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The relationship between syllable repertoire similarity and pairing success in a passerine bird species with complex song

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ABSTRACT

Repertoire size, i.e. the number of unique song elements that an individual possesses, is thought to be an important target of female preference. However, the use of repertoire size reflects how researchers work with complex songs; while it does not necessarily describe biological functions, as listeners of song may also rely on song composition. Specific syllables may have coherent consequences for mate attraction because they are costly to produce, mediate syllable sharing or indicate the dialect of origin. We tested for the relationship between song composition and pairing success in the collared flycatcher (*Ficedula albicollis*). We applied a tree-clustering method to hierarchically classify males based on the degree of repertoire overlap, and then used a phylogenetic approach to assess the degree by which pairing speed matches the hierarchically structured song data. We found that males using similar syllables also find a breeding partner at a similar speed. Partitioning the variance components of pairing speed, we detected that the consequences of particular syllables for mating are repeatable across males. When assessing the role of repertoire similarity in mediating direct syllable sharing, we derived a positive relationship between the physical distance between pairs of males and their repertoire overlap implying that neighboring males avoid copying each other's song. Finally, we were unable to demonstrate that syllables related to higher mating success are more common in the population, which would support mechanisms based on female preference for local songs. Our results imply that individual-specific song organization may be relevant for sexual selection.

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1. Introduction

The complexity of bird song has become one of the key models of sexual selection (Catchpole and Slater, 1995; Gil and Gahr, 2002; Searcy and Nowicki, 2000; Searcy and Yasukawa, 1989). Complex songs serve as indicators of male quality, which should translate into mating and ultimately breeding success, as females prefer reproducing with males of high quality (throughout the paper we refer to song complexity in a general sense). However, our understanding of the role of song complexity in sexual selection is somewhat conflicting, because research on this topic gave inconsistent results (review in Byers and Kroodsmas, 2009; meta-analyses in Garamszegi and Møller, 2004; Soma and Garamszegi, 2011). Although some studies provided evidence for the reproductive advantages of males with complex songs, others failed to support this underlying prediction and showed

non-significant or even negative associations between the studied traits. These conflicting patterns question whether universal roles are applicable to every species and whether the complexity of song *per se* is the subject of selection.

Repertoire size is a widely used measure of song complexity. It describes the number of unique vocal elements, such as song or syllable types, that individuals can possess (Botero et al., 2007; Garamszegi et al., 2002; Kroodsmas, 1982; Wildenthal, 1965). Working with repertoire size models might be practical, as from the observer's perspective, the counting of syllables can be easily achieved by the visual inspection of sonograms. In this framework, it is generally inferred that males with large repertoire size have complex songs thus are of superior quality (Catchpole and Slater, 1995). However, the comparison of males based on syllable repertoire size occurs independently of the composition in syllables of songs. Therefore, the total amount of song material that two birds produce may appear remarkably similar in terms of repertoire size, but dramatic differences may be present in terms of composition if these individuals organize their songs from completely different syllables (Kroodsmas, 1982). Beyond the

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estimation of repertoire size, it might also be of interest to compare syllable composition across individuals and to test for the role of specific syllables, if a universal syllable coding system is used for the whole population. In such cases, researchers should define syllable categories that reflect the same acoustic feature in different individuals, which is a challenging task in species with complex songs. Hence, for the estimation of repertoires' size it may be more suitable to work with non-universal syllable codes with unique meaning in each individual, but it precludes any compositional comparison between these individuals.

Focusing on repertoire size may be convenient for a researcher, but it does not necessarily describe biological functions. Little is known about how females in different species sample the vocal performance of singing males (Bensch and Hasselquist, 1992; Riebel, 2009; Riebel et al., 2005), but it is highly unlikely that they count and compare the full repertoire of all singing males similarly to how researchers work with complex songs (Garamszegi et al., 2005a). Mate sampling is usually constrained in time and space (Uy et al., 2001; Wiegmann and Mukhopadhyay, 1998), thus it is hard to imagine that females in species with very complex and discontinuous songs are responsive to small repertoire size differences that can only be detected by the extensive analysis of several songs (Botero et al., 2007). Instead, females probably rely on patterns that can be evaluated during short sampling sessions by focusing for example on short-term song complexity (e.g. or versatility). If the characteristics of syllables and individual-specific song organization count, the qualitative differences between males can also be evident from few songs. Altogether, females can therefore use preferences for either overall repertoire size or short-term estimates of song complexity, but also can base their choice on song composition. While, in addition to repertoire size, a remarkable number of studies used different estimates for song complexity, such as versatility or singing style (Kipper and Kiefer, 2010), compositional differences between males are rarely considered. In some cases, the strict focus on repertoire size might be misleading for making strong implications for sexual selection.

There are several mechanisms that can mediate a relationship between song organization and mating success independently of repertoire size. For example, particular syllables may play a role in driving female preference. In the canary (*Serinus canaria*), fast, frequency-modulated syllables has been described as being attractive for females (Leitner et al., 2001; Vallet et al., 1998; Vallet and Kreutzer, 1995), probably because only some males can afford to build their repertoires from syllables that impose a high cost on them. Only males that use these “sexy” syllables in their songs may realize successful mating, while others missing these syllables from their repertoires will not be chosen by females. However, mechanisms, which are independent of the costs associated with the production of syllables, may also be in effect. Specific song organization allows syllable sharing, which can be important for males in their competition for mates, as the amount of syllables shared with neighbors can influence the male's territory tenure (e.g. Beecher et al., 2000; Briefer et al., 2010; Hultsch and Todt, 1981; Laiolo and Tella, 2005). In addition, selection for singing the same song content might also allow females to directly compare the song performance of males (Logue and Forstmeier, 2008). Finally, females may develop preferences for local and/or common songs or dialects (Gonzalez and Ornelas, 2005; Riebel et al., 2002; Wegrzyn and Leniowski, 2010). Some syllables might even function as a group signature, and song organization can thus help females to distinguish between males of different origins (Briefer et al., 2008; Wegrzyn et al., 2010). All these mechanisms might either single-wise or in combination affect the mating success of males. Therefore, males that more frequently use certain syllables in their repertoires could be similarly attractive to females.

Here, to study the function of bird song in female attraction, we depart from the traditional method developed for song complexity, and instead of measuring repertoire size, we focused on individual-specific song organization. We used the collared flycatcher (*Ficedula albicollis*) as a model for this purpose. Previous studies aiming at exploring the determinants of various song features in this species failed to find strong roles for repertoire size in sexual selection despite the fact that males can produce up to 50 syllable types, of which most are specific to individuals (Garamszegi et al., 2004, 2006, 2008). Interestingly, males with higher repertoire size seem to be less attractive for females, as they pair later than males with songs composed of fewer syllable types (Hegyí et al., 2010). Contrary, features of song output, such as song rate and song length have shown many biologically meaningful correlations (Garamszegi et al., 2004, 2006). Therefore, the role of song complexity and the extensive between-individual variation in song organization remains cloaked in mystery in this species.

We predicted a relationship between repertoire similarity of males and their mating success, if particular syllables or song composition (and not repertoire size) are/is preferred by females. To test this prediction, we applied a tree-clustering method to hierarchically classify males based on the overlap of their repertoires. Subsequently, we used a phylogenetic statistics to test for the concordance between the tree structure describing the repertoires' similarities of males and pairing success. Moreover, we partitioned the variance components of pairing speed, and checked for consistent variation at the within-syllable and across-male contexts, i.e. we examined if the mating value of particular syllables is similar in all males that sing them. If increased mating success is caused by certain “sexy” syllables that are costly to produce, the physical properties of syllables should predict their attraction value. We also assessed whether syllable sharing results from vocal interactions between males, i.e. if the similarity in syllable composition depends on the distance between males. If song content would have evolved to enhance syllable sharing, we expect that males singing close to each other would have higher chance to share syllables than males singing further apart. Finally, if preferences for local songs result in a similar song composition with more common syllables conferring advantages, we predicted that the frequency of the use of syllables would be positively correlated with mating success.

2. Methods

2.1. Song recordings

Song recordings for this study originate from three consecutive years (2005–2007) when the song display of 63 unpaired male collared flycatcher have been successfully recorded in the Pilis Field Station, Hungary (for the general conditions of recording and protocols, see Garamszegi et al., 2004, 2006, 2008). All recorded birds had been captured and subsequently ringed to allow identification. We used unique ring numbers to verify that each male was included in our sample only once (for 4 males recordings were available from more than one year, thus we randomly removed one year data from these males for the analyses). Males originated from three different breeding plots (*k6*, *k7*, *k13*) that are located at a few hundred meters from each other. Our intense capturing protocols within and between breeding seasons indicate that individuals often move between plots even within a year (J. Török et al. unpublished data). Hence, males from different plots are likely to interact vocally, thus we considered them as members of the same population. Year- and plot specific sample sizes are $N_{2005}=14$, $N_{2006}=27$, $N_{2007}=22$ and $N_{k6}=37$, $N_{k7}=12$, $N_{k13}=4$.

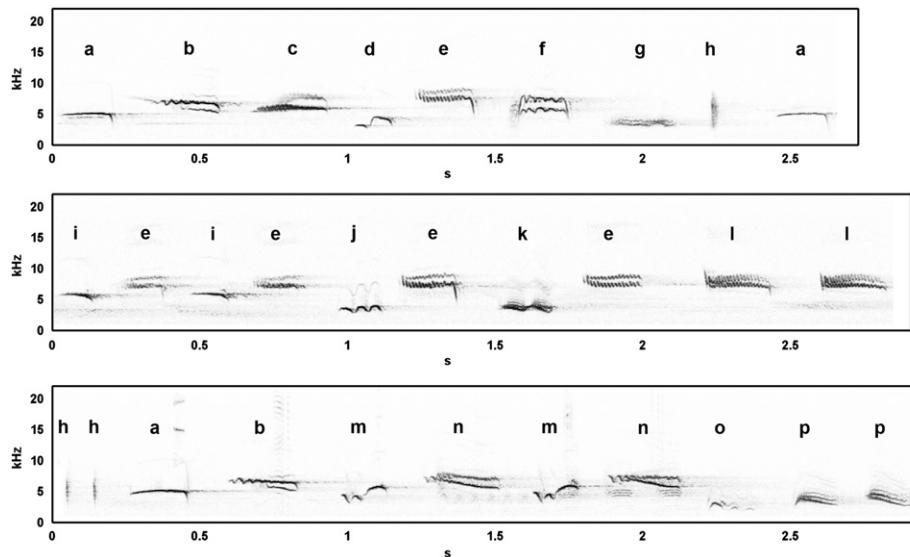


Fig. 1. Spectrographic representation of three songs from three different collared flycatcher males. Elements within songs are called syllables, which are labeled with letters. Each letter type corresponds to a syllable type from the population's repertoire stored in a syllable library. The alphabet is comparable between different males.

The song performance of the collared flycatcher consists of 2–3 long songs, which are uninterrupted series of acoustic elements so-called syllables with pauses no longer than 1 s (Fig. 1). Each song contains 4–37 syllables with a mean \pm s.e. = 10.41 ± 0.28 . From the recorded material, 20 consecutive songs of good quality (i.e. with a low level of background noise and no other bird singing in parallel) were selected and submitted to further spectrographic analyses. Previous studies relying on longer and repeated recordings of the same males showed that this sample of 20 songs is sufficient to reliably describe individual variation in song composition. Particularly, we arrived to this conclusion, because i) the vast majority of syllables known by a male are produced already within 15 songs and analyzing longer samples reveals few new syllables detected (Garamszegi et al., 2002), and ii) because sampling the same male in different days using 20 songs provides significantly repeatable estimates for repertoire size (Garamszegi et al., 2004). Additionally, we can also infer that such samples provide representative estimates for the frequency of syllable usage along the rare-common continuum, because the probability of a syllable of being sung in the first 10 songs is a good predictor of the probability with which it can be detected from the next 10 songs (Pearson's correlation $r=0.677$, $P<0.001$; as averaged across 207 syllables and 56 males—only those syllables were used, which had non-zero probability in both subsamples of 10 songs).

To obtain information on individual-specific syllable composition, we assigned syllables into distinct categories based on a semi-automatic scoring system. We wrote a computer program in *Matlab 7.0* (see Appendix) for this purpose based on time and frequency measures taken on each syllable (Fig. 2). Before allowing the program to measure these variables, the start- and the endpoint of each syllable were marked manually. Next, the program automatically measured the frequency at the most emphasized (i.e. at the highest relative amplitude) 30 points within the syllable (Fig. 2). Maximum frequency, minimum frequency and mean frequency were determined from these 30 measurements, while frequency modulation was calculated as the mean of the absolute value of the difference between the 30 frequency values measured. The program also measured frequencies at the marked start- and endpoints of the syllable, and at the middle of the syllable. Finally, the length of the syllables was also determined. Syllable categories were defined based on the particular combination of the 8 measured parameters.

This approach allowed us to establish a universal coding system that uses syllable categories that are comparable across males. In this semi-automatic coding process, the algorithm follows a decision tree method. This procedure provides a suggestion for the most appropriate matches between syllables, but always requests a final decision from the investigator to assign each syllable into a group. The automatically enhanced syllable assignments allowed us to create a universal syllable library, in which syllable codes are collectively applicable to all individuals. The current syllable library suggests that the 63 males sang 361 syllable types altogether. As in every repertoire framework, without having information on cues from the listener's perspective, we also assumed that the syllable categorization based on spectrographic parameters is biologically reliable and females operate with similar categories. Note that our study is based on the visual scoring of syllables, as the subjective judgement of an observer was needed in the final decision during the semi-automatic procedure. However, we can rule out that syllable categories are loaded with observer bias, because we observed high inter-observer reliability when different people (LZG and SZ) scored the sonograms (Pearson's correlation between the scores of observers: $r=0.890$, $N=56$, $P<0.001$).

2.2. Pairing success

We estimated pairing success of males using relative pairing date, which is the number of days elapsed from the arrival from the wintering sites until the start of egg laying. Arrival date was estimated as the date of first capture of the given male on the breeding site, and was standardized across years using the population medians as a reference for zero values. We assumed that this measure is a good surrogate of real arrival date, because we monitor our breeding plots for newly arrived, displaying birds in a standard way. It is therefore likely that males used in this study were recorded just upon their arrival. 42 males were captured while feeding young during the chick-rearing period. Data on pairing date were available only for this sample of males (it is possible that birds that were not found breeding actually bred somewhere else, but their brood remained undetected). To test if this sample is not biased, we compared the song composition between males that later bred and males that were not found breeding subsequently. The difference in the frequency of syllable

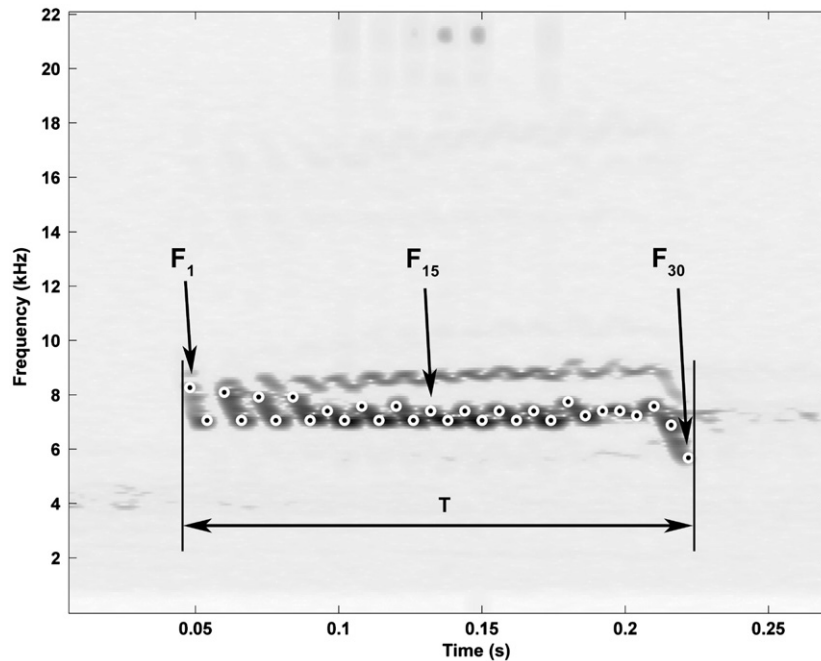


Fig. 2. Spectrographic representation of a syllable from the collared flycatcher's song, on which the 30 measured points (F_1 – F_{30}) are shown. These are frequency estimates that are taken at the most emphasized (i.e. at the highest amplitude) points of the syllable. From these 30 measurements maximum frequency, minimum frequency and mean frequency were selected, as well as frequencies at the start- and endpoints of the syllable, and at the middle of the syllable. Frequency modulation was calculated as the mean difference between the 30 frequency values measured. From the example shown: $F_{start}=F_1$; $F_{middle}=F_{15}$; $F_{end}=F_{30}$; $F_{mean} = (\sum_{i=1}^{30} F_i)/30$; $F_{max} = \max_{i=1 \rightarrow 30} F_i$; $F_{min} = \min_{i=1 \rightarrow 30} F_i$; and $F_{modulation} = (\sum_{i=1}^{29} |F_{i+1} - F_i|)/29$. These seven frequency parameters together with the length of the syllable served the basis for the categorization of syllables.

use between the two groups of males was significant only for 4 out of 189 syllables when comparing the frequencies for each syllable with *t* tests (only syllables that were found in at least two individuals were used for this test, see below), which is not distinguishable from random chance. Hence, we infer that the sample of males with information on pairing date is a representative sample. Relative pairing date is generally used to reflect how quickly a male finds a partner, because quick and early pairing is likely to be caused by female preference and successful mate attraction (Buchanan and Catchpole, 1997; Catchpole, 1980; Garamszegi et al., 2005b; Gil and Slater, 2000; Reid and Weatherhead, 1990; Searcy, 2004). Arrival date may bias pairing date, because early arriving males should wait longer to pair than late arriving males, as early in the season there may be few females available. To control for the confounding effect of arrival date, we calculated residual pairing date from the corresponding linear regression of relative breeding date on arrival date.

2.3. Association between males based on syllable sharing

The similarity in song composition between males can be described by the degree of syllable overlap between males. To visualize the association between males based on syllable composition, we hierarchically classified males using clustering methods. This approach arranges the individuals along a tree, in which the nodding patterns and branch lengths reflect the compositional similarity between males. Closely associated individuals have songs that are composed of many syllables that are shared by these individuals, but not by other birds, while distantly related individuals possess different syllables and have dissimilar songs.

For this hierarchical classification of individuals based on repertoire similarity we used tree-clustering methods with average agglomeration method (Podani, 2000). We first estimated the overlap in syllable composition between each pair of males using a coefficient of compositional similarity, defined as $s = 1 - 0.5 * \sum |o_{ik} - o_{jk}|$, where

o_{ik} is the relative occurrence of syllable *k* (measured as the number of times when syllable *k* is detected in the song record/total number of syllables analyzed in the song record) in individual *i*, while o_{jk} is the relative occurrence of the same syllable in individual *j*. Using this definition, our measure of repertoire similarity incorporates the frequency by which syllables are sung. Accordingly, the sharing of syllables that are rarely presented are downplayed, while the overlap between the repertoires of males with regard to the use of common syllables is taken into account with larger weights. Although previous analyses used similarity matrixes that disregarded the relative frequency of syllables (e.g. Briefer et al., 2010; Hultsch and Todt, 1981), we applied a method that considers this frequency of detection because the resulting similarity index is more relevant from the listener perspective. For instance, two males that share 50% of their syllables, but sing these shared syllables only occasionally, would be perceived by a listener as having highly different song composition. On the other hand, if the shared syllables are very commonly used, the similarity in song composition would appear higher than 50%.

Compositional similarity varies between 0 and 1, with 1 representing the complete sharing of syllables. Since the clustering methods require distance between objects, similarity values were back-converted to distances by subtracting compositional similarity from 1 (i.e. we used $0.5 * \sum |o_{ik} - o_{jk}|$ from the similarity formula). Such distances were computed for all possible pair-wise relationships.

We only considered syllables that were found in at least two individuals (189 syllables out of 361 syllables), because syllables observed in a single individual are of little relevance for the similarity approach we follow in our analyses (see above).

2.4. Concordance between the similarity of syllables and pairing success

To evaluate the possibility that males that share certain syllables are more likely to have similar pairing latency than others sharing other syllables, we used a phylogenetic modeling

approach. In phylogenetic studies, it is often an important task to estimate the degree by which the phylogenetic relatedness of species affects the interspecific variation of the focal trait (Blomberg et al., 2003). Analogically, we can ask whether compositional similarity of songs is associated with the inter-individual variation in pairing success. The Phylogenetic Generalized Least Squares (PGLS) method can estimate the degree of concordance between a tree structure and a continuous variable in the form of lambda (λ) statistics (Martins and Hansen, 1997; Pagel, 1999). This method was originally developed for dealing with phylogenetic inertia within an interspecific data by incorporating a matrix of the expected covariances among species due to shared phylogenetic structure (Freckleton et al., 2002; Pagel, 1997). Given the analogy, we used this method for our purposes at the within species level. Accordingly, we evaluated the likelihood surface depicted by a range of models that assume different degrees of hierarchical structure in the pairing success data. Models relying on $\lambda=0$ are equivalent with pairing success varying independently of syllable sharing, while models constrained with increasing (up to 1) λ imply stronger role for song composition mediating mating success.

For this modeling we followed a Maximum Likelihood, for which we relied on the algorithm incorporated in *BayesTraits* (Pagel et al., 2004). For this, we used the hierarchical tree of males that summarizes the repertoire similarity between the subject individuals based on syllable composition. This tree was fitted to the individual-specific data on pairing date under the assumption of “random walk” scenario (Model A). We allowed the model to take a “phylogenetic scaling factor” (λ) that results in the highest model likelihood. To estimate the relationship between syllable sharing and pairing success, we assessed the importance of the λ adjustment that was needed to reach the maximum concordance between the hierarchical structure of males based on song composition and the variance in pairing success. To quantify this association, we compared the model likelihood of a model that constrained λ to be zero with the model likelihood of a model that allowed λ to be estimated. For this appraisal, we relied on likelihood ratio statistics, that compares the log-likelihood of the model corresponding to a null hypothesis (H_0 : $\lambda=0$) over the model for the alternative hypothesis (H_1 : λ =estimated value), where the likelihood ratio = $-2 \log_e[H_0/H_1]$. The likelihood ratio statistic is asymptotically distributed as a chi-squared variate with degrees of freedom equal to the difference in the number of parameters between the two models (1 in this case).

To evaluate the robustness of our results, we also performed analyses based on randomized data. We randomly reshuffled observed estimates of pairing success across males and tested whether this random distribution of data can follow by chance the hierarchical structure of males based on syllable sharing. We repeated this randomization 1000 times, and investigated the frequency by which the above concordance patterns based on real observations can be reconstructed with the random data.

We also tested for the consistent attraction value of syllables. If each syllable has a specific consequence for mating success, this should be transparent across males that possess them in their repertoire. As such, pairing success should vary systematically within syllables and across males. To uncover such a systematic variation, we assigned a pairing success value to each syllable on each occasion it was possessed by a male using the relative pairing date of the same male. We entered these multiple observations across males in an ANOVA model, and partitioned the component of variations at the between and within-syllable category levels. To account for differences caused by rare and frequent syllables, we used statistical weights in the ANOVA model that relied on probability of syllables of being sung in each male.

From this model, we calculated mean relative pairing date across males for each syllable, and related these syllable-specific

values to the spectrographic parameters of the same syllables to test if the structural properties of syllables determine their value for mate attraction.

To test the prediction that female preference for common songs representing local dialects causes similarities in the song composition of successfully pairing males, we also correlated the frequency of use of syllables at the population level with their mating success values. To estimate the frequency of syllable use, we considered the probability of syllables of being sung by particular males and also the number of males using the syllables.

2.5. Controlling for plot effects

We repeated the analyses for a single large plot to check whether the observed association between pairing success and song composition could be due to variation across space and not be caused by female preference for certain syllables. For this purpose, we focused on the largest sample originating from a single breeding plot ($k6$, $N=33$ with information on relative pairing date). Within this sample, pairing success was not strongly varied in the between-year context ($F_{2,32}=1.266$, $P=0.296$), thus year effects were unlikely to bias the data. We predicted that if plot effects do not confound the relationship between mating success and syllable overlap, their relationship would be apparent in the single plot subsample.

2.6. The proximity of song displays and compositional similarity

If the compositional similarity between males occurs due to the direct sharing of syllables between neighbors, the degree of similarity is expected to depend on the spatial distance between males in comparison. Two males singing in close proximity and in synchrony with each other should have higher chance to take over syllables from each other than other pairs of males that sing at longer distances. To test this possibility, for each male, we have chosen another male with temporally overlapping courtship activity, and calculated the compositional similarity between their songs as well as the physical distance between their nest box (using GPS coordinates). We considered two males to have overlapping singing activity, if they were recorded within a time window of maximum 10 days. This window was determined based on our long-term experience about the average duration of male's singing period ($\sim 2-7$ day). If more than one male could be paired with a focal male within this window, we choose the one that sang at the closest distance. We continued this assignment of males into pairs in the remaining pool of males, which resulted in independent sharing indices and distances (i.e. one male was used only in one pair comparison). Using these pairs of males, we tested if song similarity of males occupying territories in close proximity is higher than males that sing at longer distance from each other under the prediction that song similarity of pairs will be negatively related to their physical distance.

As males can be arranged into independent pairs in more than one combination, we repeated pair assignments 100 times by starting from different focal males. We present results that are averaged across these repeats.

3. Results

3.1. Patterns of syllable sharing across males

The hierarchical classification of males based on compositional similarity in their song is given in Fig. 3. In general, the classification suggests a modest overlap between the repertoires of individuals, as males appear distantly related. Most of the nodes occur at high linkage distance regions, which corresponds

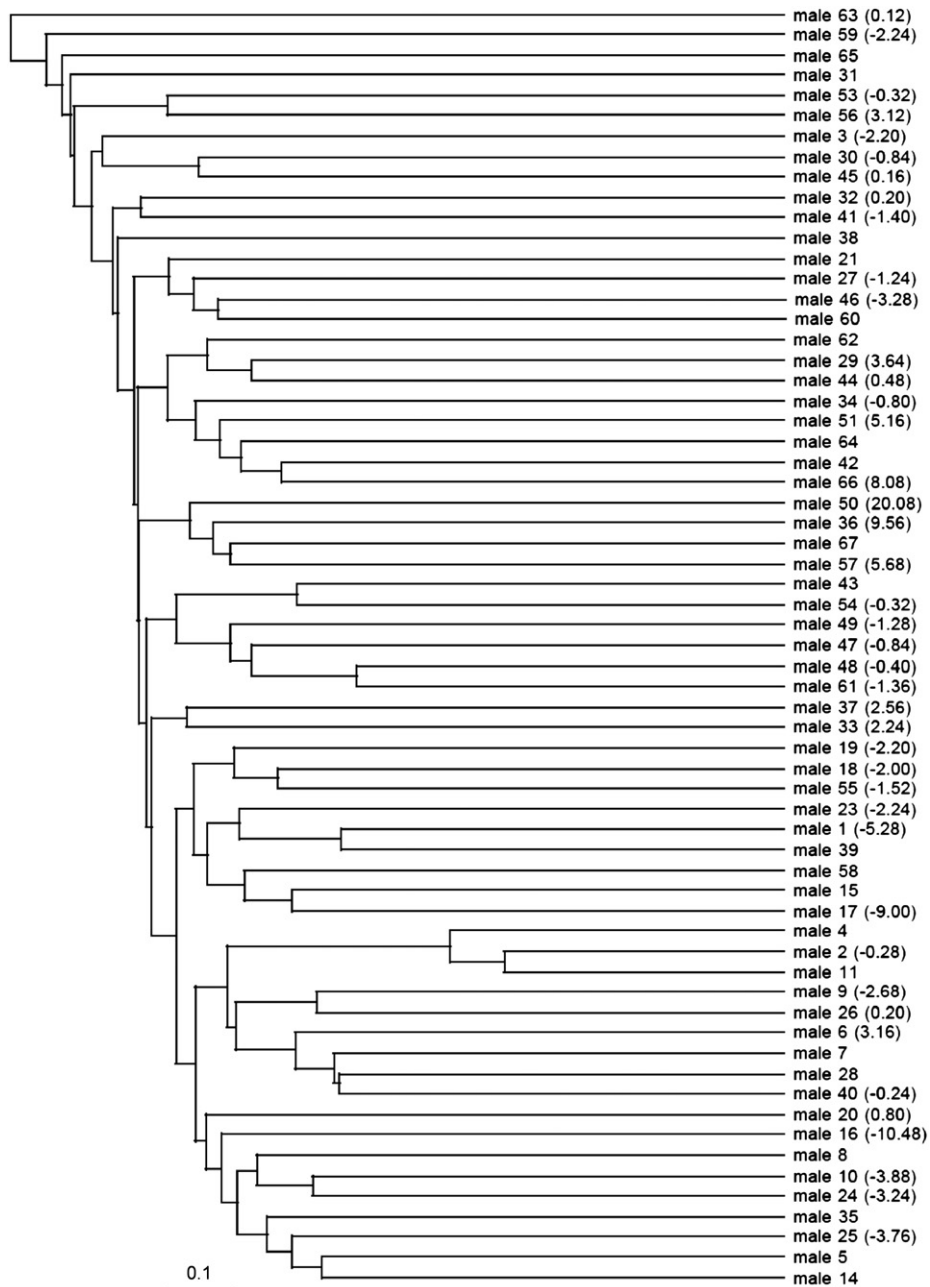


Fig. 3. Hierarchical classification of males based on the compositional similarity of their repertoires. The output from tree-clustering, in which average agglomeration method was used on the song similarity matrix of males. Numbers in parentheses show the pairing success that the given male realized after recording. Pairing success was measured as relative pairing date, thus negative values indicate quick pairing relative to the arrival date, while positive values signify slow pairing.

to a $\sim 30\%$ compositional similarity in their songs (mean \pm s.e. = 0.268 ± 0.002 , range = 0.028 – 0.686). This indicates that males do not share a large proportion of syllables that they frequently use in their courtship displays.

3.2. Song composition and pairing success

The purpose of this study was to investigate the relationship between song composition and pairing success, which would predict that the hierarchy of males based on their repertoire similarity is structured by relative pairing date. We did find a statistically significant evidence for such a structure, because the fitted PGLS model with the highest likelihood required the “phylogeny scaling factor”, λ , to take a value of 1, which implies that pairing success depended on the hierarchy of males as

depicted in Fig. 3 (model with the maximum likelihood estimate of λ , likelihood = -123.310 , $\lambda = 1.000$; model forcing λ to be zero, likelihood = -125.657 , $\lambda = 0.000$; likelihood ratio = 4.695 , $P = 0.030$). This linkage between observed pairing success and song composition is unlikely to occur by chance, because randomly mixed data do not produce such a concordance between the tree structure and the continuous predictor. The vast majority of models estimating λ for the randomized data had lower likelihoods (mean \pm s.e. = -125.548 ± 0.011 ; 95% CI: -125.526 / -125.570 , range = -125.996 – -123.012) than the model based on real observations (see above), and most of them returned with small λ values (mean \pm s.e. = 0.203 ± 0.013 ; 95% CI: 0.177 / 0.228 , range = 0.000 – 1.000) that was statistically indistinguishable from zero (there was significant evidence for non-zero λ only in 8 out of the 1000 random datasets generated).

We investigated if pairing success varied consistently within syllables and across males, which should result from that the presence of a syllable in the repertoire leads to a similar pairing date in males producing this syllable. When we weighted each syllable for the probability of being sung in each male in an ANOVA model, we found a significant support for the consistent variation of pairing success within syllables ($F_{156,1314}=2.006$, $P<0.001$).

Syllable-specific values of relative pairing date did not correlate significantly with their structural properties (syllable length: $r=0.070$, $N=157$, $P=0.384$; maximum frequency: $r=-0.070$, $N=157$, $P=0.384$; minimum frequency: $r=-0.040$, $N=157$, $P=0.617$; mean frequency: $r=-0.088$, $N=157$, $P=0.275$; frequency modulation: $r=-0.091$, $N=157$, $P=0.258$; frequency at the start of the syllable: $r=0.069$, $N=157$, $P=0.392$; frequency at the middle of the syllable: $r=-0.072$, $N=157$, $P=0.373$; frequency at the end of the syllable: $r=-0.091$, $N=157$, $P=0.258$).

We found no considerable association between the mating value of syllables and their frequency of usage ($r=-0.074$, $N=157$, $P=0.355$).

3.3. Controlling for plot effects

When we repeated our focal analyses based on a single plot subsample, the tendency for a relationship between song composition and pairing success remained. Using the phylogenetic approach, the model offering the best fit to the data relied on a λ adjustment that set its value to 1, which implied a support for the hierarchically structured variation of relative pairing date. The significance of the likelihood ratio testing for the estimated λ being different from zero was $P=0.160$ (model with the maximum likelihood estimate of λ , likelihood = -95.641, $\lambda=1.000$; model forcing λ to be zero, likelihood = -96.629, $\lambda=0.000$; likelihood ratio = 1.976), while 58 out of 1000 models relying on randomly generated data resulted in likelihood that exceeded the maximum likelihood of the model based on the observed data (likelihoods based on randomized data:

mean \pm s.e. = -96.497 ± 0.013 ; 95% CI: $-96.521/-96.471$, range = $-96.880-93.697$). Therefore, we could not obtain a significant support for our predictions, but this was likely caused by the smaller sample of males that were available for this test in this subset (the likelihood-based λ statistics is sensitive to power issues as suggested by R. Freckleton via personal communication).

We checked for the consistent variation of pairing success within syllables and across males using the subsample of males breeding on the same breeding plot. This test indicated that the presence of a syllable leads to similar pairing success in males that incorporate this syllable in their song ($F_{153,993}=1.718$, $P<0.001$).

3.4. The proximity of song displays and compositional similarity

Relying on independent pairs of males, we found a significant association between syllable sharing and the physical distance between males. However, contrary to our predictions based on direct sharing of syllables, this relationship was positive and not negative (Pearson's correlation: $r=0.452$, $P=0.044$, as averaged across 100 repeats, Fig. 4). This suggests a higher similarity in syllable composition in vocally non-interacting individuals than in interacting males.

4. Discussion

Here we demonstrated that the song of collared flycatchers with moderately high repertoire size includes syllables that are shared between males. However, the majority of syllables are not shared between birds and are thus combined in a way that results in song composition to vary in an individual-specific manner.

In spite of the relatively low rate of repertoire overlap among males, we found that males having similar song composition realize similar mating success, and the consequence of syllables for mating is repeatable across males. This could potentially imply that the presence of at least some particular syllables in the repertoire encompasses a certain probability for female attraction, e.g. males having "sexy" syllables (*sensu* Vallet and Kreutzer, 1995) experience similar advantages during mating. If there are different syllables with different meaning in the collared flycatcher's song, the consequences of these syllables for mating might be summed up in the repertoire. As a result, males sharing a given amount of syllables would provide similar information about their quality and thus would experience similar mating success.

However, we find it unlikely that the attractive syllables in the collared flycatcher have a similar function as the sexy syllables in the canary through their production costs. We did not detect that the mating value of syllables was associated to their measured acoustic parameters. Furthermore, syllable types that appear in different males but similarly enhance mating might have a completely different structure indicating that spectrographic characteristics of syllables do not necessarily determine their mating value. These results suggest that female choice might not be strictly mediated by the physical properties of syllables (i.e. production costs), and we should consider other alternative mechanisms that do not require repertoires to function through costly syllables.

Based on our correlative results, we can envisage that syllables have another particularity than shape, and that an indicator value that is not transparent on the spectrograms plays more important role in driving females' decision. The relationship between similarity in song composition and pairing success may also occur due to the advantage of direct sharing of syllables between neighbor males (e.g. Beecher et al., 2000; Briefer et al., 2010; Hultsch and

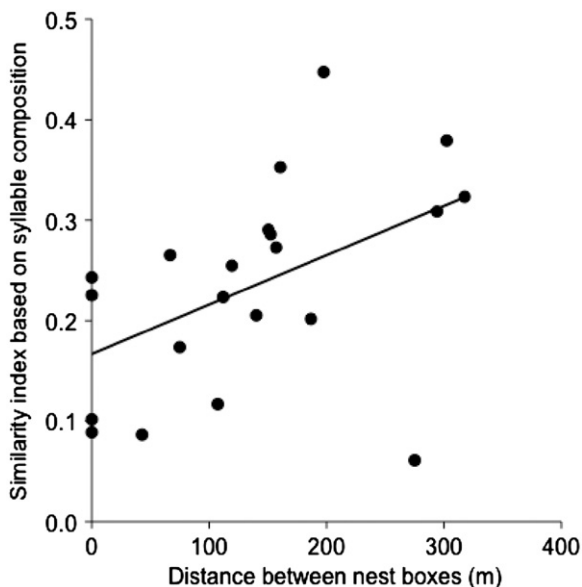


Fig. 4. Relationship between similarity in syllable composition and the physical distance between males when they sing within an overlapping time window. An example from the 100 repeats of independent pair assignment of males. Zero distance means that the males in comparison were recorded at the same nest box with few days apart (they were likely settled in neighboring boxes after several interaction).

Todt, 1981; Laiolo and Tella, 2005; Logue and Forstmeier, 2008). Such mechanisms would predict a higher degree of similarity between males that potentially interact vocally than between males that are separated in time and space and thus have no chance to take over syllables from each other. However, we found a positive relationship between the physical distance between pairs of males and their repertoire overlap. This implies that neighboring males avoid copying syllables from each other that would otherwise lead to syllable sharing. On the contrary, males occupying territories in close proximity seem to favor dissimilarity in their song content, perhaps to enhance individual recognition of pair seeking females. Alternatively, the positive relationship between syllable repertoire similarity and the distance between pairs of males might mirror the genetic determination of song content or the fact that siblings learn from common tutors. If related individuals disperse far from each other (e.g. Van de Castele and Matthysen, 2006), kin individuals with similar songs will be settled distantly from each other when breeding, while unrelated individuals with different song will occupy adjacent territories.

It is, therefore, plausible that syllable composition has been genetically or culturally selected to provide information about certain relationships between individuals and thus have genetically or culturally enhanced indicator values. For instance, if the overlap in syllable composition reflects genetic relatedness, females can avoid mating with individuals that are closely related to them (Stoddard, 1996). As for culturally selected songs, females may prefer individuals that use the local dialect composed of specific syllables, because these males may have high local experience on the breeding grounds (Nelson, 1992). For example, it has been suggested that the females of the great reed warbler *Acrocephalus arundinaceus* discriminate between philopatric and immigrant males based on the presence of specific whistles, which may provide the females with a clue about males' origin (Węgrzyn et al., 2010). Note that in this study, we were unable to obtain evidence for a relationship between the probability of syllables being sung and their mating value. Therefore, it seems unlikely that systematic female preference for songs composed of common syllables would be responsible for the association between syllable content and pairing success. Therefore, these potential mechanisms await further investigations, and the mechanism that gives a certain mating value to a syllable remains to be identified.

In this study, we applied a novel method to fit the hierarchical structure of individuals with the inter-individual variation of a continuous trait. The method relies on a phylogenetic approach, in which the tree structure represents phylogenetic relationships and the continuous trait varies across species (Blomberg et al., 2003; Pagel, 1997). In such a context, the λ statistics can be used, which allows testing for the presence of phylogenetic inertia in the data, i.e. to investigate if the interspecific variation in the trait of interest follows any phylogenetic structure (Freckleton et al., 2002). Our main question was similar to this, because song similarity can be represented by a tree structure that results from the hierarchical classification of males based on compositional similarity in their repertoires. Therefore, we used the λ statistics in the inter-individual context to assess the relationship between song composition and mating success. We envisage that several similar biological questions can be formulated, in which subjects are not independent from each other as they share certain degree of relationship (e.g. due to their origin or to other properties that can be shared). In such cases, subjects can be hierarchically classified and an appropriate λ statistics can be applied to examine the association between the resulting tree structure and the biological predictor in focus. Consequently, the methodology we applied here may be adopted in a broad context.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtbi.2011.11.011.

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