arrival and the start of egg laying. The arrival date may bias the pairing date because early-arriving, high-quality males should wait longer to pair than late-arriving, poor-quality males as early in the season there may be few females available. To control for the confounding effect of arrival date, we included arrival date in the analyses, where relative pairing date was used.

Estimates of health status

When capturing birds the first time, we determined the abundance of the chewing louse species *Hirundoecus malleus* from the small holes that this species chews in feathers. We counted the total number of holes in the wing and tail feathers. These counts were subsequently summed to reflect overall chewing lice load. Chewing lice are abundant ectoparasites of the barn swallow, and their main damage consists of plumage destruction affecting the energy cost of flying and thermoregulation (Barbosa et al., 2002; Møller, 1991, 1994). Previous studies using extraction of live chewing lice and counts of holes in feathers showed a strongly positive correlation between the two estimates of abundance (Møller, 1991).

We used two hematological variables to assess health status, namely, sedimentation rate of erythrocytes that may reflect blood protein and immunoglobulin content, and hematocrit, which is the percent of whole blood that is comprised of red blood cells. These measures are generally assumed to reflect health because infection status may affect both sedimentation rate and hematocrit (Harrison GJ and Harrison LR, 1986). To measure blood sedimentation rate, capillary tubes containing blood samples were placed vertically for 4 h at 4°C after having been stored horizontally in a cooling bag in the field. The level of blood cell sedimentation was measured by calculating the ratio between the volume of the part of the capillary not occupied by blood cells and the total volume of the blood in the capillary. The sedimentation rate of erythrocytes reflects the amount of proteins in the blood, including immunoglobulins that are produced as a response to inflammatory and infectious diseases (e.g., Sharma et al., 1984). Hence, high values signify inferior health status. After measuring sedimentation rate, blood samples were centrifuged for 10 min at 10,000 rpm, and hematocrit was expressed as the volume of the part of the capillary occupied by red blood cells per total volume of blood in the capillary. Acute or chronic anemia as indicated by low hematocrit values can result, for example, from blood and gastrointestinal parasite infections or bacterial infections (Harrison GJ and Harrison LR, 1986). Therefore, males with a relatively poor health status may be predicted to have low hematocrit values. Because blood was collected in more than one capillary for many individuals, we could calculate the within-individual repeatability of our hematological variables; these were found to be high (sedimentation rate: $R = .915$, hematocrit: $R = .917$). When multiple measurements were available, we calculated mean sedimentation rate and hematocrit. Hematocrit and sedimentation rate are not independent variables, as confirmed by their strong, negative association in a linear regression with sedimentation rate as dependent and hematocrit as independent variable ($F_{1,113} = 54.993$, $p < .001$, slope $= -0.370$ [SE $= 0.050$]). To reflect the amount of proteins in the blood independent of the proportion of red blood cells, we performed multiple regression analyses in which both sedimentation rate and hematocrit were included.

None of the variables reflecting health status varied significantly between years (chewing lice load: Mann-Whitney U test, $Z = -0.929$, $p = .353$, $N = 122$; sedimentation rate: $t_{113} = 0.858$, $p = .179$; hematocrit, $t_{119} = 1.549$, $p = .124$). There was no significant association between chewing louse load and any hematological variable (Spearman correlations: sedimentation rate: $R = -.020$, $p = .829$, $N = 114$; hematocrit: $R = -.163$, $p = .076$, $N = 120$). Hence, different tests were largely independent, and different measures of parasitism and health status seem to involve different mechanisms.

Song recordings and spectrogram analysis

Breeding territory and preferred perching places of males were determined based on regular observations from a hide at the breeding barns. Song of individuals was recorded by L.Z.G. from 20 February to 10 April 2000–2001, starting when they established breeding territories. We used a Sony TCD-D8 DAT tape recorder at low sensitivity that was connected to a MD 21 U microphone attached near (20–30 cm) the nest or the perching place of a male where it sang. With this setup we were able to make relatively high-quality recordings directly of

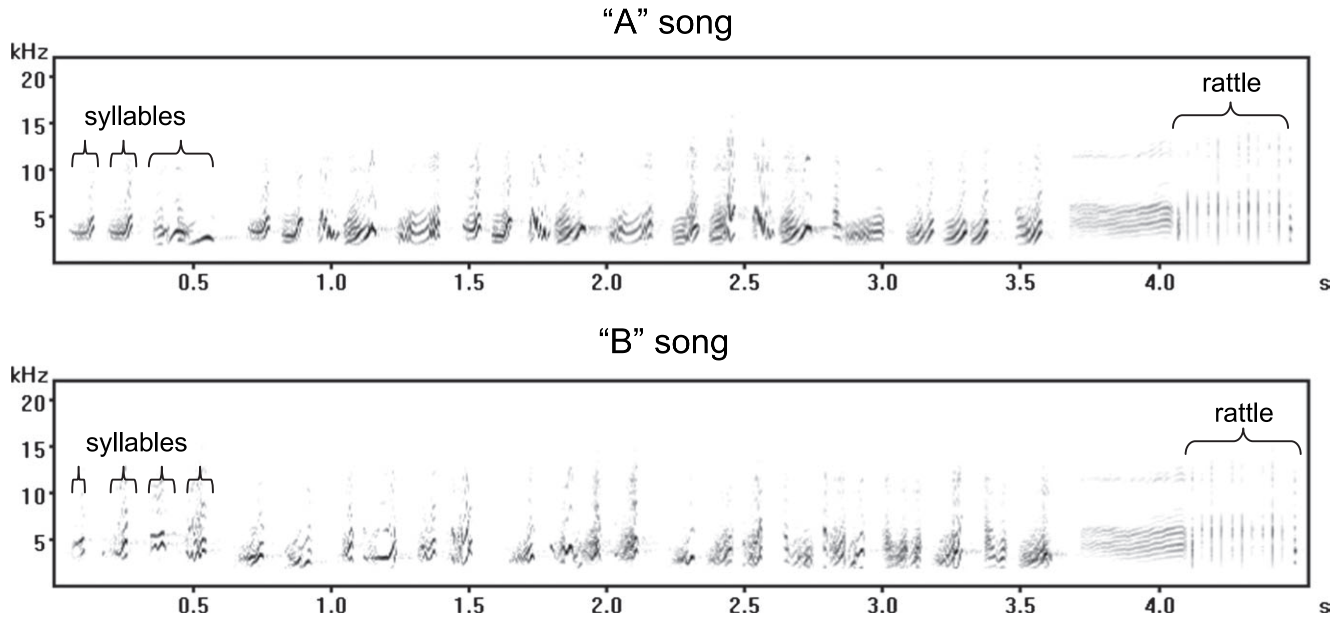

Figure 1
The spectrographic presentation of type "A" and type "B" songs of a male barn swallow.

the selected male, avoiding the echo effects and other disturbing noises within the barn. Recordings were made during the most active singing period (usually 0700–1200 h, but no later than 1300 h) and only under relatively good weather conditions. We obtained high-quality recordings (minimal background noise and high sound intensity) for 28 males in 2000, and we rerecorded 13 of these birds in 2001. These males were used in the longitudinal analysis. In the cross-sectional analyses of individuals, we used data from 2000 that were extended by adding two independently recorded birds in 2001, resulting in a maximum sample size of 30 (neither of the song traits considered showed year-dependent effects in corresponding analyses of variance [ANOVAs] in which we controlled for individual effects: year effects, $F < 0.967$, $p > .345$).

We assumed that breeding stage did not confound our results in relation to features of song because all males considered in this study were recorded in the period from 17.06 days (SE = 2.53) prior to clutch initiation date to the day when the last egg was laid. Within this period, individual song features do not change considerably in the barn swallow (Galeotti et al., 2001, see also variance analysis). The mean period between song recording and capture (when measurements and blood sampling occurred) was 9.09 days (SE = 1.47).

Male barn swallows typically sing two basic song types differing in the structure of elements that compose them, except from the final rattle (Galeotti et al., 1997, Figure 1). Song type "A" is a rapid twittering consisting of a variable number of complex syllables or elements covering a broad range of frequencies and arranged in very variable sequences. A typical syllable of song type "A" has a well-pronounced fundamental frequency and several harmonics. Type "B" song is more stereotyped and composed of many contact calls and other simple vocalizations with no clear fine-scale structure. Galeotti and coworkers usually considered song type "A" in their analysis. However, except for the analysis of repertoire size (see below), we pooled the raw song data irrespective of song type, and using both song types "A" and "B", we calculated the mean of each variable for each individual. This pooling was carried out because ANOVAs indicated that the same song variables (song length, number of syllables per song, song performance, song peak amplitude frequency, syllable rate, rattle length, number of impulses in the rattle, and rattle peak amplitude; see definitions for these variables in Table 1) calculated separately for different song types varied consistently within individuals (ANOVAs: song type "A", $F_{27,121} > 1.835$, $p < .05$ [two individuals had only "B" songs available]; song type "B", $F_{29,161} > 2.680$, $p < .001$), and their mean values averaged for different song types were significantly and positively correlated within individuals ($r = .444$–$.806$; all $p < .05$). Hence, a song trait in a given individual that is expressed in a certain way in song type "A" has a high probability of being expressed in a similar way in song type "B". The benefit of combining "A" and "B" songs for our analysis was that we could include more songs per individual, and we could also increase our sample size as two individuals had only "B" songs available. However, we included individual compositional variation due to song type in the analysis by defining a variable based on the percentage of different song types (see below).

Barn swallow song consists of sequences of various song types organized in bouts. These bouts may involve 1–12 songs in our population. Time intervals between songs within a bout are not longer than the mean time interval between elements of the song. When the raw recordings were digitalized and tailored for spectrographic analyses, we counted the number of bouts that consisted of at least two songs and also the number of single songs. We also counted the number of complete and incomplete songs depending on the presence of a harsh syllable, the rattle. The rattle is the typical terminal syllable of complete songs, which is a sequence of 5–15 impulses in our population, that is sung at high frequency. The rattle is prominent during pair formation, and it may have an important function in male-male competition (Galeotti et al., 1997).

We further analyzed songs spectrographically with the computer program "Avisoft-SASLab Pro" (Specht, 1999). The best resolution was achieved in the 0–10 kHz frequency range, with a sampling rate of 20,000 samples/s, bandwidth

Table 1
Definitions of variables used to characterize the song of the barn swallow

Variable	Definition
Song/bout ratio (S/B)	The ratio between the total number of songs found in a record and the number of bouts consisting of more than one song
Percentage of complete songs (%) (CO%)	The percentage of complete songs where a complete song is considered to be a song that is terminated with a rattle
Percentage of song type "A" (%) (A%)	The percentage of song type "A" within the available record
Number of songs per bout (NSO)	The number of songs within bouts consisting of at least two songs
Bout duration (s) (BLE)	The length of a bout in seconds consisting of at least two songs
Song duration (s) (SLE)	The length of the song in seconds
Number of syllables per song (NSY)	The number of syllables within songs
Song performance (%) (PER)	The time when the amplitude exceeds a threshold value of 20% of the maximum value (percentage of the total song duration), which can be used to estimate the ability of a male to maintain a high sound amplitude during singing (see Forstmeier et al., 2002)
Song peak amplitude frequency (kHz) (MPA)	The frequency (kHz) of that harmonic over which most energy was distributed (see also Galeotti et al., 1997; Nowicki, 1989)
Syllable rate (n/s) (SYR)	The number of syllables divided by the duration of song (number/second)
Repertoire size (REP)	Syllable repertoire size of "A" songs
Rattle duration (s) (RAL)	The length of the rattle in seconds
Number of impulses per rattle (NIR)	The number of impulses within the rattle
Rattle peak amplitude frequency (kHz) (RPA)	The frequency (kHz) of that harmonic over which most energy was distributed within the rattle

200 Hz, frequency resolution 80 Hz, and a time resolution of 32 ms. These settings are identical to those used by Galeotti et al. (1997, 2001) and Saino et al. (1997a). We analyzed 2.698 (SE = 0.387) bouts of song per male for a total of 116 bouts, 7.814 (SE = 0.525) songs per male for a total of 336 songs, and 7.977 (SE = 0.484) rattles per male for a total of 344 rattles.

During the song analysis we measured 13 song variables that are all defined in Table 1, and their means and standard errors together with their pairwise correlations are given in Table 2. The majority of these variables (or their derivates) describe the same vocal phenomena that were defined by Galeotti et al. (1997, 2001) and Saino et al. (1997a). Our choice of variables to describe song features was based on considerations concerning their sexual function, as observed in the barn swallow or other species, or on assumptions about costs or constraints that might influence song (see also Galeotti et al., 1997, 2001; Saino et al., 1997a). We believe that with our choice of variables we capture a wide range of features that may be associated with song composition at the bout, song and rattle levels, and also with song complexity and song performance.

In our repertoire framework we used song type "A" only because song type "B" is more stereotyped in sequence than "A" songs. In addition, "B" songs are usually composed of many contact calls and simple vocalizations (Cramp, 1988; Galeotti et al., 1997). Based on the visual inspection of sonagrams, we classified syllable types largely based on the syllable catalogue presented by Galeotti et al. (1997) for an Italian population. Syllables that could not be categorized by that scheme were defined using new syllable categories. We counted the total number of syllable types found in five complete "A" songs of a male. Cumulative plots that were drawn between the number of unique syllable types found and the number of songs sampled showed that after analyzing 2–3 songs we probably reached the real repertoire size (plateau effect) (see also Galeotti et al., 1997, 2001). In a pilot study using a capture-recapture approach (see Garamszegi et al., 2002) and syllable sequences for both the Italian and the Spanish populations, we found that syllable counts based on five songs can give reliable estimates of the true repertoire size in the barn swallow (Galeotti P and Garamszegi LZ, unpublished data). Repertoire size was only estimated in individuals for which at least five "A" songs were available ($N = 16$).

Statistical analyses and choice of variables

Variables were checked for normality and homoscedasticity. When necessary, statistical transformation was applied in order to meet the parametric criteria. Hence, song variables with the exception of song/bout ratio, percentage of songs, and percentage of song type "A" were $\log_{10}$-transformed. However, the assumptions of parametric statistics were violated for some variables even after transformation. In such cases, we used nonparametric tests.

We used one-way ANOVA (or its nonparametric analogue, Kruskal-Wallis ANOVA) on the individual song measurement data to test for consistent variation within males and within year (for some males we had multiple recordings from the same year). In 11 out of 13 song variables (for repertoire size we only had one measurement in a year), we found significant evidence for the variance being larger between than within individuals (Table 2). However, when taking the number of statistical tests into account by adjusting significance levels (see below), significance remained only for eight variables. Where data structure and the corresponding variance analysis allowed, we also calculated within-year repeatability for each trait; this was found to vary between 0.098–0.631, which was comparable to repeatabilities obtained for similar sexually selected song traits in other species (Forstmeier et al., 2002; Garamszegi et al., 2004; Gil and Slater, 2000). Because, after Bonferroni correction, we did not find significant evidence for consistent variation within individuals for the ratio of the number of songs to the number of bouts, the percentage of complete songs, the percentage of song type "A," the number of songs per bout and syllable rate, we excluded these variables from the subsequent analyses.

The correlation matrix for song traits showed a very strong association between song length and the number of syllables within songs and between rattle length and the number of impulses (Table 2). These relationships may appear because the variables probably reflect the same biological phenomena. Therefore, we excluded the number of syllables within songs and the number of impulses within rattles from the subsequent analyses. After Bonferroni correction we did not find any other significant correlations between song traits. To

Table 2

Means (SE) and repeatabilities of variables used to describe song features of the barn swallow and their correlations

	Mean (SE)	Repeatability	BLE	SLE	NSY	PER	MPA	REP	RAL	NIR	RPA
Song/bout ratio (S/B)	0.168[a] (0.02)	—	—	—	—	—	—	—	—	—	—
Percentage of complete song (%) (CO%)	60.68[a] (2.60)	—	—	—	—	—	—	—	—	—	—
Percentage of song type "A" (%) (A%)	35.05[a] (2.16)	—	—	—	—	—	—	—	—	—	—
Number of songs per bout (NSO)	2.90[a] (0.19)	—	—	—	—	—	—	—	—	—	—
Bout duration (s) (BLE)	**12.63** (0.96)	0.170		0.132	0.072	0.040	0.171	0.014	0.275	0.042	0.023
Song duration (s) (SLE)	**4.73** (0.08)	0.422			**0.958**	0.093	−0.476	0.470	0.007	−0.018	−0.033
Number of syllables per song (NSY)	**24.14** (0.47)	0.370				0.175	−0.434	0.490	−0.002	0.013	0.027
Song performance (%) (PER)	**14.25** (0.37)	0.149					−0.397	0.065	−0.003	−0.082	−0.018
Song peak amplitude frequency (kHz) (MPA)	**3.69** (0.32)	0.124						−0.350	−0.067	−0.095	−0.223
Syllable rate (n/s) (SYR)	6.15 (0.05)	0.098							—	—	—
Repertoire size (REP)	22.94[b] (1.24)	—							0.047	0.065	−0.088
Rattle duration (s) (RAL)	**0.41** (0.01)	0.556								**0.927**	0.348
Number of impulses per rattle (NIR)	**10.33** (0.12)	0.631									0.439
Rattle peak amplitude frequency (kHz) (RPA)	**6.77** (0.37)	0.495									

[a] consistent variation between individuals was tested with Kruskal-Wallis ANOVA.

[b] Only a single measurement is available for each individual, and thus we did not test for consistent variation within individuals. Significant effects ($p < .05$) are underlined, while significant effects after Bonferroni adjustment (see Methods) are underlined and given in bold.

The reliability of means was tested by comparing between-individual variation with within-individual variation (ANOVA or Kruskal-Wallis ANOVA).

Repeatabilities are given if the corresponding ANOVA showed significant effects of individuals. Correlations are Pearson correlations.

reduce the number of statistical tests performed with multiple song features, a common practice is to apply principal component analysis (e.g., Galeotti et al., 1997; Garamszegi et al., 2004; Gil and Slater, 2000). However, we avoided this approach, because the principal component axes explained relatively little variance, with component loadings of minor importance and no obvious biological meaning.

We applied Bonferroni correction using a significance level of 5% to reduce the number of cases with significance arising by chance because of multiple tests. All the tests are two tailed. Due to varying data availability resulting from different constraints, sample size can differ between analyses.

RESULTS

Cross-sectional analysis of individuals in relation to age

Chewing louse parasitism was significantly higher in yearlings than in older males (Mann-Whitney test: $Z = 3.603$, $N = 122$, $p < .001$, Figure 2a), whereas there was no significant evidence for hematological variables being related to age (t tests: hematocrit, $t = -1.164$, df = 119, $p = .247$; sedimentation rate, $t = 1.514$, df = 113, $p = .133$).

Similar analyses for song traits showed that three out of seven variables varied significantly between age classes (Table 3), and this is a significantly higher probability than could be expected by chance (Fisher exact $p = .021$). After Bonferroni correction, only results for song peak amplitude frequency and song duration remained significant, indicating that older males have longer songs with lower mean peak amplitude frequency than yearlings (Figure 2b,c).

Tail length also differed between age classes, being significantly lower in yearling than in adult males (t test: $t = -3.182$, df = 119, $p = .001$, Figure 2d). Hence, parasite load and sexual signaling appears to be age dependent.

Longitudinal analysis of individuals: directional selection or individual changes

Chewing louse infection, tail length, and two song traits varied between age classes, with differences between yearlings and older birds. Using individuals for which we had measurements from their first and second breeding season, we tested whether these differences were due to consistent individual changes during the transition between ages of 1 and 2 years. If the apparent relationship between age and chewing louse load is due to the fact that individuals in their first year of breeding experience higher parasite pressure than in their second year, we should expect a uniform decline in ectoparasite load in the cohort in a matched-pair comparison. This prediction was supported (Wilcoxon matched-pairs test: $Z = 2.107$, $p = .035$, $N = 22$, Figure 3a). Based on recapture data, we did not find that 1-year-old males that had more chewing lice survived less well than yearlings with fewer lice (Mann-Whitney U test: $Z = 0.280$, $N = 55$, $p = .780$).

We checked for similar longitudinal changes in song traits that showed significant differences between yearlings and 2-year-old birds. In the previous cross-sectional analysis, songs of yearlings were found to be of shorter duration than the songs of older birds. Hence, in a longitudinal analysis, we predicted a consistent increase in song duration with aging, which tended to be the case (paired t test: $t_4 = 2.001$, $p = .093$, Figure 3b). Due to the small sample size ($N = 5$) the power of this test was 0.393, indicating that the probability of committing type II errors was large. However, significant

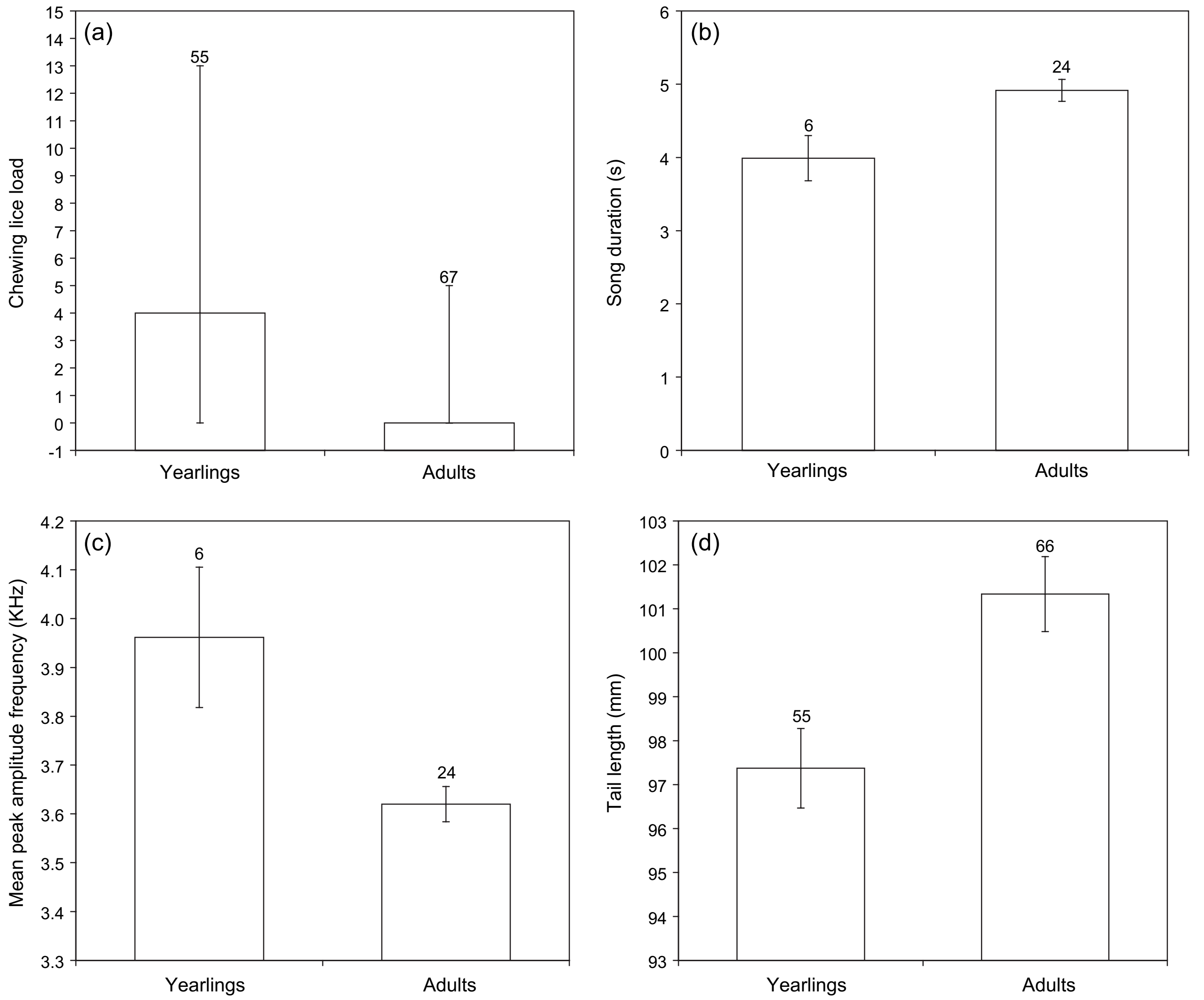

Figure 2
Parasite load and expression of sexual traits in yearling and adult male barn swallows. (a) Median (±25% quartiles) chewing louse load as assessed by the number of holes in feathers, (b) mean (±SE) song duration (s), (c) song peak amplitude frequency (kHz), and (d) tail length (mm). Numbers are sample sizes.

within-individual declines were detected for mean peak amplitude of songs (paired *t* test: $t_4 = -4.008$, $p = .016$, Figure 3c). Among the six yearlings for which we had song data, all but one bird survived till the next breeding season, and thus we did not test whether recapture probability was associated with song length and mean peak amplitude frequency.

Tail length increased with aging, as individuals had significantly longer tails in their second year of breeding than on their first breeding attempt (paired *t* test: $t_{20} = 8.952$, $p < .001$, Figure 3d). On the other hand, there was no significant evidence for yearling males with shorter tails having lower probability of being recaptured the next year than yearlings with longer tails (*t* test: $t_{53} = 2.832$, $p = .409$). In general, we found more evidence that age-related patterns of chewing lice infestation and trait elaboration were caused by consistent individual changes rather than phenotype-dependent mortality.

Relationship between song, tail length, and health status

Pairwise comparisons of song traits with variables reflecting health status indicated that duration of song was significantly negatively associated with chewing louse load (Table 4; Figure 4a). Because these variables were both dependent on age, it is plausible that their emerging association is due to systematic age-effects. However, when we calculated partial correlation coefficients, we found that the relationship between song duration and chewing louse load remained significant when controlling for age effects (partial correlation based on Spearman rank order correlation coefficients treating age as a continuous variable: song duration and chewing louse load, $r = -.685$, $p < .001$, $N = 30$).

We detected significant positive associations between peak amplitude frequency of the rattle and hematological variables, with the strongest pattern appearing for sedimentation rate (Table 4; Figure 4b). We also applied multiple regression to uncover the relationship between these traits by entering peak

Table 3

The difference in features of song between yearling and adult male barn swallows

	t	df	p
Bout duration	0.710	22	0.485
Song duration	**−2.896**	**28**	**0.007**
Song peak amplitude frequency	**3.352**	**28**	**0.002**
Song performance	−0.375	28	0.711
Repertoire size	−2.783	14	0.015
Rattle duration	0.868	28	0.393
Rattle peak amplitude frequency	0.678	28	0.504

Cross-sectional analyses based on *t* tests.

Significant effects ($p < .05$) are underlined, while significant effects after Bonferroni adjustment (see Methods) are underlined and given in bold. These effects are presented in Figure 2.

amplitude frequency of the rattle as the dependent variable while using sedimentation rate and hematocrit as independent variables. The resulting model produced findings similar to the pairwise comparisons in Table 4 ($F_{2,28} = 10.109$, $p < .001$; slope for sedimentation rate: 0.250 [SE = 0.088], $p = .009$; slope for hematocrit: −0.226 [SE = 0.181], $p = .223$). This relationship was independent of male age because previous analyses revealed no significant evidence for sedimentation rate and peak amplitude frequency of the rattle being related to age.

There were no significant associations between features of song and tail length ($-.298 < r < .201$, $p > .116$), and, similarly, variables reflecting health were not related significantly to tail length ($-.056 < r < .055$, $p > .548$).

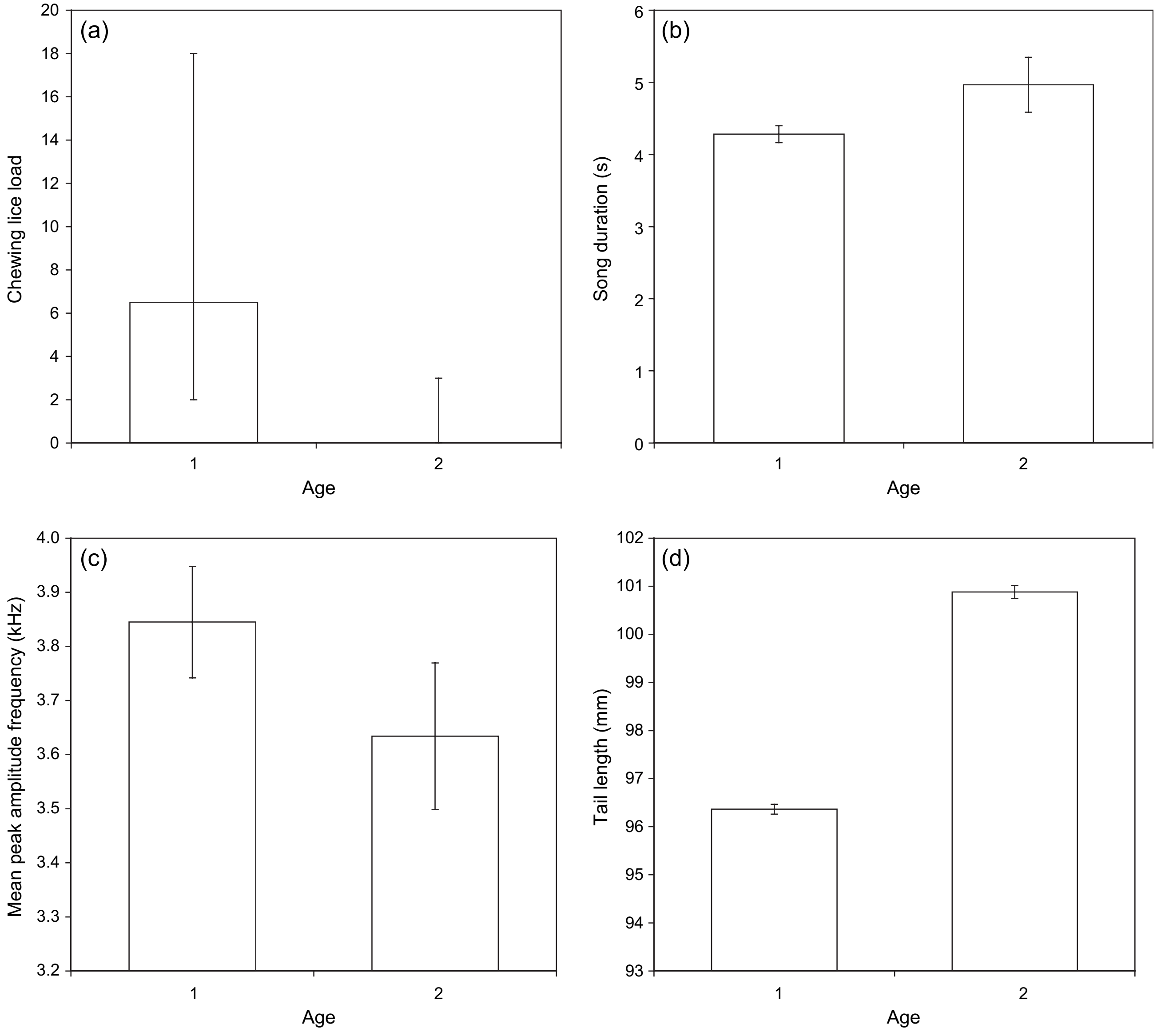

Figure 3

Longitudinal analysis as revealed by the matched-pair comparison of individuals during their first (Age 1) and second (Age 2) breeding attempts. (a) Chewing louse load as assessed as the number of holes in feathers, (b) song duration (s), (c) song peak amplitude frequency (kHz), and (d) tail length (mm). For chewing louse load, medians (±25% quartiles) for 22 males are given. For song duration and mean peak amplitude frequency, means (±SE) for five individuals are presented. Sample size for tail length is 21.

Table 4

Pearson correlations or Spearman rank order correlations for the relationship between features of song and chewing louse load and hematological variables, respectively

	Chewing louse load	Sedimentation rate	Hematocrit
Bout length	−0.064[a]	0.030	0.112
Song duration	**−0.571**[a]	−0.081	0.090
Song peak amplitude frequency	0.304[a]	−0.155	0.118
Song performance	−0.128[a]	−0.108	0.010
Repertoire size	−0.277[a]	−0.463	0.333
Rattle length	0.027[a]	0.086	−0.242
Rattle peak amplitude frequency	0.070[a]	**0.635**	−0.513

[a] Spearman rank order correlations.

Significant effects ($p < .05$) are underlined, while significant effects after Bonferroni adjustment (see Methods) are underlined and given in bold.

Pairing success and indicators of health

Males that sang long songs paired significantly earlier relative to their arrival than males that sang short songs, whereas there was no significant association between relative pairing date and peak amplitude frequency of the rattle (Pearson correlations: song duration, $r = -.443$, $N = 27$, $p = .021$, Figure 5; peak amplitude frequency of the rattle, $r = -.160$, $N = 27$, $p = .427$). These relationships were not confounded by arrival date because a multiple regression with relative pairing date and arrival date as independent variables revealed a significant association between song duration and relative pairing date independent of arrival date (song duration, $F_{2,25} = 3.800$, $p = .038$; slope for relative pairing date: −0.172 [SE = 0.083], $p = .050$; slope for arrival date: −0.103 [SE = 0.134], $p = .452$; peak amplitude frequency of the rattle, $F_{2,25} = 1.158$, $p = .332$; slope for relative pairing date: −0.041 [SE = 0.030], $p = .184$; slope for arrival date: 0.057 [SE = 0.048], $p = .248$).

DISCUSSION

Here, we have shown that ectoparasitism (chewing louse infestation), features of song (song duration and mean peak amplitude frequency of songs), and tail length exhibit age-related patterns in the barn swallow. By applying a combination of cross-sectional and longitudinal analyses of individuals within and between years, we found that such age dependence is more likely to be caused by consistent individual changes with age rather than phenotype-dependent survival. We detected significant association between song duration and chewing lice load and also between mean peak amplitude frequency of the rattle and sedimentation rate. Age-dependent variation in traits was hypothesized to drive, without causal linkage, apparent phenotypic correlations between song and parasitism. However, the negative relationship between song duration and chewing lice load remained significant when the potentially confounding effect of age was statistically controlled. The association between peak amplitude of the rattle and sedimentation rate should not result from consistent variation due to age because these traits had previously been found to be unrelated to age. These results fit with the predictions of the theory of parasite-mediated sexual selection as the covariance between trait elaboration and parasitism appears not to result from systematic variation between age classes. Therefore, certain song traits hold the potential to reliably signal parasite prevalence or health status independent of age.

A correlational study of an Italian barn swallow population showed that certain song features of male barn swallows may reflect age (Galeotti et al., 2001). Specifically, yearling males produced shorter songs. Moreover, yearlings used a lower

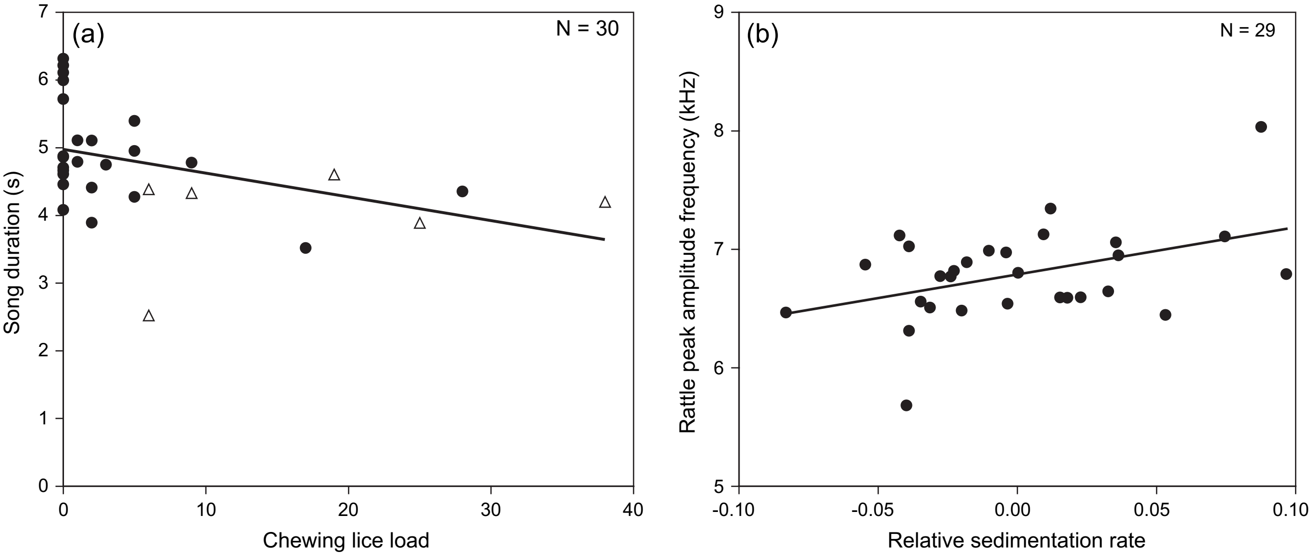

Figure 4

Relationship between song and parasitism. (a) Song duration (s) and chewing louse load as assessed by the number of holes in feathers. Because these traits are age dependent, different styles are used for different age classes: filled circles are for adults and open triangles for yearlings (b) Peak amplitude frequency of the rattle (kHz) and relative sedimentation rate corrected for hematocrit as estimated in a multiple regression model with sedimentation rate and hematocrit as independent variables. Units for relative sedimentation rate are residuals from this regression. For illustration, linear regression lines are shown, but results for chewing lice load are given in the main text based on nonparametric statistics (Spearman rank order correlation).

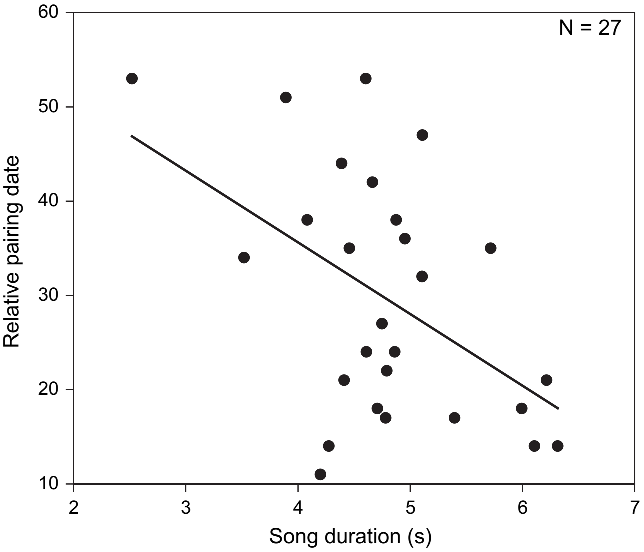

Figure 5
Relationship between song duration (s) and relative pairing date indicating pairing success. Relative pairing date is the time elapsed from arrival till the start of egg laying. Linear regression line is shown (see main text for statistics).

number of syllable types in their songs, and never repeated the same syllable sequences, while, in contrast, older males used more syllable types in their songs and emitted longer songs (Galeotti et al., 2001). Here, we were partly able to replicate these results for song duration, as we demonstrated that songs are significantly longer in adult birds. This may be the result of systematic increase after the first year of breeding, although, probably due to insufficient power, we obtained a nonsignificant tendency to exhibit such a pattern in the corresponding longitudinal analysis. We think that the findings of the present study for song duration captured biological phenomena in relation to age very similar to those found by Galeotti et al. (2001). In addition, we detected an age-dependent mean peak amplitude frequency of songs, which declined in the second year of breeding. In conclusion, the expression of song in the barn swallow appears to be strongly age dependent.

Parasitism caused by the directly transmitted chewing louse *H. malleus* decreased after the first year of breeding. The causality determining this relationship remains undetermined. We assume that systematic differences in the number of chewed holes in feathers between yearling and adult birds are not due to systematic differences in molt patterns because first-year breeders follow the same molting strategies as older birds (Svensson, 1984). It is possible that individual hosts mount more efficient defense mechanisms against these parasites after the first year of breeding (see Møller and Rózsa, 2005, for a review on immune responses to chewing lice). Comparative analyses of richness of chewing lice genera in relation to T-cell–mediated immune response of their avian hosts revealed a positive relationship for the suborder Amblycera of lice to which *H. malleus* belongs, members of which contact or penetrate the skin of the host, but not for the suborder Ischnocera, members of which contact only the dead tissue of feathers (Møller and Rózsa, 2005). Recent literature on wild birds including the barn swallow suggests that humoral immune response deteriorates at a relatively old age (Cichoñ et al., 2003; Saino et al., 2003). Immunosenescence may cause a higher parasite load at old age, as observed in senescent barn swallows (Møller and de Lope, 1999). The link between immune response and ectoparasite infection implies that a decrease in chewing lice loads among barn swallows between 1 and 2 years of age is a result of an improvement in host defenses early in life (Møller and de Lope, 1999).

Foraging by chewing lice imposes important costs on their hosts because feathers often break at sites where chewing lice have made holes (Kose and Møller, 1999), thus causing an increase in aerodynamic costs for the host. However, the exact fitness costs caused by high chewing lice loads remain to be determined in the barn swallow. We found no statistical evidence for recapture probability being affected by chewing lice load. It remains to be investigated whether these contagious ectoparasites cause other costs to the birds, such as reduced reproductive success due to high nestling mortality. If chewing louse infestation entails particular fitness costs for hosts, the theory of parasite-mediated sexual selection predicts that female hosts should prefer mates that are better at coping with such parasites (Møller et al., 1999). Some evidence suggests a preference for chewing louse–free mates both at the social and the extrapair levels, indicating that individuals searching for a partner may attempt to avoid the fitness costs caused by chewing lice (Møller, 1991). Under these circumstances, sexually selected characters may be expected to reveal resistance against such parasites, as reported for *H. malleus* in the barn swallow (Møller et al., 2004).

A previous analysis suggested that the song of the barn swallow may be such a trait (Saino et al., 1997a). Here, we detected significant correlations between estimates of health status and certain song traits. Additionally, we showed that males displaying longer songs are more successful in pairing as indicated by earlier pairing date relative to arrival date. These results are consistent with the hypothesis that female barn swallows use song duration, which has the potential to reveal ectoparasite burden caused by chewing lice, in their preference for ectoparasite-free males.

Because we also found a relationship between male age and chewing louse infestation and between age and song duration, such age-specific effects may drive the apparent relationship between ectoparasite load and song duration without a causal link between the two. Accordingly, a correlation between male age, song duration, chewing lice load, and mating success may arise from females preferring signals of male age. Age would then be a proximate indicator of mate quality, and there would be no benefit in terms of heritable parasite resistance (Thomas et al., 1995). Alternatively, older males may be preferred for more direct benefits such as experience, parental ability, or skill in predator avoidance, and a relationship between song and parasite load is then incidental (Thomas et al., 1995). However, that was not the case in our study because when we factored out age effects statistically, the negative relationship between song duration and chewing louse load remained significant. Hence, yearling and adult male swallows may signal the absence of chewing lice in a similar way by singing longer songs. Although song duration appears to increase with age, a considerable amount of variation between individuals may partly reflect differences in age-independent components such as genetic or maternal effects. Therefore, adults have longer songs, but an adult male with superior resistance against chewing lice may be expected to have longer songs than average males. A recent study has shown significant genetic variation in levels of ectoparasitism caused by the chewing louse *H. malleus* despite the fact that these are directly transmitted parasites (Møller et al., 2004). Parent-offspring regressions based on extrapair and within-pair offspring revealed that similarity in chewing louse abundance between relatives was caused by shared genes. Hence, in accordance with the "good-genes" theory of sexual selection (Williams, 1966), females that prefer males that signal low chewing louse infestation will acquire parasite resistance for their offspring. Advertisement of such resistance genes should be independent of age.

The rattle of the song of the barn swallow functions as an effective deterrent during male interactions, signaling a high probability of escalated contests (Galeotti et al., 1997). The negative association between body condition and peak amplitude frequency of the rattle shows that the frequency attributes of the rattle are important components of such signaling (Galeotti et al., 1997). Here, we found a positive association between sedimentation rate, indicating inferior health status, and mean peak amplitude frequency of the rattle. Hence, if the rattle directed to other males is sung in highly competitive contexts, males may be able to assess the health and vigor of their rivals by the frequency attributes of the rattle. Rattle features reflecting immune quality can be used to assess the likelihood of success of a territorial challenge. Apparently, such intrasexual communication of immune condition is independent of age because rattle characteristics and hematological profile were similar in yearlings and adults.

Much research has assessed the importance of tail length in parasite-mediated sexual selection in the barn swallow, suggesting that males having longer tails are in general better at coping with parasites (e.g., Møller, 1990, 1991; Saino and Møller, 1994, 1996). A previous study found a weak but significant negative correlation between tail length and abundance of chewing lice (Møller, 1991). We did not replicate this finding in the present study, which was conducted in another population, but given that the correlation generally is weak, it is plausible that the relationship exists but went undetected in our sample. Alternatively, it is possible that the signal conveys different information in different populations or in different years.

In conclusion, features of song in the barn swallow not only indicate the age of the singer but also provide information about health status. However, we showed for the first time that the relationship between attributes of song and health was not confounded by age, thus providing support for health status–signaling mechanisms used in both mate choice and male-male competition.

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REFERENCES

Able DJ, 1996. The contagion indicator hypothesis for parasite-mediated sexual selection. Proc Natl Acad Sci USA 93:2229–2233.

Absil P, Pinxten R, Balthazart J, Eens M, 2003. Effect of age and testosterone on autumnal neurogenesis in male European starlings (*Sturnus vulgaris*). Behav Brain Res 143:15–30.

Adret P, Margoliash D, 2002. Metabolic and neural activity in the song system nucleus robustus archistriatalis: effect of age and gender. J Comp Neurol 454:409–423.

Alatalo RV, Gustafsson L, Lundberg A, 1986a. Do females prefer older males in polygynous bird species? Am Nat 127:241–245.

Alatalo RV, Lundberg A, Glynn C, 1986b. Female pied flycatchers choose territory quality and not male characteristics. Nature 323: 152–153.

Andersson M, 1994. Sexual selection. Princeton: Princeton University Press.

Armstrong GL, Conn LA, Pinner RW, 1999. Trends in infectious disease mortality in the United States during the 20th century. J Am Med Assoc 281:61–66.

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

Bernard DJ, Eens M, Ball GF, 1996. Age- and behavior-related variation in volumes of song control nuclei in male European starlings. J Neurobiol 30:329–339.

Birkhead TR, Buchanan KL, Devoogd TJ, Pellatt EJ, Székely T, Catchpole CK, 1997. Song, sperm quality and testes asymmetry in the sedge warbler. Anim Behav 53:965–971.

Birkhead TR, Fletcher F, Pellatt EJ, 1998. Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. Behav Ecol Sociobiol 44:179–191.

Borgia G, Collis K, 1989. Female choice for parasite-free male satin bowerbirds and the evolution of bright male plumage. Behav Ecol Sociobiol 25:445–453.

Buchanan KL, Catchpole CK, Lewis JW, Lodge A, 1999. Song as an indicator of parasitism in the sedge warbler. Anim Behav 57:307–314.

Buchholz R, 1995. Female choice, parasite load and male ornamentation in wild turkeys. Anim Behav 50:929–943.

Cadée N, 2000. Parent barn swallow fluctuating asymmetry and offspring quality. J Avian Biol 31:495–503.

Catchpole CK, 1986. Song repertoires and reproductive success in the great reed warbler *Acrocephalus arundinaceus*. Behav Ecol Sociobiol 19:439–445.

Cichoñ M, Sendecka J, Gustafsson L, 2003. Age-related decline in humoral immune function in Collared Flycatchers. J Evol Biol 16: 1205–1210.

Clayton DH, 1991. The influence of parasites on host sexual selection. Parasitol Today 7:329–334.

Cosens SE, Sealy SG, 1986. Age-related variation in song repertoire size and repertoire sharing of Yellow Warblers. Can J Zool 64:1926–1929.

Cramp S, 1988. The birds of the Western Palearctic. Oxford: Oxford University Press.

Cuervo JJ, de Lope F, Møller AP, Moreno J, 1996. Energetic cost of tail streamers in the barn swallow (*Hirundo rustica*). Oecologia 108: 252–258.

Cuervo JJ, Møller AP, 2001. Components of phenotypic variation in avian ornamental and non-ornamental feathers. Evol Ecol 15: 53–72.

Cuervo JJ, Møller AP, de Lope F, 2003. Experimental manipulation of tail length in female barn swallows (*Hirundo rustica*) affects their future reproductive success. Behav Ecol 14:451–456.

de Lope F, Møller AP, 1993. Female reproductive effort depends on the degree of ornamentation of their mates. Evolution 47:1152–1160.

Eens M, Pinxten R, Verheyen RF, 1992a. No overlap in song repertoire size between yearling and older Starlings *Sturnus vulgaris*. Ibis 134: 72–76.

Eens M, Pinxten R, Verheyen RF, 1992b. Song learning in captive European starlings, *Sturnus vulgaris*. Anim Behav 44:1131–1143.

Espmark YO, Lampe HM, 1993. Variations in the song of the pied flycatcher within and between breeding seasons. Bioacoustics 5: 33–65.

Folstad I, Karter AJ, 1992. Parasites, bright males, and immunocompetence handicap. Am Nat 139:603–622.

Forstmeier W, Kempenaers B, Meyer A, Leisler B, 2002. A novel song parameter correlates with extra-pair paternity and reflects male longevity. Proc R Soc Lond B 269:1479–1485.

Galeotti P, Saino N, Perani E, Sacchi R, Møller AP, 2001. Age-related song variation in male barn swallows. Ital J Zool 68:305–310.

Galeotti P, Saino N, Sacchi R, Møller AP, 1997. Song correlates with social context, testosterone and body condition in male barn swallows. Anim Behav 53:687–700.

Garamszegi LZ, Boulinier T, Møller AP, Török J, Michl G, Nichols JD, 2002. The estimation of size and change in composition of avian song repertoires. Anim Behav 63:623–630.

Garamszegi LZ, Møller AP, Török J, Michl G, Péczely P, Richard M, 2004. Immune challenge mediates vocal communication in a passerine bird: an experiment. Behav Ecol 15:148–157.

Getty T, 2002. Signaling health versus parasites. Am Nat 159:363–371.

Gil D, Cobb JLS, Slater PJB, 2001. Song characteristics are age dependent in the willow warbler, *Phylloscopus trochilus*. Anim Behav 62:689–694.

Gil D, Slater PJB, 2000. Song organisation and singing patterns of the willow warbler, *Phylloscopus trochillus*. Behaviour 137:759–782.

Hamilton WD, 1990. Mate choice near and far. Am Zool 30:341–352.

Hamilton WD, Zuk M, 1982. Heritable true fitness and bright birds: a role for parasites. Science 218:384–387.

Harrison GJ, Harrison LR, 1986. Clinical avian medicine and surgery. London: W. B. Saunders Company.

Hasselquist D, Bensch S, vonSchantz T, 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. Nature 381:229–232.

Hecking-Veltman J, Tenter AM, Daugschies A, 2001. Investigations on the prevalence of endo- and ectoparasites in stray cats in the area of Mönchengladbach, Germany. Praktische Tierarzt 82:563–574.

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

Kroodsma DE, 1982. Learning and the onthogeny of sound signals in birds. In: Acoustic communication in birds (Kroodsma DE, Miller EH, eds). London: Academic Press, Inc.; 1–23.

Manning JT, 1985. Choosy females and correlates of male age. J Theor Biol 116:349–354.

Marchetti K, Price T, 1989. Differences in the foraging of juvenile and adult birds: the importance of developmental constraints. Biol Rev 64:51–70.

McGregor PK, Krebs JR, Perrins CM, 1981. Song repertoires and lifetime reproductive success in the great tit (*Parus major*). Am Nat 118:149–159.

Møller AP, 1990. Effects of a hematophagous mite on the barn swallow (*Hirundo rustica*)—a test of the Hamilton and Zuk hypothesis. Evolution 44:771–784.

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

Møller AP, 1994. Sexual selection and the barn swallow. Oxford: Oxford University Press.

Møller AP, Christe P, Lux E, 1999. Parasitism, host immune function, and sexual selection. Q Rev Biol 74:3–20.

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

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

Møller AP, Rózsa L, 2005. Parasite biodiversity and host defenses: chewing lice and immune response of their avian hosts. Oecologia 142:169–176.

Møller AP, Saino N, Taramino G, Galeotti P, Ferrario S, 1998. Paternity and multiple signaling: effects of a secondary sexual character and song on paternity in the barn swallow. Am Nat 151:236–242.

Møller AP, Tegelström H, 1997. Extra-pair paternity and tail ornamentation in the barn swallow *Hirundo rustica*. Behav Ecol Sociobiol 41:353–360.

Mountjoy DJ, Lemon RE, 1995. Extended song learning in wild European starlings. Anim Behav 49:357–366.

Müller-Graf CDM, Collins DA, Packer C, Woolhouse MEJ, 1997. Schistosoma mansoni infection in a natural population of olive baboons (*Papio cynocephalus anubis*) in Gombe Stream National Park, Tanzania. Parasitology 115:621–627.

Nottebohm F, Nottebohm ME, 1978. Relationship between song repertoire and age in canary, *Serinus canarius*. Z Tierpsych 46:298–305.

Nowicki S, 1989. Vocal plasticity in captive black-capped chickadees: the acoustic basis and rate of convergence. Anim Behav 37:64–73.

Perrier C, de Lope F, Møller AP, Ninni P, 2002. Structural coloration and sexual selection in the barn swallow *Hirundo rustica*. Behav Ecol 13:728–736.

Poulin R, 1992. Determinants of host-specificity in parasites of freshwater fishes. Int J Parasitol 22:753–758.

Price T, Schluter D, Heckman NE, 1993. Sexual selection when the female directly benefits. Biol J Linn Soc 48:187–211.

Proudman CJ, Holmes MA, Sheoran AS, Edwards SER, Trees AJ, 1997. Immunoepidemiology of the equine tapeworm *Anoplocephala perfoliata*: age-intensity profile and age-dependency of antibody subtype responses. Parasitology 114:89–94.

Redpath SM, Appleby MB, Petty SJ, 2000. Do male hoots betray parasite loads in Tawny Owls? J Avian Biol 31:457–462.

Rehbein S, Lutz W, Visser M, Winter R, 2002. Investigation of the parasite fauna of game animals of Northrhine-Westfalia. 3. Endoparasites of red deer. Z Jagdwissensch 48:69–93.

Saino N, Ferrari RP, Romano M, Rubolini D, Møller AP, 2003. Humoral immune response in relation to senescence, sex and sexual ornamentation in the barn swallow (*Hirundo rustica*). J Evol Biol 16:1127–1134.

Saino N, Galeotti P, Sacchi R, Møller AP, 1997a. Song and immunological conditions in male barn swallows (*Hirundo rustica*). Behav Ecol 8:364–371.

Saino N, Møller AP, 1994. Secondary sexual characters, parasites and testosterone in the barn swallow, *Hirundo rustica*. Anim Behav 48: 1325–1333.

Saino N, Møller AP, 1996. Sexual ornamentation and immunocompetence in the barn swallow (*Hirundo rustica*). Behav Ecol 7:227–232.

Saino N, Primmer CR, Ellegren H, Møller AP, 1997b. An experimental study of paternity and tail ornamentation in the barn swallow (*Hirundo rustica*). Evolution 51:562–570.

Searcy WA, Andersson M, 1986. Sexual selection and the evolution of song. Annu Rev Ecol Syst 17:507–533.

Searcy WA, McArthur PD, Yasukawa K, 1985. Song repertoire size and male quality in song sparrow. Condor 87:222–228.

Searcy WA, Nowicki S, 2000. Male-male competition and female choice in the evolution of vocal signaling. In: Animal signals: signaling and signal design in animal communication (Espmark Y, Amundsen T, Rosenqvist T, eds). Trondheim: Tapir Academic Press; 301–315.

Sharma SC, Bhatia BB, Pachauri SP, 1984. Blood cellular and biochemical studies in chicken experimentally infected with *Toxoxara canis*. Int J Parasitol 8:159–163.

Specht R, 1999. Avisoft SasLab Pro: a PC-program for sonagraphic analysis. Users guide. Berlin: Avisoft Bioacustics.

Svensson L, 1984. Identification guide to European passerines, 3rd ed. Stockholm: British Trust for Ornithology.

Thomas F, Renaud F, DeMeeüs T, Cezilly F, 1995. Parasites, age and the Hamilton-Zuk hypothesis: inferential fallacy? Oikos 74:305–309.

Trivers RL, 1972. Parental investment and sexual selecrtion. In: Sexual selection and the descent of man (Cambell B, ed). Chicago: Aldine; 139–179.

Wang NG, Hurley P, Pytte C, Kirn JR, 2002. Vocal control neuron incorporation decreases with age in the adult zebra finch. J Neurosci 22:10864–10870.

Weatherhead PJ, Metz KJ, Bennett GF, Irwin RE, 1993. Parasite faunas, testosterone and secondary sexual traits in male red-winged blackbirds. Behav Ecol Sociobiol 33:13–23.

Williams GC, 1966. Adaptation and natural selection. Princeton: Princeton University Press.

Yasukawa K, 1981. Male quality and female choice of mate in the red-winged blackbird (*Agelaius phoeniceus*). Ecology 62:922–929.