

Nutritional correlates and mate acquisition role of multiple sexual traits in male collared flycatchers

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Abstract The information content of a sexual signal may predict its importance in a multiple signal system. Many studies have correlated sexual signal expression with the absolute levels of nutrient reserves. In contrast, the changes of nutrient reserves associated with signal expression are largely unknown in the wild due to technical limitations although they are important determinants of signal information content. We compared two visual and eight acoustic sexual traits in male collared flycatchers to see whether the nutritional correlates of expression predict the role of the signal in sexual selection. We used single point assays of plasma lipid metabolites to estimate short-term changes in nutritional state in relation to sexual trait expression during courtship. As a measure of sexual selection, we estimated the relationship with pairing latency after arrival in a 4-year dataset. Males which found a mate rapidly were characterized by large wing and forehead patches, but small song

strophe complexity and small figure repertoire size. Traits more strongly related to pairing latency were also more closely related to changes in nutrient reserves. This indicates a link between signal role and information content. Small wing patches and, surprisingly, complex songs seemed to indicate poor phenotypic quality and were apparently disfavoured at mate acquisition in our population. Future studies of the information content of sexual traits, especially dynamic traits such as song, may benefit from the use of plasma metabolite profiles as non-invasive indicators of short-term changes in body condition.

Keywords Body condition · Courtship · Lipid metabolites · Signalling costs · Song complexity

Introduction

Sexual signals often reflect individual quality. In case of multiple conspicuous traits, it is necessary to clarify their relative information content and role (Candolin 2003). Multiple signals may serve different functions (Møller and Pomiankowski 1993; Pryke et al. 2001) or may have a role in the same sexual selection process (Robson et al. 2005). However, it is debated whether multiple signals conveying similar information to the same receiver can co-exist (Schluter and Price 1993; Møller and Pomiankowski 1993; Johnstone 1996). The debate is unresolved partly because it is problematic to find common currencies to assess the information content of multiple traits (Kokko 2001; Hunt et al. 2004; Tomkins et al. 2004). As an important aspect of information content, the relationship of trait expression with body condition could be a basis for such a comparison (Rowe and Houle 1996), but body condition may prove difficult to define, estimate and interpret (Cotton et al. 2004).

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Sexual signals may correlate with physiological or nutritional condition because (1) condition determines sexual trait expression (Møller et al. 1999; Cotton et al. 2004), (2) sexual trait expression causes changes in body condition (Rohwer and Ewald 1981; Balmford et al. 1993; Mennill et al. 2003) or (3) a common background factor determines both body condition and signal expression (Houle and Kondrashov 2002; Roberts et al. 2004). It is often difficult to distinguish among these explanations. For instance, even if the given trait is static and the measurement of body condition does not coincide with trait development time, all three explanations may still hold, if body condition is repeatable. The causal pathway from condition to signal expression is often tested experimentally (Hill and Montgomerie 1994; Veiga and Puerta 1996), but the consequences of signal expression for body condition remain elusive because signal expression is very difficult or impossible to manipulate in many cases. This limitation is important because the nutritional costs of sexual signals may contribute to their reliability (Grafen 1990; Kokko et al. 2002).

Monitoring short-term changes in body condition in relation to actual sexual signal expression in the courtship period may at least partly clarify this point. Although a positive correlation between sexual trait expression and change in body condition during courtship has multiple potential explanations, negative relationships are most likely due to high sexual signal expression levels reducing body condition. This is because the reverse causal pathway, with males in reduced condition increasing their level of advertisement, would go against the central paradigm of signal honesty and has seldom been demonstrated. In other words, a robust negative relationship between sexual signal expression and body condition change indicates a costly signal. The few studies of changes in male nutritional state during the courtship stage seem to indicate that rates of mass loss reflect the costs of courtship to males (Andersson 1994; Thomas et al. 2003; Garamszegi et al. 2004). However, such studies usually refer to relatively long time periods or laboratory conditions (but see Thomas 2002), because courting males are often difficult to capture repeatedly, and their behaviour may change in response to repeated disturbance. Data on short-term changes in nutritional state in the wild, which are most informative regarding the costs of plastic traits such as certain song attributes, are largely lacking.

Studies of migrating birds have shown that a single measurement of plasma lipid metabolite levels is a sensitive and reliable indicator of recent changes in lipid reserves, and indirectly, changes in body mass and body condition. In other words, a single body mass measurement provides a snapshot view of actual body condition, while a single lipid metabolite measurement does not indicate actual body condition but it reliably reflects the recent short-term

change of body condition (over a few hours) and therefore provides very different information than body mass (Jenni-Eiermann and Jenni 1994; Jenni and Schwilch 2001). High levels of triglycerides (TG) indicate recent food consumption and ongoing lipid deposition, while high levels of the ketone body beta-hydroxy-butyrate (HBA) indicate recent glucose shortage due to fasting or starvation (Jenni-Eiermann and Jenni 1994; Jenni and Schwilch 2001). Later studies have extended the applicability of the method to periods of up to several days (Williams et al. 1999), and several different life stages (Jenni and Jenni-Eiermann 1996) and diet compositions (Smith et al. 2007). Lipid metabolite data, as easily obtained descriptors of body condition change, could represent a convenient way of assessing the direct and indirect costs of sexual signalling. However, despite numerous studies of breeding individuals (Masello and Quillfeldt 2004; Owen et al. 2005; Kern et al. 2007), there are apparently no published data on lipid metabolites in males during the courtship period.

In the present study, we examine white plumage patch sizes and several song characteristics of male collared flycatchers (*Ficedula albicollis*) in relation to sexual selection and lipid metabolite levels during courtship. A recent study has successfully used the lipid metabolite method in breeding males and females of the sister species pied flycatcher (Kern et al. 2007). As a first step, we examine the role of the sexual traits in mate acquisition using 4 years of data. We record pairing latency (latency from capture and release at arrival to the first egg of the breeding attempt), which is a strong determinant of reproductive success due to the inflexibility of migration dates (Both and Visser 2001; Both et al. 2006; Török et al. 2004), and also a sensitive indicator of female mate choice (Qvarnström et al. 2000; Garamszegi et al. 2004). We predict that different traits will show different relationships with pairing latency, in line with their different roles in sexual selection. In the second step, we look at correlations between sexual trait expression and metabolite levels during courtship. These results will indicate how closely short-term changes of body condition follow the expression of a given sexual trait (Williams et al. 1999). We assume that a trait with a stronger link to metabolites will be more informative with respect to body condition dynamics.

In the third step, we compare sexual traits with strong versus weak links to mating latency with regard to their metabolic correlates to see whether an aspect of information content predicts one pathway of sexual selection in this multiple signal system (Møller and Mousseau 2003; Garamszegi et al. 2006a). We predict strong mating latency selection on sexual traits that strongly reflect individual metabolic scope. The phenotypic plasticity and information content of all sexual traits examined here have been intensively studied before (reviewed in Garamszegi et al.

2004; Hegyi et al. 2007). These studies considerably facilitate the interpretation of our present findings and also allow us to compare the conclusions of our metabolite-based approach to information content with those of previous studies employing more conventional methods. Our study also illustrates the benefit of statistically analysing effect sizes over the traditional, significance-based binary thinking ('relationship' versus 'no relationship'; Garamszegi 2006; Nakagawa and Cuthill 2007; Garamszegi et al. 2009).

Materials and methods

Study population

The collared flycatcher is a small (12 g), long-distance migratory, hole-breeding, insectivorous passerine. Two intensively studied white plumage ornaments, the forehead and the wing patch, together with the moderately elaborate song, seem to constitute a system of signals with a specific correlation structure and partly independent information content (Garamszegi et al. 2004; Hegyi et al. 2007). The fieldwork was conducted in Pilis Mountains, Hungary (47° 43'N, 19°01'E), on one of the nestbox plots used for long-term studies (Török et al. 2004; Hegyi et al. 2006). Males arrive in the first half of April, occupy nestboxes and start singing. Females arrive approximately a week later and settle with a chosen male.

Field methods

We collected data on sexual traits in 2004 to 2007. We closely monitored male activity on the focal plot ($n=99$ nestboxes), so the date when the song of males was recorded closely corresponded to their arrival date. In support of this assumption, arrival dates were specific to individual males (results not shown). We recorded song in the most active morning singing period (usually 0500–1000 h, but no later than 1200 h), and only in relatively good weather. The recording equipment comprised of a Sony TCD-D8 DAT tape recorder, connected to an MD 21U microphone mounted on a parabolic antenna. To stimulate and standardize singing behaviour, we exposed males to a dummy female for 5 min, but we removed this just before recording. The recordings represent normal singing after one of the frequent female visits, so we assumed that the stimulations would not disrupt the normal singing schedule or alter the metabolic profile of focal males. To make sure that we analyse only unpaired males, we conservatively omitted any records where a female accompanied the male during recording. Records were at least 10 min long to obtain a sufficient sample of songs

without strong background noise. We caught males with nestbox traps within an hour of recording and removed them from the trap immediately. We ringed them with a standard numbered metal ring. We determined age (yearling or adult) based on unambiguous plumage characters (colour of remiges and size of wing patch) and took measurements of body mass (spring balance, nearest 0.1 g) as well as tarsus length, forehead patch height and width, and the visible length of white on the outer vanes of primaries 4–8 of the right wing (calliper, nearest 0.1 mm). In 2005 and 2007, we also collected a small blood sample (up to approx. 100 μ l) by brachial venipuncture within a few minutes after capture. We released males after sampling. We checked the plots multiple times a week during the breeding season. Because the speed of nest building is variable and it is therefore not suitable for backdating, we chose first egg date as a measure of breeding time. We calculated first egg date only for nests that were found before or during egg laying, and not later on. This measure is relatively accurate because both laying gaps and partial clutch predation are rare in the study population. We caught parents in the chick-rearing period, which allowed us to link the sampled courting males that later bred in the study area to a nest and thereby learn their breeding date. Work at the study site, including trapping, blood sampling and ringing birds, was licensed by Duna-Ipoly National Park and the regional nature conservation authority (permits DINP 2573/2/2004, KTVF 15951/2005, KTVF 22021/2006, KTVF 16360-2/2007).

Analyses of plasma metabolites

We collected blood into heparinized capillary tubes. We stored the closed capillaries in a cooling box at approx. 4°C until returning to the laboratory within a few hours. We then centrifuged the samples for 10 min at 10,000 rpm and froze the separated plasma at –20°C. Samples were transported on dry ice to the Schweizerische Vogelwarte, Sempach, Switzerland, for metabolite analyses within a few months of collection. We determined metabolites from the plasma using standard test combinations modified for small amounts of plasma (5 μ l per determination): enzymatic UV tests for beta-hydroxy-butyrate (Wako Diagnostics) and enzymatic colorimetric tests for triglycerides (Invicon). We assayed a subset of the samples in duplicates and found high assay repeatabilities for both metabolites (0.806 ± 0.068 SE, $n=14$ for TG; 0.959 ± 0.062 SE, $n=12$ for HBA). The metabolite levels we measured reliably estimate lipid reserve changes for a time range that encompasses the time of our song recordings (Jenni-Eiermann and Jenni 1994; Williams et al. 1999). Males start singing very early in the morning, so they most likely fed before even the earliest recordings, and certainly had

opportunities to feed before capture and blood sampling. The metabolite measurements are therefore not confounded by overnight fasting (also see the lack of time of day effect below).

Sexual signals

We calculated forehead patch size as the product of maximum height and width, and wing patch size as the sum of the measured white segments (Hegyi et al. 2002; Török et al. 2003). Males sing 2- to 4-s-long strophes consisting of varying elements called figures. We estimated song rate as the number of strophes per minute in the available records. We used 20 consecutive strophes with minimal background noise for sonographic analyses with the program Avisoft SasLab Pro (Specht 1999). For each strophe, we extracted the following parameters: strophe length in seconds, complexity (number of unique figures per number of figures) tempo (number of figures per second), peak amplitude frequency (frequency of the maximum amplitude in the song, kHz), minimum frequency (kHz) and maximum frequency (kHz). We averaged these parameters over strophes to get values characteristic of the male. Finally, we used the number of figure types in the 20 songs to estimate figure repertoire size in a capture–recapture analysis that controlled for heterogeneous figure detection probability (Garamszegi et al. 2002). We used the capture–recapture model that best fitted the data (Otis et al. 1978) for repertoire size estimation (Garamszegi et al. 2002, 2005). We did not use several other potential song parameters here to avoid the inclusion of mathematically interdependent variables into the trait level comparative analyses. For all plumage and song traits analysed here, the repeatability of signal expression estimates is comparable to that found in other studies of behavioural and plumage attributes, and differences in trait repeatability cannot cause the results reported below (see details in Hegyi et al. 2002; Török et al. 2003; Garamszegi et al. 2004).

Statistical analyses

As mentioned above, data on sexual traits and sexual selection were collected in 4 years, whereas metabolite data are available for only two of these years. We expressed pairing latency as the number of days between capture at courtship and the first egg date of the breeding attempt. When excluding individuals already sampled in previous years, a total of 73 males were sampled for morphology and song. Of these, 54 were recaptured as breeders in the same year, and only these were considered in the analysis of pairing latency. However, the quality of 11 song records from 2004 was too poor to estimate automatically generated strophe parameters, which left $n=43$ pairing latency

estimates for peak amplitude frequency, minimum frequency and maximum frequency. Nine of the 34 blood samples were too small to assay two metabolites, so there were 34 data points for TG and 25 for HBA concentration. Metabolite levels were not related to year- and age-standardized body mass ($n=25$ or 34 , $\text{abs}(r)<0.156$) or time of day (first or second order, $df=1$, 24 or 1 , 19 , $F<2.073$), so we did not correct them for these variables.

We first ran general linear models on all variables with year and age as fixed factors as well as their interaction, and standardized (mean of zero, standard deviation of one for each group) all variables showing significant effects for year (TG, capture date, pairing latency, peak amplitude frequency, maximum frequency), age (wing patch size) or year and age (repertoire size, tempo, minimum frequency). We used the standardized variables to assess the relationships of sexual traits with relative pairing latency by calculating a partial r from multiple regressions with capture date and sexual trait expression as predictors. Pairing latency strongly declines with capture date, probably due to a time constraint on late females to pair up rapidly. We also calculated Pearson correlations between trait expression and plasma metabolite levels. The standardization of signals was different for the relationships with metabolites, because of the more restricted dataset (tempo for year, song rate and repertoire size for age, wing patch size for year and age). Due to the bimodal distribution of mating latency effects (see ‘Results’), the link between the metabolic and sexual selection correlates of signals was assessed using Yates-corrected chi-square test and logistic regression. All statistical tests were calculated in Statistica 5.5 (Stat Soft, Inc.).

The sexual traits we consider here show varying degrees of correlation. There were positive relationships of at least medium effect size ($r=0.3$; details not shown) between wing patch size and forehead patch size, song length and repertoire size, maximum frequency and repertoire size, and peak amplitude frequency and maximum frequency. There were medium or stronger negative relationships between repertoire size and minimum frequency, song length and minimum frequency, song length and strophe complexity, and minimum frequency and maximum frequency. Apparent mating advantage for one trait may occur due to its correlation with a sexually selected trait, but mating advantage estimates may also be downward biased due to trait correlation if the traits are evaluated in a hierarchical fashion. We therefore used the comparative method suggested by Garamszegi et al. (2006a) to correct the trait attribute patterns for the correlation structure of the sexual traits. The results did not change, so we report the uncorrected analyses in the present paper for simplicity.

Our study focusses on the sexual selection and metabolic effects of sexual traits and the parallelism of the two, and

we chose to calculate the effect sizes for each trait in separate models due to the different sample sizes and the risk of overparameterization in a multivariate procedure. However, stepwise multivariate models led to very similar results although with a lower sample size, so we consider our present findings robust to statistical methodology. The trait comparison approach uses absolute effect sizes irrespective of their significance (Garamszegi 2006; Garamszegi et al. 2006a). Accordingly, we do present significance values but nevertheless draw our conclusions based on effect sizes and their 95% confidence intervals (Nakagawa and Cuthill 2007).

Results

TG and HBA levels were positively related to each other, but the relationship had a broad confidence interval and was not significant ($n=25$, $r=0.260$, CI lower= -0.151 , CI upper= 0.594 , $p=0.210$). The distribution of relationships of the ten sexual traits with pairing latency was clearly bimodal (Table 1; Fig. 1). A group of four traits (forehead patch size, wing patch size, song repertoire size and strophe complexity) approached or reached the conventional medium effect size ($r=0.3$; Cohen 1988). All four effects were also significant. The smallest of these effects ($r=0.27$) was approximately twice as large as the largest effect for the remaining six traits (Fig. 1). Therefore, two plumage and two song characters were designated as selected in terms of pairing latency, with early pairing favouring large forehead patch size and wing patch size, but small repertoire size and strophe complexity (Fig. 2). Of these four traits, strophe complexity was strongly negatively related to HBA levels (Table 1, Fig. 3a), while the relationships of repertoire size with TG levels (positive; Fig. 3b) and wing patch size with HBA levels (negative; Fig. 3c) were around the medium effect size but had broad confidence intervals and were therefore not significant ($p<0.15$). In contrast, forehead patch size showed little relationship with either metabolite. Due to the even distribution of metabolite effect sizes, they were considered as a continuous variable.

When comparing traits under strong versus weak pairing latency selection, the two strongest correlations with TG levels belonged to two strongly selected traits (negative with wing patch size, positive with song repertoire size; Table 1). Similarly, it was two strongly selected traits that showed the strongest relationships with HBA levels (wing patch size and strophe complexity, both negative; Table 1). In total, three of four strongly selected traits were related to at least one metabolite with an approximately medium or higher effect size, compared with none of the six weakly selected traits (Yates corrected $\chi^2=3.350$, $df=1$, effect size $r=0.579$, CI lower= -0.080 , CI upper= 0.886 , Fisher exact $p=0.033$). Similarly, the maximum absolute correlation (log trans-

Table 1 Effect sizes (Pearson r) for the relationships of ten sexual traits with mating latency (multiple regressions) and plasma lipid metabolite levels (correlations)

Effect	Trait	r	CI lower	CI upper	n	p
PL	FPS	-0.270	-0.504	0.000	53	0.047
PL	WPS	-0.290	-0.520	-0.021	53	0.027
PL	RAT	0.032	-0.240	0.300	53	0.813
PL	REP	0.333	0.069	0.553	53	0.011
PL	LEN	-0.030	-0.298	0.242	53	0.823
PL	TEM	-0.136	-0.392	0.139	53	0.307
PL	PAF	-0.074	-0.360	0.224	45	0.624
PL	MIF	0.025	-0.271	0.316	45	0.876
PL	MAF	0.035	-0.261	0.325	45	0.819
PL	COM	0.341	0.078	0.560	53	0.009
HBA	FPS	0.131	-0.278	0.500	25	0.531
HBA	WPS	-0.297	-0.619	0.111	25	0.149
HBA	RAT	0.204	-0.249	0.584	21	0.375
HBA	REP	-0.056	-0.487	0.396	20	0.814
HBA	LEN	0.164	-0.288	0.556	21	0.477
HBA	TEM	-0.160	-0.553	0.292	21	0.490
HBA	PAF	-0.250	-0.615	0.204	21	0.274
HBA	MIF	0.084	-0.361	0.498	21	0.718
HBA	MAF	-0.259	-0.621	0.194	21	0.257
HBA	COM	-0.649	-0.844	-0.301	21	0.001
TG	FPS	0.048	-0.300	0.385	33	0.789
TG	WPS	-0.236	-0.536	0.117	33	0.180
TG	RAT	-0.113	-0.479	0.287	26	0.576
TG	REP	0.300	-0.108	0.621	25	0.137
TG	LEN	0.204	-0.199	0.548	26	0.307
TG	TEM	-0.003	-0.390	0.385	26	0.988
TG	PAF	0.126	-0.275	0.489	26	0.530
TG	MIF	0.020	-0.370	0.404	26	0.923
TG	MAF	-0.112	-0.479	0.288	26	0.578
TG	COM	-0.114	-0.480	0.286	26	0.571

CI 95% confidence interval, HBA beta-hydroxy-butyrate, PL pairing latency, TG triglycerides, FPS forehead patch size, WPS wing patch size, RAT song rate, REP song repertoire size, LEN song length, TEM song tempo, PAF peak amplitude frequency, MIF minimum frequency, MAF maximum frequency, COM strophe complexity

formed) of a trait with metabolites predicted its role (strong or weak) in mate acquisition (Fig. 4; logistic regression, $\chi^2=3.037$, $df=1$, effect size $r=0.551$, CI lower= -0.120 , CI upper= 0.877 , $p=0.081$). The large effect size and still marginally non-significant p value of the latter relationship is due to the small dataset ($n=10$ traits).

Discussion

Here we examined a system of multiple secondary sexual traits to see whether relationships of trait expression with

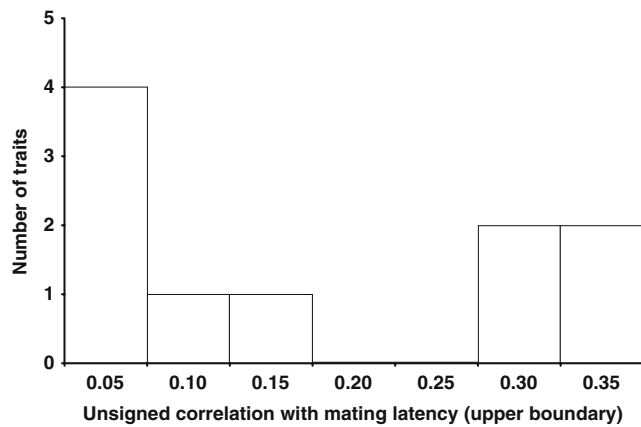
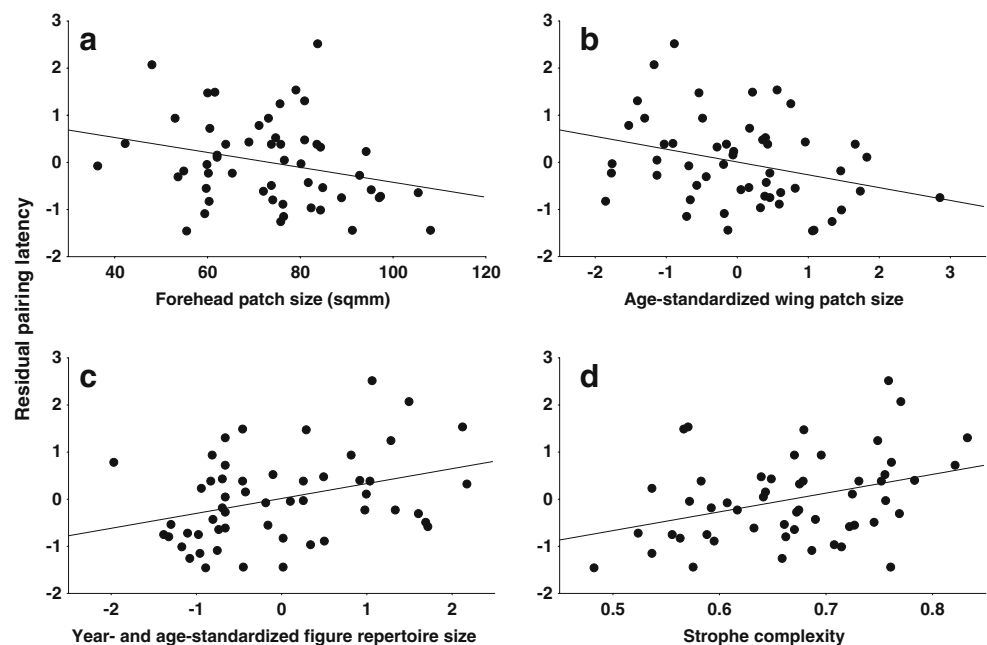


Fig. 1 The distribution of absolute (unsigned) effect sizes for relationships between male sexual trait expression and pairing latency

nutrient reserve changes during courtship predicted their importance in one component of sexual selection, mate acquisition. Such questions would be very difficult to address by conducting single measurements of body condition (Cotton et al. 2004). However, assaying plasma lipid metabolites allowed us to track the short-term temporal trajectory of body condition (i.e. rate and direction of change) based on a single blood sample (Williams et al. 1999), and this led to novel findings regarding the connection between sexual trait role and information content. We detected strong sexual selection in terms of pairing latency on several sexual traits, and the relationships of trait expression with lipid reserve dynamics also varied among sexual traits. It seemed that the traits more robustly predicting mate acquisition were those in which trait expression more strongly indicated the depletion rates of nutrient reserves.

Fig. 2 Pairing latency of males in relation to the expression of four sexual traits. **a** Forehead patch size, **b** wing patch size, **c** figure repertoire size, **d** strophe complexity. *Least square regression lines* are shown. Residual pairing latency is a value corrected for capture date in a linear regression



White plumage patches are often found to be sexually selected (e.g. Höglund et al. 1990; Kose et al. 1999; McGlothlin et al. 2005; Bókony et al. 2006). A smaller number of studies also found links between white patch expression and nutritional or physiological condition (e.g. Hanssen et al. 2006; McGlothlin et al. 2007). The relatively direct measurements of mate acquisition success presented here for the first time in this population suggest that both forehead patch size and wing patch size are important in this process, which is in line with previous indirect estimates (Hegyi et al. 2006, 2007). White plumage patches may indicate individual quality through, for example, predation costs (Dale and Slagsvold 1996; but see Palleroni et al. 2005), developmental stability (Kose and Møller 1999) or aggression costs (Qvarnström 1997). In the present study, wing patch size correlated non-significantly negatively with HBA levels ($r=-0.297$). This relationship, if confirmed by additional data, will indicate that birds with large wing patch size show weaker signs of glucose shortage and fasting than small-patched males. This relationship cannot reflect a causal effect of nutritional state on wing patch size because this plumage trait is replaced once a year, in summer (Hegyi et al. 2007). Therefore, the correlation can be due to a common effect of individual quality on nutritional dynamics and plumage ornamentation, or the effect of plumage ornamentation on nutritional dynamics. Large wing patch size provokes male territorial aggression (Garamszegi et al. 2006b) and this could reduce food intake (see the negative tendency between wing patch size and TG here), but the negative effect was apparently counterbalanced by another effect, possibly the high genetic quality of large patched males

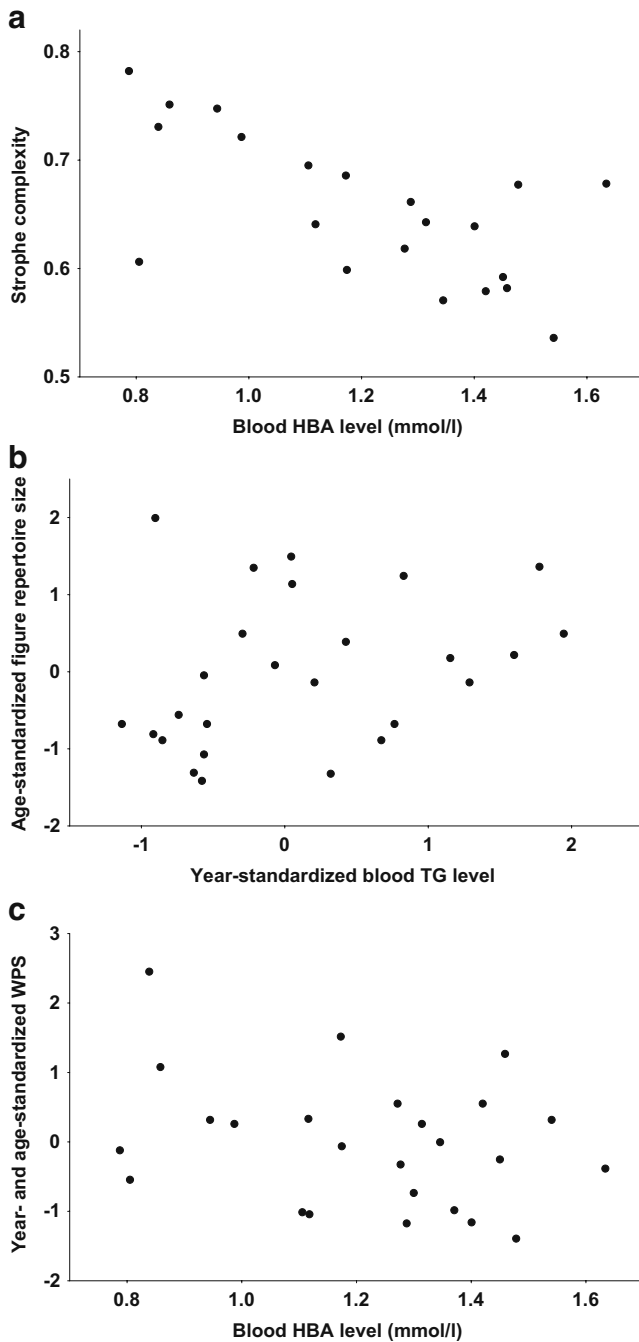


Fig. 3 Relationships of sexually selected traits with plasma lipid metabolite status; **a** song strophe complexity in relation to HBA levels; **b** song figure repertoire size in relation to TG levels; **c** wing patch size (*WPS*) in relation to HBA levels. Note that sexual selection in terms of mate acquisition favours small strophe complexity, small figure repertoire size and large wing patch size

(Török et al. 2003; Hegyi et al. 2007) which may influence foraging ability (Hill 1991) or nutrient use efficiency (Blackmer et al. 2005). Forehead patch size, on the other hand, was weakly related to lipid metabolites ($abs(r) < 0.15$), which supports the view that it is a relatively static trait less informative of individual quality (Hegyi et al.

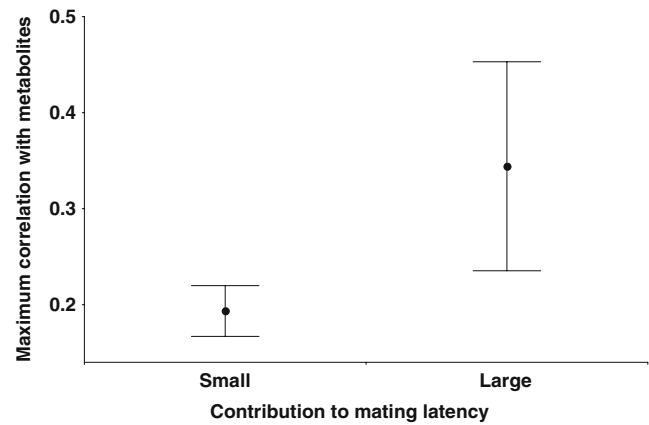


Fig. 4 The maximum unsigned correlation of sexual traits with metabolite levels, in relation to their apparent role in mate acquisition (means \pm SE)

2006, 2007) and less important in territorial conflicts (Garamszegi et al. 2006b) in this population.

In contrast to white patches, there is ample evidence that many aspects of bird song are sexually selected (Catchpole and Slater 1995; Vehrencamp 2000). Song traits can be roughly classified as performance traits or complexity traits (Gil and Gahr 2002). Performance traits, for example song rate, are expected to show direct sensitivity to actual body condition (Birkhead et al. 1998; Berg et al. 2005). The link between song complexity and physiological or nutritional condition is less direct, but both correlative and experimental evidence increasingly presents complexity traits as indicators of phenotypic quality (Buchanan et al. 1999; Spencer et al. 2004; but see Weatherhead et al. 1993; Forstmeier et al. 2002). Earlier pairing in our population strongly favoured two complexity traits. Surprisingly, males singing smaller repertoires and less complex strophes paired up earlier. Moreover, strophe complexity was strongly negatively related to HBA levels (large effect size) and repertoire size was non-significantly positively related to TG levels (medium effect size). Therefore, males singing larger song repertoires and more complex strophes seemed to experience lower rates of nutrient reserve depletion during courtship. Costly sexual signals may positively correlate with point estimates of body condition, but they are expected to negatively correlate with nutrient reserve changes during trait expression (Andersson 1994; Thomas et al. 2003). In our case, however, if females were looking for males capable of high nutrient expenditure rates, they should look for those with smaller repertoires and less complex strophes. The mate acquisition patterns we recorded are consistent with this. The coherent picture outlined by our nutritional and sexual selection results contradicts much recent research on song and individual quality.

A complex song indicates individual quality (Buchanan et al. 1999; Spencer et al. 2004) and is favoured by mate

choice in several species (Eens et al. 1991; Buchanan and Catchpole 1997) including the sister species pied flycatcher (Lampe and Saetre 1995), and our results go against these findings. How can we explain this? It could be that males compensated for their low song complexity by singing more and thereby exhausted themselves. Indeed, males with less complex strophes sang longer songs (see ‘Materials and methods’). However, this does not explain the patterns we found because (1) song length was related to neither metabolites nor pairing latency, (2) song rate was not related to song complexity, metabolites or pairing latency, and (3) correcting the analyses for sexual trait correlations (see ‘Materials and methods’) did not alter the conclusions. Another explanation could be direct, negative condition dependence, with tired males singing less complex songs. However, repertoire size and strophe complexity are complexity traits and not performance traits (Gil and Gahr 2002), so they are probably not directly linked to body condition. The causal pathway could be the reverse. Singing less complex songs may have caused greater reserve expenditure rates if these songs contained specific, costly song or strophe types, which could also explain sexual selection for simple songs (Rehsteiner et al. 1998; Vallet et al. 1998; Ballentine et al. 2004; Cardoso et al. 2007). Alternatively, low repertoire size or strophe complexity could function as costly indicators of individual quality if they revealed song learning accuracy (O’Loughlen and Rothstein 1995; Nowicki et al. 2002; Searcy et al. 2002). In the latter case, individual quality would be the common cause of less complex songs and greater reserve expenditure rates.

In a previous study of our population (Garamszegi et al. 2004), in line with our present results, strophe complexity was positively related to song rate reduction after an immunological challenge, negatively related to the testosterone levels of non-manipulated males and positively related to pairing latency, while repertoire size was not related to any of these variables (Garamszegi et al. 2004). We therefore conclude that, when correcting for age effects on the song traits, high-quality and preferred males in our population are characterized by small repertoire size and low strophe complexity. Further studies of song composition are currently underway to illuminate the details of this apparent sharp difference in the quality indicator value and role of song parameters between two congeneric passerine species (Lampe and Saetre 1995; Garamszegi et al. 2004).

The relatively few studies that examined the information content and role of multiple conspicuous traits often concluded that the characters that more accurately reflected individual quality were more important as signals (Kodric-Brown 1993; Møller and Mousseau 2003; Scheuber et al. 2004; but see Garamszegi et al. 2006a). This may represent either the response of female preferences to sexual trait information content, or the evolution of quality dependence in response to

trait exaggeration caused by sexual selection (Wilkinson and Taper 1999). If, however, the production or wearing costs of sexual traits predict their importance in sexual selection, this strongly indicates that females evolved to prefer informative traits (Grafen 1990; but see Kokko 2001). Our results suggest such a situation. Mate acquisition apparently disproportionately favours song traits in which the favoured level of expression seems to be accompanied by relatively higher levels of nutritional stress (song repertoire size and strophe complexity; also see Garamszegi et al. 2004). The exact reasons for which less complex songs indicate high reserve expenditure remain to be investigated. Wing patch size, in contrast, is a relatively static trait, and its relationship with metabolites probably reflects a combination of quality indicator value and wearing costs (second and third mechanisms from the ‘Introduction’, Török et al. 2003; Garamszegi et al. 2006b; Hegyi et al. 2007). Taken together, we found evidence that sexual selection in terms of mate acquisition is stronger on characters that, for any reason, relatively more strongly indicate the metabolic scope of males. The exception is forehead patch size, which is sexually selected but unrelated to metabolites. Mate choice for this trait may confer genetic benefits in terms of offspring attractiveness (Hegyi et al. 2006).

To conclude, our pairing latency estimates support previous indirect evidence on the information content and role of plumage signals in the study population (Hegyi et al. 2007). Our results further suggest that singing smaller figure repertoires and strophes of lower complexity is accompanied by higher nutrient expenditure and is also sexually selected in this population (Garamszegi et al. 2004) although the mechanism awaits further investigation. When comparing effect sizes across the sexual traits, we detected a link between the nutritional correlates and the role of components of a multiple signal system (Grafen 1990; Vehrencamp 2000). Finally, the results illustrate that assessing changes in male nutritional state in the courtship period may provide important insights and indicate that plasma lipid metabolites can be useful tools in such studies (Jenni-Eiermann and Jenni 1994).

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