

The design of complex sexual traits in male barn swallows: associations between signal attributes

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Abstract

Variation in the expression of sexually selected traits among individuals is widely investigated on the premise that these traits evolved to signal male quality. Significant repeatabilities of sexual signals and their associations with condition, mating success, survivorship and age may be the signatures of sexual selection. However, little is known about the relationship between these sexual attributes. Here we studied 28 acoustic and visual traits in the barn swallow, *Hirundo rustica*, that may potentially function in sexual selection. Based on effect sizes calculated at the between-individual level, we assessed the relationship between repeatability, condition-dependence, attractiveness, age-dependence and viability indicator value of sexual traits using sexual signals as the units of analyses. Those traits that showed high within-year repeatability also showed high between-year repeatability, indicating that between-individual variation is consistent within and among seasons. In addition, age-dependence of traits, probably causing between-year variation, was negatively related to between-year repeatability. Condition-dependence was negatively correlated with effect sizes for the extent to which traits predicted viability. Therefore, traits that are positively related to immediate condition are those that are negatively related to survival, which may be the signature of a trade-off between current and future reproductive success ultimately reflecting signal reliability. No other significant relationship was found between trait attributes. We conclude that multiple sexual signals reflect different aspects of male quality in the barn swallow.

Introduction

Sexually selected traits may reflect (i) immediate individual condition that is dependent on environmental effects; (ii) individual-specific attributes that show how individuals can cope with these environmental effects on immediate body condition or (iii) the genetic quality of males that is independent of condition (Pomiankowski, 1987; Grafen, 1990; Iwasa & Pomiankowski, 1994,

1999). Therefore, the three main, nonexclusive types of benefit females get from mating with elaborately ornamented males are (i) direct benefits, (ii) good genes and (iii) offspring attractiveness. Accordingly, correlations between these aspects of male quality, sexual signals and mating success and their repeatability may help to reveal why certain traits are favored by sexual selection while others are not. Below, we briefly examine the implications of these correlations between aspects of male quality for sexual selection.

Correlation with body condition, i.e. the condition-dependence of sexual traits can reflect male genetic or environmental quality, or both (Rowe & Houle, 1996). Female preferences may have evolved for

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condition-dependent, costly sexual ornaments because using such signals may provide female with benefits directly affecting reproductive output (e.g. parasitism, parental quality), and potentially also confer indirect advantages in terms of the genetic background of body condition. Accordingly, it is widely assumed that condition-dependence is a common feature of sexual traits (Andersson, 1994; Johnstone, 1995, but see also Cotton *et al.*, 2004).

Correlation with age may also be important in the context of sexual selection, because signal expression in males may reflect the bearer's ability to survive, or an attribute that improves with experience, but it can also happen that signals are just more reliable in older males (Trivers, 1972; Manning, 1985; Hansen & Price, 1995; Proulx *et al.*, 2002). Hence, by mating with older males, females may obtain 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 (Yasukawa, 1981; Alatalo *et al.*, 1986b; Marchetti & Price, 1989) or absence of pathogens (Saino *et al.*, 2003). Alternatively, they may obtain genetic benefits for the offspring, if the age-dependence of attractiveness reflects heritable viability differences among males (Trivers, 1972; Török *et al.*, 2003).

By correlations with survival, age effects on sexual displays due to differences in viability can be separated from age effects caused by consistent phenotypic changes with aging (e.g. experience, senescence). In general, females seem to prefer to mate with males showing elaborate traits that indicate high survival probability (Jennions *et al.*, 2001). However, theoretically, if the cost of female preference is low, and mating skew is pronounced, males with the highest genetic quality may invest heavily in sexual signals so they experience lower survival than males of poorer quality (Grafen, 1990; Kokko, 1998, 2001; Brooks, 2000; Eshel *et al.*, 2000). Therefore, the relationship between viability and sexually selected trait elaboration can be negative under certain circumstances (Kokko, 1997, 1998).

Repeatability of sexually selected traits is integral to the process of sexual selection, as for reliable signalling, the given trait is required to show consistent variation among individuals. Within-individual consistency of a sexual display is beneficial (i) for the signaller as only repeatable attractiveness leads to fitness differences among males (Boake, 1989; Wagner & Sullivan, 1995) and (ii) for the receiver, as the degree and pathway of additive genetic determination and condition-dependence determine the benefits from choosiness (Rowe & Houle, 1996). The extent to which variation within individuals contributes to total variation in a population may be described by the repeatability of a trait, which reflects the ratio of genetic and individual-specific environmental variance to total phenotypic variance (Falconer & Mackay, 1996). Repeatability provides an upper limit to heritability (Falconer &

Mackay, 1996). Thus, traits with low or negative repeatabilities are unlikely to provide genetic benefits to choosy females. Sexual features with high among-individual variation and repeatability are expected to be potential targets of sexual selection (Gil & Slater, 2000b; Gil & Gahr, 2002; Sattman & Cocroft, 2003). However, phenotypically plastic traits with low repeatability may also be sexually selected, if they indicate important fitness benefits (West-Eberhard, 2003).

If secondary sexual traits are dependent on condition or experience, or indicate heritable viability and consistently vary among individuals, females may be expected to evolve preference for such traits (Kirkpatrick & Ryan, 1991; Kokko *et al.*, 2003). Therefore, indicators of male quality should predict the outcome of female choice, and trait elaboration should correlate positively with estimates of mating success. In agreement with this, it has frequently been demonstrated that males displaying the most extravagant sexual displays are those that are able to pair earlier, become polygynous by acquiring multiple breeding partners, or achieve higher levels of extra-pair paternity (Searcy & Yasukawa, 1996; Møller & Alatalo, 1999; Parker & Ligon, 2003). However, success in mating does not necessarily reflect female preference, as it may be the result of male–male competition, which is also expected to favour quality-indicating male traits (Searcy & Andersson, 1986; but see Berglund *et al.*, 1996; Qvarnström & Forsgren, 1998).

Although, the repeatability, condition- and age-dependence, viability-indicator value and mating success consequences of sexual traits are well founded on theoretical and empirical grounds, we generally lack information on how these different signal attributes are linked to each other. However, in accordance with the principles of sexual selection, it would be logical to expect relationships between signal design, information content and female preference. Information conveyed by signals is expected to influence both sexual and other selection forces acting on them, so signal information content has multiple implications to signal evolution. For example, if females prefer the most reliable signals, one may predict a positive association between repeatability and mating success. Age-dependence or condition dependence should be negatively related to repeatability, because phenotypic changes due to immediate condition or aging represent phenotypic plasticity, on different time scales, which increases within-individual relative to among-individual variation. However, if ornaments are genetically correlated across age classes, individuals with relatively large ornament will be relatively large within each age class, which can create high repeatability even if trait expression strongly differs between ages. Mating success attributed to male traits may be expected to positively associate with the condition-dependence, age-dependence and viability indicator value of traits, because females are generally thought to enjoy fitness benefits by preferring these attributes. However, if signal

design is primarily important in male contest, and the relationship between success in mating and male–male competition is weak or negative (Berglund *et al.*, 1996; Qvarnström & Forsgren, 1998), the relationship between mating success, repeatability, age- and condition-dependence should be weak or negative, respectively. If a trait is evolved to signal male quality in general, and it simultaneously reflects male age, survivorship and condition to some degree (see Møller & Pomiankowski, 1993; Candolin, 2003), this should mediate a relationship between these attributes. On the other hand, different traits can signal different aspects of quality, with one signalling condition, and another indicating experience or viability for example (see Møller & Pomiankowski, 1993; Candolin, 2003), which should cause an absence of covariation between trait attributes. Age-dependence of traits may arise due to phenotypic plasticity caused by aging effects or due to phenotype-dependent survival. The latter scenario should mediate a positive relationship between age-dependence and survival value of traits, while the former predicts that these attributes are independent. If age-dependence is a side effect of aging, it can result from a strategic, age-dependent adjustment of trait expression, or from plastic changes due to sensitivity to condition, which in fact should mediate a relationship between age-dependence and condition-dependence. Condition-dependence of a trait may predict its survival value, if individuals optimize their investment in costly traits that affect their survival (Rowe & Houle, 1996). In general, these are fundamental predictions of sexual selection, because associations between trait attributes have important implications for signal evolution. However, these predictions are difficult to test, because they necessitate analyses at the level of variables, in which parallel information on several sexual traits can be powerfully compared for different signal attributes. This difficulty may have contributed to the fact that these key questions still remain unanswered.

Here we investigated associations between sexual trait attributes by focusing on 28 phenotypic traits in males of the barn swallow, *Hirundo rustica*. We considered plumage coloration, tail length, body size and features of song that may all play a role in sexual selection in this species (Møller, 1994b; Galeotti *et al.*, 1997; Saino *et al.*, 1997a,c, 2002; Møller *et al.*, 1998; Perrier *et al.*, 2002; Garamszegi *et al.*, 2005). First, we monitored variation in trait expression among individuals, and determined associations between these traits. We also quantified the relationship of all individual traits with age, body condition, survival and mating success. From the resulting correlation matrix, we derived effect sizes corresponding to the strength of age-dependence, condition-dependence, survival indicator value and relationship with mating success for particular sexual signals. Second, we worked at the level of variables to determine the relationship between different trait attributes relying on effect sizes of particular relationships.

Materials and methods

Variables measured at the individual level

The barn swallow is a widely studied migratory, insectivorous passerine, and its sexual behaviour has been described repeatedly in the literature (e.g. Cramp, 1988; Møller, 1994b; Galeotti *et al.*, 1997; Møller & Tegelström, 1997; Saino *et al.*, 1997d; Møller *et al.*, 1998). Data for the current study were collected in a locally abundant population near Badajoz (38°50'N, 6°59'W), Spain in 2000–2001.

Relying on standardized field procedures, males were captured and measured for morphological variables, whereas their song was recorded in natural conditions (see Perrier *et al.*, 2002; Garamszegi *et al.*, 2005). We used these measurements and recordings to quantify the expression of 28 potential secondary sexual traits (tail length, body size, four structural plumage colours and 22 features of song) in males that all may function in mate choice and male–male competition. Definitions, means and sample sizes are given in Table 1 and Fig. 1. In general, our choice of variables was based on considerations concerning their sexual function, as typically observed in this intensively studied species (Møller, 1994b; Galeotti *et al.*, 1997; Saino *et al.*, 1997a,c, 2002; Møller *et al.*, 1998; Perrier *et al.*, 2002; Garamszegi *et al.*, 2005) and from speculations about costs and constraints, which might influence trait expression. In particular, tail length in the barn swallow is the classical model of sexual traits (Møller, 1988, 1994b; Møller *et al.*, 1998), while plumage coloration has recently been suggested to function in sexual selection (Perrier *et al.*, 2002). Previous studies discovered the role of particular song traits in mate choice and male–male competition in detail (Galeotti *et al.*, 1997, 2001; Møller *et al.*, 1998; Garamszegi *et al.*, 2005). We believe that with these song variables, we capture a wide range of features that may be associated with song composition at different temporal resolutions (along an axis of bout-song-rattle in Fig. 1), and also with song complexity and song performance.

Male age was coded on a binomial scale, and was set to zero for males caught in their first breeding year and to one for older males. Birds were assumed to be yearlings when first captured without a ring, with all older birds already ringed in a previous year. The reliability of this assignment of age was demonstrated by earlier findings (Perrier *et al.*, 2002; Saino *et al.*, 2003). Age of birds captured with a ring was determined based on the year of their first capture.

When males were first captured, we recorded their body mass. Body mass relative to body size, as reflected by keel length, was used to assess body condition. The control for body size was achieved in ANCOVA or in partial correlation designs in which body size was entered as a covariate (García-Berthou, 2001).

Table 1 Definitions and means \pm SE (N) of variables used to characterize sexual signals (plumage and song traits) in the barn swallow, as given by data from 2000. The detailed description of traits and their standard measurements can be found in Møller (1994b)), Perrier *et al.* (2002), and in Fig. 1 with the associated references. Structural coloration refers to dorsal plumage. We used external keel length as a predictor of overall body size (see Senar & Pascual, 1997). Due to varying data availability resulting from different constraints, sample size differs among traits.

Variable	Definition	Means \pm SE (n)
Morphology		
Tail length	The mean of the length of left and right outermost tail feathers (mm)	99.4 \pm 0.66 (100)
Body size	Keel length (mm)	21.6 \pm 0.07 (101)
Structural coloration		
UV reflectance	Sum of reflectance in the spectrum interval 300–400 nm	4943 \pm 137 (99)
Blue reflectance	Sum of reflectance in the spectrum interval 400–475 nm	4526 \pm 121 (99)
Blue chroma	Blue reflectance/total reflectance (reflectance in 400–475 nm/reflectance in 300–700 nm)	0.24 \pm 0.01 (99)
Hue	Wavelength where maximal reflectance occurred (nm)	233.1 \pm 0.77 (99)
Song		
Song rate	The total number of songs within the recording/duration of the recording in min	5.78 \pm 0.41 (28)
'A' ratio	The number of 'A' songs/total number of songs within the recording	0.36 \pm 0.03 (28)
'C' ratio'	The number of complete songs/total number of songs within the recording	0.64 \pm 0.03 (28)
Bout ratio	The number of bouts/(the number of bouts + number of single songs presented outside the bouts)	0.35 \pm 0.0 (28)
Bout length	The duration of bouts (s)	15.72 \pm 2.25 (23)
No. of songs	The number of songs found within bouts	3.32 \pm 0.47 (23)
Bout 'A'	The number of 'A' songs/number of songs within bouts	0.13 \pm 0.06 (19)
Bout 'C'	The number of complete songs/number of songs within bouts	0.37 \pm 0.04 (23)
Song length	The duration of songs (s)	4.75 \pm 0.16 (28)
Overall repertoire size	The cumulative number of syllable types of five 'A' songs	22.9 \pm 1.06 (16)
Syllable repertoire	The number of syllable types within songs	17.8 \pm 0.69 (16)
Versatility	The number of syllable types within songs/the number of syllables within the song	0.75 \pm 0.02 (16)
Mean peak amplitude frequency	The frequency of the harmonic over which the most energy was distributed in the entire song (kHz)	3.70 \pm 0.05 (28)
Song peak amplitude frequency	The frequency of the maximum amplitude of the song (kHz)	6.94 \pm 0.08 (28)
Song performance	The percentage of song length when the amplitude exceeds a threshold value of 20% of the maximum (%)	14.2 \pm 0.67 (28)
Syllable tempo	The number of syllables/the duration of song in sec	6.15 \pm 0.07 (28)
Rattle length	The duration of the rattle (s)	0.41 \pm 0.01 (28)
Impulse tempo	The number of impulses within the rattle/the duration of the rattle in sec	25.4 \pm 0.25 (28)
Rattle peak amplitude frequency	The frequency of the maximum amplitude of the rattle (kHz)	6.78 \pm 0.08 (28)
P length	The duration of syllable P (s)	0.43 \pm 0.014 (28)
P peak amplitude frequency	The frequency of the maximum amplitude of syllable P (kHz)	5.66 \pm 0.07 (28)
P performance	The percentage of P length when the amplitude exceeds a threshold value of 20% of the maximum (%)	78.1 \pm 2.55 (28)

We estimated the mating success of males by their pairing date relative to their arrival, which was defined as the number of days elapsed between the arrival of a male and the start of egg laying by its mate. Arrival date of a bird was estimated as the date of its first capture across the weekly based capturing sessions. A previous study showed that arrival date does not bias pairing date, and hence we did not control for arrival date when relative pairing date was used (Garamszegi *et al.*, 2005).

We captured 102 and 69 males in the two field seasons, respectively. Male recapture probability was calculated based on the capture–recapture histories of birds in the 2 years. Long-term studies of the barn swallow have revealed that previously encountered birds that are not captured in a given year are very unlikely to be recaptured in the future because recapture probabilities

exceed 98% (Møller & Szép, 2002). A similar value has been estimated for our Spanish population (F. de Lope, A. P. Møller and T. Szép unpublished data). Hence, recapture probability reliably reflects survival in this species without a need for application of mark-recapture modelling. Accordingly, those males from 2000, which were not recaptured in 2001, were considered dead.

Statistical analyses at the levels of individuals and variables

Variables were checked for normality and homoscedasticity. When necessary, statistical transformation was applied in order to meet the parametric criteria.

Within-year repeatability was computed based on the repeated measurement of the same trait of the same male

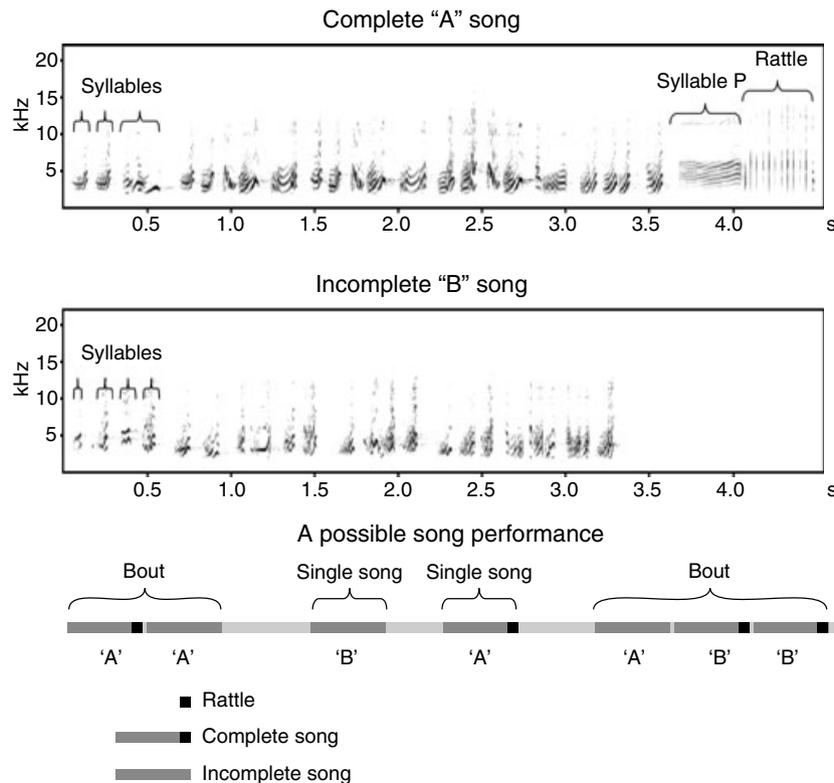


Fig. 1 The spectrographic representation and the corresponding terminology used to describe the song of the barn swallow. Males sing two basic song types, song types 'A' and 'B', differing in the structure of syllables that compose them. The classification of these song types as complete or incomplete relies on the presence or absence of the terminal harsh syllable, the rattle, which is a sequence of impulses. The song display consists of sequences of these song types organized in single songs or complex bouts. Bouts involve at least two songs with the time intervals between them not being longer than the mean time interval between syllables of the song. Repertoire size is defined by the number of different syllable types known by individuals, which can be reliably estimated based on five 'A' songs. See Galeotti *et al.* (1997, 2001) Garamszegi *et al.* (2005) and Saino *et al.* (1997c) for more details on definitions. Here we also considered the characteristics of the penultimate syllable (called syllable *P*) of complete songs, which we found to be typical and biologically important. Recordings for 28 males used in the current study were made with a Sony TCD-D8 DAT tape recorder connected to a MD 21 U microphone in 2000. Songs were analysed spectrographically with the computer program 'AVISOF-T-SASLAB PRO' (Specht, 1999) using 0–10 kHz frequency range, 20 000 samples/s sampling rate, 200 Hz band width, a frequency resolution of 80 Hz and a time resolution of 32 ms (see also Garamszegi *et al.*, 2005).

within the same breeding season (separately for 2000 and 2001) following the approach of Lessells & Boag (1987). However, this estimation could be done for song variables only. Although within a single recording, multiple songs were available, tail length, body size and structural coloration were measured only once within a single breeding season. Theoretically, the repeatability of these traits could be considered ~ 1 , as they are not expected to change within a season. However, measurement error and plumage abrasion can cause a certain level of variation within individuals, and hence we used the observed within-year repeatabilities of these traits from other sources (Møller, 1991, 1994b; Perrier *et al.*, 2002). Because these within-year repeatabilities correspond to different years for plumage coloration and to a different population for tail length, we factored out statistically these potential sources of bias by controlling for trait type in the analyses or by focusing on song traits

only (see below). Because the estimation of repertoire size requires a sample of multiple songs (see Fig. 1 and Table 1), only one value could be calculated for each male in each breeding season. Consequently, we could not obtain within-year repeatability for this trait. We assessed the reliability of within year repeatabilities of 21 song traits for which data were available. We compared within-year repeatabilities calculated for the two breeding seasons separately by correlating them between years and across song variables in order to check whether the same trait had consistent repeatabilities in different years ('repeatability of repeatability'). When estimating between-year repeatability of secondary sexual traits, we correlated the mean expression of the trait in two consecutive seasons. The between-year repeatabilities are from 1999 to 2000 for spectral traits, and from 2000 to 2001 for all other traits, and these differences are taken into account when we controlled for trait type

(song vs. plumage traits) statistically (see below). However, given that within- and between-year repeatabilities are from different sources for different trait types, comparisons of these repeatabilities between plumage and song traits should be interpreted with caution. Note that these potentially confounding effects should appear in association with repeatabilities only, because other trait attributes were directly assessed from the available data originating from a single year (2000).

Based on the individually averaged values in 2000, we estimated the pair-wise relationship between trait expression and age, survival, condition and mating success for each sexual trait. In these analyses, we relied on data from 2000, for which we had a large sample size, to avoid the confounding effect of year. Variables that were significantly related to male age were standardized for age before correlating them with mating success, condition and survival (note that repeatabilities were calculated based on the nonstandardized values). To archive this control of age, we transformed age class to a common mean of 0 and standard deviation of 1. The strength of the relationships between sexual traits and other phenotypic traits was estimated as the Pearson's correlation coefficient between the variables of interest. Pearson's product-moment correlation coefficients are commonly used as a standardized measure of effect size (i.e. effect size correlations), under the null hypothesis that the mean underlying effect size equals zero (Hedges & Olkin, 1985; Rosenthal, 1991; Cooper & Hedges, 1994).

In cases where we controlled for confounding effects, such as body size in the analyses of body condition, we included body size as a covariate only if it was significant when entered to the model together with mass (this seemed necessary, as multivariate analyses are unreliable with such small sample sizes). As male age and survival are categorical variables, we estimated their association with the expression of sexual signals by comparing the nonstandardized values of sexual traits between yearlings and older males, and between survivors and nonsurvivors. From the corresponding *t*-tests, Cohen's *d* effect size was calculated that was subsequently converted to effect size correlations estimated as Pearson's *r*. Based on the effect sizes for the relationship between the investigated traits and other characteristics of males, we created a data matrix at the level of variables. In this matrix, each trait attribute is sampled across 28 traits. Relying on this effect size matrix, we determined the general associations between repeatabilities, age- and condition-dependence, survival indicator value and mating success across sexual traits. Effect sizes and repeatabilities for all traits are given in Table 2.

A priori, it is difficult to decide for each trait whether selection arising from male age, condition, survival and mating success acts in a positive or negative direction, e.g. whether high or low values of the traits represent superior male quality. Hence, in the trait-level comparative study, it would cause confusion and meaningless

results if the trait attributes were pooled irrespective of the direction of female preference for the respective traits. For instance, if the measured values of both traits A and B are positively correlated with survivorship, but female preferences for them act in opposite directions, then effects of the preference for female fitness are in fact opposite for the two traits. Therefore, we determined the sign of each effect according to the importance of the trait in sexual selection as reflected by its correlation with pairing date. First, we assessed the direction of female preference for each potential sexual trait as the direction of change accompanied by a reduction of pairing latency. Second, under this directional scenario, we converted the signs of the correlations of trait expression with condition, age and survivorship so that positive relationships coincided with the direction of female preference. In the above example, if the correlation with pairing latency was positive for trait A and negative for trait B, we assumed that the relationship with survival was negative for trait A and positive for trait B.

However, traits that do not affect pairing date, but have weak correlation with pairing date generating random signs with no biological meaning may produce confounding effects. Additionally, there may be potentially different roles in effect for song and plumage traits (and repeatabilities may be potentially inflated by between source effects). Therefore, we applied two analytic approaches. In the first analysis, we involved all traits, and assessed the importance of differences between song and plumage traits in an ANCOVA model that included type of traits (song vs. plumage) as a cofactor. In the second approach, we only considered song traits, which are sexually selected, as mirrored by their absolute effect size for relative pairing date being larger than 0.2 (using alternative criteria, such as effect size limit of 0.1 or 0.3 did not affect the conclusions). Focusing on these song traits (see Table 2), we correlated trait attributes to each other.

Relationships between different trait attributes may be confounded by correlations between sexual traits. Co-variation between secondary sexual traits renders estimates of effect sizes statistically dependent observations, violating an important statistical assumption. Therefore, relationships between secondary sexual trait attributes should be controlled for such statistical dependence of the traits. Due to missing data points (see Table 1), we could not perform multivariate analyses (e.g. partial correlations) involving all sexual traits to estimate effect sizes that are independent of each other. Hence, we applied an alternative solution that was motivated by an analogous problem in comparative biology (Garamszegi, 2006). The relationship between different variables causes a lack of statistical independence similar to that arising from the use of species-specific values in comparative analyses (Felsenstein, 1985). In such inter-specific studies, phylogenetic approaches are applied to eliminate the confounding

Table 2 Attributes of secondary sexual characters in the barn swallow, in terms of their repeatabilities (within-year and between-year), and their association with condition, age, viability and mating success. Pearson's effect sizes are given, with signs converted according to the direction of the mating preference (see main text for details). Song traits with effect sizes for mating success larger than 0.2 (given in bold) were considered as preferred by females and were used in the analyses of associations between trait attributes (Table 3). Within-year repeatabilities of song traits were averaged between 2000 and 2001, as they were significantly repeatable. Traits that were age-dependent were standardized to remove age effects when calculating condition dependence, viability indicator value and mating success (but not when calculating repeatabilities and age dependence).

Variable	Within-year repeatability	Between-year repeatability	Condition dependence	Age dependence	Viability indicator value	Mating success
Morphology						
Tail length	0.998	0.932	0.197	0.416	-0.159	0.327
Body size	0.846	0.332	-0.514	-0.167	0.269	0.241
Structural coloration						
UV reflectance	0.420	0.132	0.207	0.230	0.093	0.280
Blue reflectance	0.480	-0.034	0.412	0.023	-0.142	0.181
Blue chroma	0.680	0.245	-0.025	0.271	0.397	0.118
Hue	0.610	0.441	-0.096	0.397	0.220	0.285
Song						
Song rate	0.142	-0.329	-0.022	0.079	0.228	0.150
'A' ratio	0.139	0.115	-0.497	0.230	-0.006	0.067
'C' ratio	-0.013	0.091	0.369	0.095	0.191	0.185
Bout ratio	0.075	-0.302	-0.024	0.296	0.079	0.468
Bout length	0.305	0.397	0.290	0.158	-0.021	0.168
No. of songs	0.478	0.173	0.326	0.437	-0.163	0.100
Bout 'A'	0.240	0.591	0.248	-0.719	0.248	0.055
Bout 'C'	0.313	0.269	0.325	0.327	-0.197	0.191
Song length	0.273	-0.230	0.018	0.550	0.038	0.394
Overall repertoire size	Data not available	0.578	0.039	-0.647	0.000	0.358
Syllable repertoire	0.731	0.664	0.401	0.700	0.214	0.123
Versatility	0.256	0.411	0.178	0.181	-0.124	0.220
Mean peak amplitude frequency	0.244	-0.029	-0.340	0.570	0.390	0.346
Song peak amplitude frequency	0.302	0.742	-0.377	-0.036	0.000	0.249
Song performance	0.159	0.035	0.333	-0.058	-0.475	0.190
Syllable tempo	0.094	0.584	0.509	-0.102	-0.224	0.195
Rattle length	0.575	0.742	-0.277	-0.173	0.082	0.146
impulse tempo	0.336	0.487	-0.102	0.098	-0.082	0.446
Rattle peak amplitude frequency	0.489	0.602	-0.453	-0.145	0.018	0.290
P length	0.487	0.839	-0.203	-0.434	0.150	0.129
P peak amplitude frequency	0.429	0.352	0.126	-0.093	-0.274	0.393
P performance	0.241	0.456	-0.540	-0.297	0.210	0.345

effect of common ancestry depicted in a phylogenetic tree that causes statistical dependence for the data at hand. We utilized a similar approach to deal with the confounding associations between variables. First, we constructed a 'phenetic' tree that described the relationships between variables based on the correlations of traits (Fig. 2). This was done by the hierarchical classification of variables using joining- or tree-clustering methods with a single linkage (Podani, 2000). Tree clustering methods uses dissimilarities or distances between objects to group objects of similar kind into respective categories. Just as phylogenetic distance matrices are transformed to phylogenetic trees (Sibley & Ahlquist, 1990), if the distance between variables is reflected by their relationship, a correlation matrix of variables could be used as a distance matrix in a cluster analysis. Therefore, from the correlation matrix of traits, we estimated the distances

between variables as $1 - |r|$, and clustered them accordingly. We used the numeric (unsigned) correlation coefficients, as we were interested in controlling for the strength of different associations regardless of the direction of the patterns. In the resulting tree, the tips were the secondary sexual traits, and their distance, i.e. correlation were represented by differences in branch lengths. For example, strongly correlating variables were closely related to each other, and the distance between them was small. Second, we imported this tree into the phylogenetic program CAIC (Purvis & Rambaut, 1995) that calculated standardized linear contrasts that were independent of correlations between variables. We used these statistically independent contrasts to test for associations between different trait attributes, while holding co-variation between secondary sexual traits constant. Note that CAIC was especially developed for

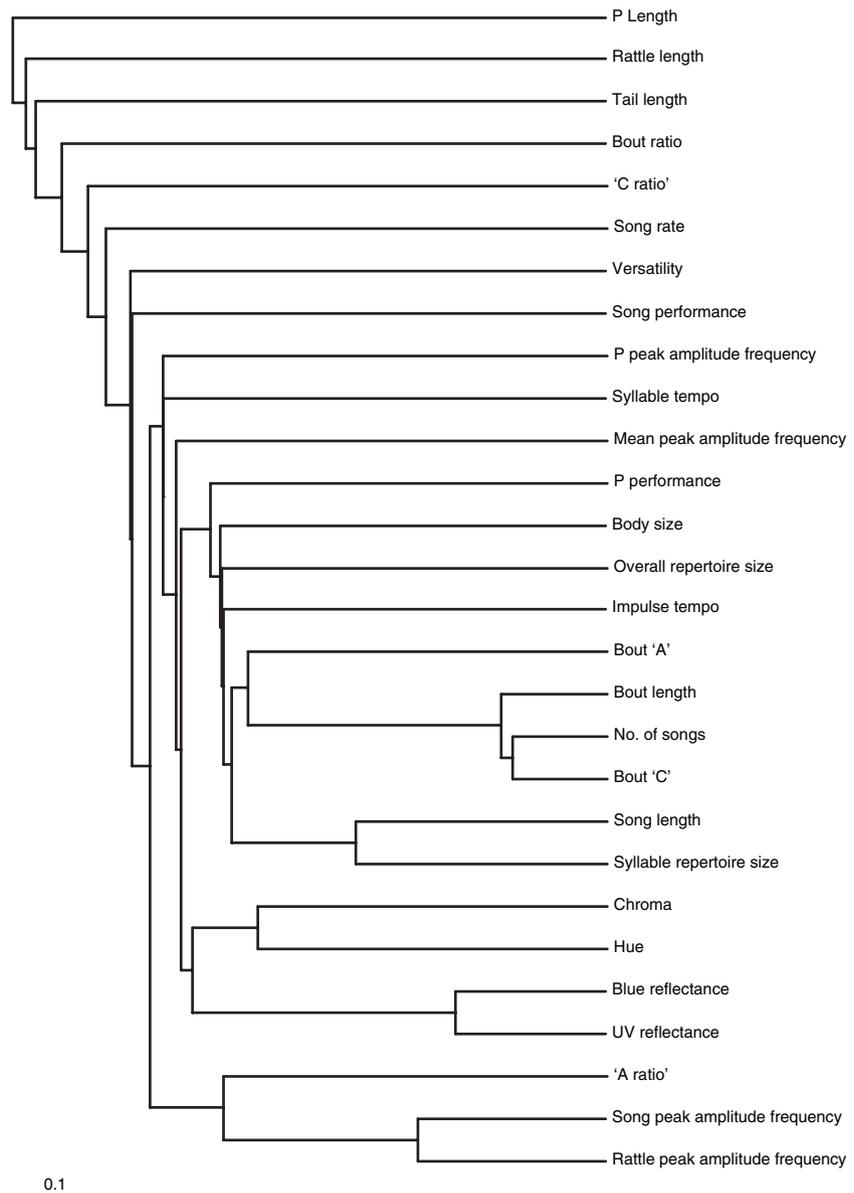


Fig. 2 Hierarchical classification of 28 different secondary sexual traits in the barn swallow based on pair-wise correlation coefficients (r) between them. Result from tree clustering with a single linkage method. The unit for the tree is $1 - \text{abs}(r)$ reflecting the distance between traits and it is given at the bottom left.

phylogenetic analyses, and may be sensitive to specific assumptions, which may render our control for the association between variables conservative. However, we do not know other programs that could be used for our specific purposes. Values reported are mean \pm SE.

Results

Repeatabilities

Within-year repeatabilities varied from -0.013 to 0.998 with a mean \pm SE of 0.382 ± 0.045 (Table 2; negative values can occur when $F < 1$ in the corresponding ANOVA). Within-year repeatabilities of plumage traits

were significantly larger than those of song traits (Fig. 3, $t_{24} = 3.585$, $P = 0.002$; plumage traits, 0.638 ± 0.101 ; song traits, 0.300 ± 0.039 ; keel length excluded). When using song traits there was a significant positive correlation between within-year repeatabilities assessed in different years ($r = 0.520$, $n = 21$, $P = 0.015$). This pattern indicates that within-year repeatabilities are consistent, i.e. the repeatability measured for a trait is similar in different years. We expect this effect to be stronger, if relatively inflexible plumage traits with very high within-year repeatabilities were included. Hence, within-year repeatability is a trait-specific attribute, and we calculated the mean of the within-year repeatabilities of song traits obtained in the two years.

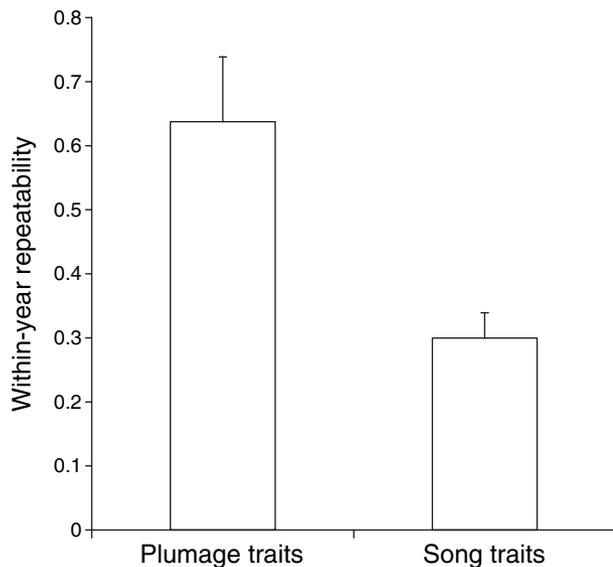


Fig. 3 Differences in within-year repeatabilities of secondary sexual traits between plumage ($n = 5$) and song traits ($n = 21$), which are listed in Table 1 (keel length excluded). Means and standard errors are given.

Between-year repeatabilities ranged from -0.329 to 0.932 with a mean \pm SE of 0.332 ± 0.064 , which is similar to what we observed for within-year repeatabilities (Table 2). A paired comparison revealed no statistically consistent difference between within- and between-year estimates (paired $t_{26} = 1.028$, $P = 0.313$). Between-year repeatabilities did not differ significantly between plumage and song traits (Fig. 4; $t_{24} = 0.082$, $P = 0.935$; plumage traits, 0.343 ± 0.166 ; song traits, 0.329 ± 0.074 ; keel length excluded). Within-year and between-year repeatabilities were significantly positively correlated ($r = 0.504$, $n = 27$, $P = 0.007$). This relationship remained significant when co-variation between traits summarized in Fig. 2 was held constant (regression of statistically independent contrasts through the origin: $F_{1,25} = 5.95$, $P = 0.022$). Similar results were obtained when we controlled for differences between plumage and song traits in an ANCOVA (Fig. 4; within-year repeatability: effect for between-year repeatability, $F_{1,23} = 16.92$, $P < 0.001$; effect for trait type, $F_{1,23} = 20.23$, $P < 0.001$), or when we focused on the 10 sexually selected song traits that are related to mating success via pairing date ($r = 0.566$, $n = 10$, $P = 0.088$; Fig. 4). This association shows that a trait with a consistent variation within a season also has consistent variation across seasons.

There was a tendency for age-dependence being negatively related to between-year repeatability (Fig. 5; $r = -0.370$, $n = 28$, $P = 0.053$; holding the correlation between traits constant by a regression of statistically independent contrasts through the origin: $F_{1,26} = 3.30$,

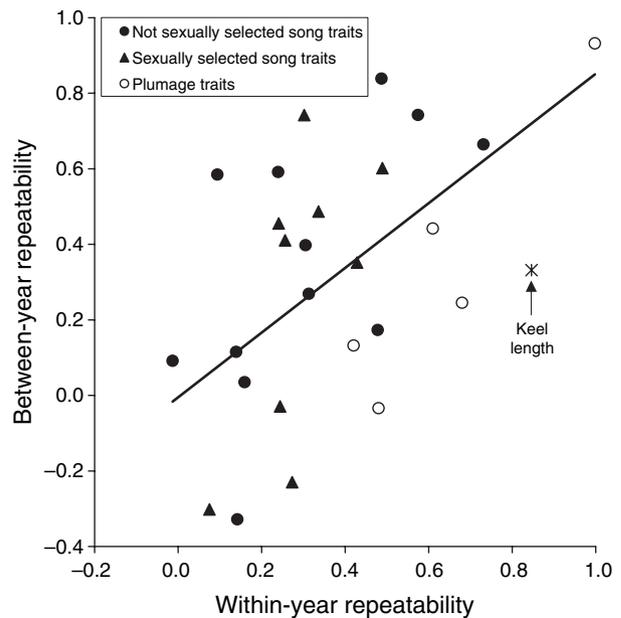


Fig. 4 Relationship between within-year and between-year repeatabilities of secondary sexual traits in the barn swallow. Different symbols are used for different signal types (see main text for details). Each data point represents a variable defined in Table 1. The line is the linear regression line for the entire sample with the equation $Y = 0.02 + 0.82X$ (keel length is excluded). Note that we used data for between-year repeatability of plumage traits from other sources. When focusing on song variables only, the relationship is $r = 0.581$, $n = 21$, $P = 0.006$.

$P = 0.081$). However, when we controlled for variation between plumage and song traits and excluded keel length, this relationship became significant (ANCOVA: effect for between-year repeatability, $F_{1,24} = 4.30$, $P = 0.049$; effect for trait type, $F_{1,24} = 1.99$, $P = 0.172$; Fig. 5). A similar significant association was found, when we considered sexually selected song traits only (Fig. 5; $r = -0.742$, $P = 0.014$). This relationship implies that variation between ages in song traits emerges across seasons thereby affecting between-year repeatability, if age-dependence is due to within-individual change (see Galeotti *et al.*, 2001; Garamszegi *et al.*, 2005). Other trait attributes (condition-dependence, survival indicator value and mating success) were not significantly related to either measure of repeatability using either approach ($-0.566 < r < -0.140$, $P > 0.088$).

Age- and condition-dependence, survival indicator value and mating success

Mean effect sizes for traits were not significantly different from zero (age-dependence, 0.078 ± 0.065 , condition-dependence, 0.018 ± 0.060 , survival indicator value, 0.034 ± 0.039 , see Table 2 for particular effect sizes).

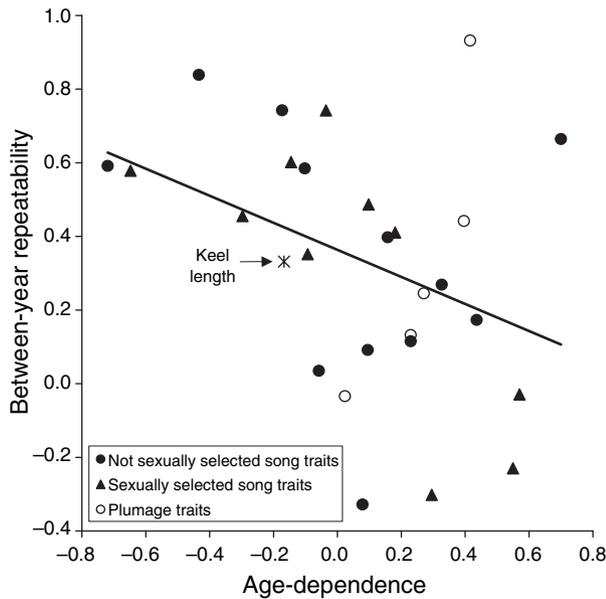


Fig. 5 Age-dependence in relation to the between-year repeatability of secondary sexual traits using different symbols for different signal types. Each data point represents the summary statistic for a variable (see Table 1). The line is the linear regression line for the 27 sexual traits with the equation $Y = 0.36 - 0.37X$. Note that we used data for between-year repeatability of plumage traits from other sources. When focusing on song variables only, the relationship is $r = -0.512$, $n = 22$, $P = 0.015$.

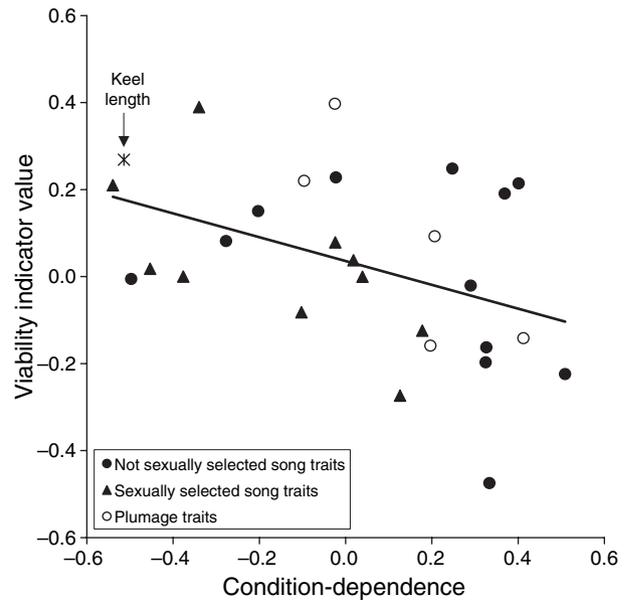


Fig. 6 Relationship between viability indicator value and condition-dependence as shown by the association of effect sizes across 27 traits of different types. The line is the linear regression line with the equation $Y = 0.04 - 0.28X$.

We checked the relationship between different trait attributes, and the only significant pattern found was a negative association between condition-dependence and survival indicator value (Table 3, Fig. 6). This relationship was independent of co-variation between secondary sexual traits, as indicated by the regression of statistically independent contrasts through the origin ($F_{1,26} = 6.49$, $P = 0.017$), and was also present when we focused on the 10 sexual selected song traits only (Table 3). Therefore, it seems that traits that are more strongly associated with survival are those that are less-dependent on male condition.

Discussion

In this study of 28 potential secondary sexual traits in male barn swallows, we found that estimates of within-year repeatability of different traits were consistent across years, and they also predicted between-year repeatability. Within-year repeatabilities of song traits are about half as large as within-year repeatabilities of plumage traits, which may be expected when considering the development and stability of these traits within a season. However, such patterns should be interpreted with caution, because differences in within-year repeatabilities between different signal types may be year-specific, as repeatability data for song and plumage traits are not from the same years. At least for the song traits we were able to demonstrate that within-year repeatabilities are

Table 3 Relationship between different attributes of sexual traits in the barn swallow, including age- and condition-dependence, survival indicator's value and role of traits in mating success. The first statistics are for the ANCOVA analyses that involve 27 traits (keel length excluded) and consider differences in plumage and song traits. The second statistics are Pearson's correlations of effect sizes calculated by using 10 sexually selected song traits (see Materials and methods, Table 1 and Fig. 2 for further details).

	Age-dependence	Condition-dependence	Survival indicator value
Age-dependence			
Condition-dependence	$F_{1,24} = 0.33, P = 0.57$ $r = 0.12, P = 0.74$		
Survival indicator value	$F_{1,24} = 0.03, P = 0.87$ $r = 0.30, P = 0.40$	$F_{1,24} = 5.63, P = 0.03$ $r = -0.63, P = 0.05$	
Mating success	$F_{1,24} = 0.32, P = 0.58$ $r = 0.17, P = 0.64$	$F_{1,24} = 1.45, P = 0.24$ $r = 0.23, P = 0.53$	$F_{1,24} = 0.26, P = 0.61$ $r = 0.03, P = 0.95$

repeatable between different years, and thus we can infer that year effects may be negligible. Between-year repeatabilities for the two types of signals are similar, because intra-individual variation in plumage traits increases across seasons leading to small between-year repeatabilities compared to their within-year repeatabilities. Apparently, variation in between-year repeatabilities of traits was linked to variation due to age. However, there were no significant associations between repeatabilities and other trait attributes, such as condition-dependence, survival indication value and mating success. Correlations of effect sizes across variables suggested that condition-dependence of sexually selected song traits may be inversely related to their survival indicator value, and this pattern was independent of covariance between sexual traits. Accordingly, those traits that are positively associated with survival seem to be those that are negatively affected by condition. The observed patterns are independent of co-variation between traits and of whether we focused on all traits or on the 10 sexually preferred song traits.

Traits that are sensitive to environmental fluctuations should exhibit large within-individual variation (West-Eberhard, 2003). On the other hand, signals of individual-specific attributes shaped by early environmental, maternal or genetic effects should be stable, and within individual variation is expected to remain small even across breeding seasons. Although the relationship between repeatability and heritability is not straightforward (Dohm, 2002), the presence of reliable differences among individuals is consistent with the presence of genetic variation (Boake, 1989; Martins, 1991). Because short-term and long-term variation within individuals will reveal the importance of phenotypic plasticity in trait expression at different time scales, comparing within-year and between-year repeatabilities is informative when disentangling environmental effects and individual-specific attributes (see also Griffith & Sheldon, 2001; Hegyi *et al.*, 2002). Here we found a positive relationship between within- and between-year repeatabilities. This relationship may be mediated by two mechanisms. First, unreliable signals with inconsistent variation within individuals should have low repeatability at any time scale, because no consistent variation is expected to occur either within or across years. On the other hand, reliable signals of male quality showing high repeatabilities and consistent variation within individuals may have high between-year repeatability because of stable individual-specific determination of signal expression. This would leave minor roles for phenotypic plasticity to act on the chosen traits across seasons, resulting in high within-season repeatability accompanied with low repeatability among years. Second, as repeatabilities involve intra-individual variation but also measurement errors, it remains possible that similar repeatabilities found at different time scales are caused by similarities in the extent of measurement errors (Falconer & Mackay,

1996). We have no direct approach to assess and eliminate measurement errors, but we can intuitively assume that such errors caused random noise only and not any systematic bias in the case of song traits, because these were measured similarly, and thus can be estimated with comparable errors (e.g. the duration of structures, frequency attributes, the number of certain elements). We note that the same observers were involved in the measurements of the same traits in different years, which are thus an unlikely cause of bias (PN and C. Perrier for plumage traits, LZG and DH for song traits).

We predicted that repeatabilities should be related to other trait attributes such as condition, age and mating success. For example, secondary sexual traits sensitive to immediate condition may be expected to be subject to large intra-individual fluctuations and thus likely become unstable traits with low repeatabilities. In addition, age-dependence should decrease between-year repeatabilities (but not within-year repeatabilities), because males will vary signal expression differently in different years according to their age, while maintaining low levels of variation within years. Finally, sexual selection theory posits that characteristics with the greatest reliability as reflected by high repeatability will be those that are most important in the context of sexual selection and therefore preferred by females (Boake, 1989; Andersson, 1994; Gil & Gahr, 2002). We only found support for the second prediction, as between-year repeatabilities were negatively correlated with age-dependence (Fig. 5). However, this negative association cannot be the result of consistent aging effects alone. Note that we defined age-dependence based on the phenotypic differences between yearlings and older males, but this does not necessarily mean that individuals change the expression of their secondary sexual characters with aging. Although longitudinal and cross-sectional analyses of some male traits in this species revealed that variation between young and older males may be due to systematic aging effects (Galeotti *et al.*, 2001; Garamszegi *et al.*, 2005), other mechanisms, such as consistent age- and phenotype-dependent survival and immigration/emigration may also cause differences between yearlings and older males (see Gil *et al.*, 2001). In addition, there are traits that are strongly but negatively related to male age, which would imply that yearlings decrease the expression of these traits, if aging was the only important factor. However, these traits have very high between-year repeatabilities (Fig. 5), which is at conflict with individual changes between years. Hence, it is also likely that young males developing sexier sexual characters than older individuals pay fitness costs in terms of reduced survival (negative age-dependence), and then trait expression is fixed in the population once yearlings with extreme traits were contra-selected (high between-year repeatability, which can be measured mainly in older males).

We also investigated associations between different trait attributes using effect sizes, which can reflect the strengths of different relationships in a standard and comparable way. Although we predicted several relationships (see Introduction), we only found that condition-dependence was negatively correlated with survival indicator value (Fig. 6). This relationship shows that if a trait that affects mating success is positively associated with condition, at the same time it is negatively associated with survival prospects. This result is a further evidence that longevity is not an index of genetic quality, but a fitness component that is influenced by quality (Hunt *et al.*, 2004). If traits that draw on condition also deplete the remaining condition that can be allocated to somatic maintenance, only individuals that produce exaggerated traits that are condition-dependent do so without experiencing diminished survival. The negative association between condition-dependence and survival indicator value of sexually selected traits may thus be the guarantee for signal reliability. On the other hand, it is also possible that this negative correlation is the consequence of signal reliability. If females prefer traits that negatively affect condition of the signaller, they will prefer high quality males, which are inherently superior survivors that are able to bear these costs. Under this scenario, signal reliability, in turn, is the consequence of production, maintenance costs, and the more costly the trait, the more likely it is to evolve condition-dependence. In any case, our results are in accordance with the hypotheses of sexual selection for condition-dependent traits.

We have seen that more than one mechanism can mediate a certain pattern. In some cases, as discussed above, these mechanisms can work in the same direction and they can enhance each other. On the other hand, opposing mechanisms may be in effect, which makes generalizations difficult. Accordingly, the majority of the correlations between trait attributes were nonsignificant (Table 3). We suspect that these nonsignificant associations hide evolutionarily important mechanisms, but we failed to detect them in this correlative study of effect sizes, in which we tested for linear relationships. However, the negative results across a wide range of traits have important theoretical implications, because they suggest that instead of generally applicable roles, different mechanisms may shape the evolution of different traits. For example, it is usually believed that age-dependence is a key feature of sexually selected traits (see Introduction). The song of the barn swallow may appear to support this hypothesis, as two independent studies have shown that the expression of several song traits may vary between age classes (Galeotti *et al.*, 2001; Garamszegi *et al.*, 2005). However, here we demonstrated that the mean effect size for age-dependence of traits is not significantly different from zero, and age-dependence does not always predict pairing success. Therefore, although some traits may be related to male age in particular, age-dependence of secondary sexual charac-

ters may be less important than previously thought (see also Kokko, 1997, 1998). In addition, there can be an effect of a genetic correlation of trait expression across ages, which bring the relative position of an individual within an age-class in focus, and makes age-dependency less important. Similarly, our results revealed no general link between signal repeatability and mating success, which adds to the conflicting pattern found in other species (Wagner & Sullivan, 1995; Gil, 1998; Rivero *et al.*, 2000; Sattman & Cocroft, 2003). Sometimes, females seem to prefer reliable signals that consistently vary among individuals, but sometimes they choose traits with low repeatabilities (e.g. phenotypically plastic traits, such as drift in song of the great tit *Parus major* (Lambrechts & Dhondt, 1988)).

We hypothesized that if signals of male quality are used by potential receivers in the context of sexual selection, age- and condition-dependent sexual traits, or those that are constrained by survival indicator value, should be those that correlate with mating success. Under this scenario, we expected that such signal attributes covary positively with the predictive values of traits for mating success, when correlations were made across variables by using effect sizes. However, none of these attributes seemed to relate to mating success. Therefore, the indicator value of traits is not closely linked to their use as signals by females. Although evolutionary constraints are important determinants of multiple signalling systems, as they may involve, for example, the tuning of the sensory system in the receiver (e.g. Endler *et al.*, 2001), as well as morphological limitations on signal design (e.g. Podos, 2001), phylogenetic constraints on signal evolution were generally found to be weak (e.g. Omland & Lanyon, 2000; Martins *et al.*, 2004). Noteworthy, certain features of song in the barn swallow have been suggested to function in a male–male context (Galeotti *et al.*, 1997), which may partially affect our findings in association with mating success.

The weak associations between other trait attributes imply that traits with high indicator value for one measure of quality (e.g. age) do not necessarily reflect other measures of quality (e.g. condition). This is in line with the hypothesis, which assumes that different male traits reveal different aspects of quality (see Møller & Pomiankowski, 1993; Candolin, 2003). The correlation matrix of sexual traits as summarized in Fig. 2 shows generally weak associations between the traits themselves, which also supports this hypothesis. However, a subset of traits strongly covaried, and thus may reveal similar aspect of male quality. For example, traits of structural coloration or song bout characteristics may have similar information content (Fig. 2).

Our analyses have important implications for the study of multiple signals. Such studies usually involve only a few secondary sexual traits. Here we included 28 potential sexual traits and treated them equally in our analyses of the associated effect sizes. We used effect size in our

study as a standardized metric of the magnitude of effects for different traits, as suggested when many different traits are compared simultaneously (Nakagawa, 2004). The adopted simple methodology that uses variables as the units of analysis (Garamszegi, 2006) may help to understand general patterns concerning the relationships between trait attributes that may be overlooked when considering a few traits only. We made an effort to control statistically for correlations among phenotypic variables that are otherwise traditionally treated as statistically independent traits. While the conclusions of our analyses did not change as a consequence of this exercise, we believe that statistical dependence of observations may pose a serious problem in many analyses that are based on individual variables as observations, and that are presented in separate papers.

The findings of our study should be interpreted with caution because of the possible problems with our assessment of condition-dependence and mating success. Although relative pairing date is generally thought to reflect female preference or success in male–male competition (e.g. Møller, 1988; Buchanan & Catchpole, 1997; Gil & Slater, 2000a; Garamszegi *et al.*, 2004), other components of sexual selection, most importantly extra-pair paternity were not considered in our study. Moreover, we omitted nonmating males that may inflate estimates of average male reproductive success and diminish the variance, leading to underestimates of the potential strength of sexual selection. Similarly, condition-dependence may also be underestimated by using body mass relative to body size. This approach can be criticized, because it applies the untested assumption that body mass and body size are biologically related, and that body mass corrected for body size is a good marker for fat reserves and ultimately fitness (see Cotton *et al.*, 2004). In addition, several components of male condition, such as health status or stress levels, may not be reflected by body mass relative to body size. However, we suspect that these confounding effects occurred randomly, and these shortcomings are unlikely to have caused the nonsignificant relationship between condition-dependence and mating success. Furthermore, studies in the barn swallow have demonstrated that individual optimization with brood size manipulation elicited changes in a similar measure of condition (e.g. Saino *et al.*, 1997b), and that condition was related to survival probability (e.g. Møller & Szép, 2002). Extra-pair paternity is also generally nonrandom with respect to social mate choice, and social mate choice and extra-pair mating success are often positively related (e.g. Birkhead & Møller, 1998; Pitcher *et al.*, 2005), so that our measure of sexual selection is probably conservative rather than entirely wrong. Additionally, relative pairing date seems to be repeatable across seasons in the barn swallow (Møller, 1994a). Finally, we found an impressive number of traits to be sexually selected (Table 2), which suggests that pairing date is a sensitive measure of sexual selection in our case.

In conclusion, extensive analyses of correlations between different attributes of sexual traits in male barn swallows suggest that different traits may partially evolve to signal different aspects of male quality, and traits involving benefits in terms of mating success are not consistently those that are dependent on condition or age, or have high repeatability or high survival predicting value. Further experiments targeting the traits of great importance (i.e. with large effect sizes) may shed more light on the mechanistic link between different trait attributes.

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