Evolutionary Ecology Research, 2004, **6**: 377–396

Rapidly evolving traits and the comparative method: how important is testing for phylogenetic signal?

Frank E. Rheindt,[1]* T. Ulmar Grafe[2] and Ehab Abouheif[1]‡

[1]*Department of Biology, Duke University, Durham, NC 27708, USA and*
[2]*Department of Animal Ecology and Tropical Biology, University of Würzburg,*
97074 Würzburg, Germany

ABSTRACT

The indiscriminate application of phylogenetically based comparative methods in cross-species correlated evolution analyses has been questioned. It has been argued that traits are not always significantly correlated to their phylogenetic history, and that correcting for phylogeny in these cases may be unnecessary and may even introduce statistical error. Statistical diagnostics to test for phylogenetic signal have recently been presented. Here, we investigate the relationship between habitat and vocal characters within a tropical bird community. We show that the bird song parameters investigated are not correlated to their phylogenetic history, indicating that certain aspects of bird song can be subject to rates of evolution that are much more rapid than speciation events. In contrast, we show that the habitat parameter is significantly correlated to its phylogenetic history. Previous comparative studies, which have not taken phylogenetic signal into account, show a significant association between habitat and song. With respect to our continuous data, we demonstrate that analyses which fail to correct for phylogeny in traits that show phylogenetic signal (i.e. habitat), or those that correct for phylogeny in traits that are phylogenetically independent (i.e. bird song parameters), support an association between habitat and song. Analyses that incorporate tests for phylogenetic signal, however, reject this association, and thus call for a re-evaluation of the evidence on correlated evolution of habitat and bird song. Therefore, using tests for phylogenetic signal before and after conducting a cross-species correlated evolution analysis is crucial to the outcome of a comparative study when analysing rapidly evolving traits.

Keywords: bird song, habitat, phylogenetic autocorrelation, phylogenetically based comparative methods, rapid evolution.

INTRODUCTION

Song is a major target of sexual selection in most bird families, and has therefore received considerable attention by evolutionary biologists. The high number of avian sister taxa with

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* Address all correspondence to Frank Rheindt, Lehrstuhl für Verhaltensphysiologie und Soziobiologie (Zoologie II), Universität Würzburg, Biozentrum, Am Hubland, 97074 Würzburg, Germany. e-mail: formicarius@hotmail.com

‡ *Present address*: Department of Biology, McGill University, Montreal, Quebec, H3A 1B1, Canada.

dramatically different vocalizations (see, for example, Helbig *et al.*, 1996; Isler *et al.*, 1998) attests to the important role songs play in maintaining species integrity and suggests a relatively fast rate of song evolution. It has been proposed that environmental variables, such as differences in vegetation structure, may give rise to the differentiation of bird song between adjacent populations, and in the past 30 years numerous studies have been carried out to look into the nature of the selectional forces that habitat exerts on song evolution. Several different approaches have been taken. On the one hand, sound transmission experiments in natural habitats (Linskens *et al.*, 1976; Marten and Marler, 1977; Wiley and Richards, 1978, 1982; Richards and Wiley, 1980; Brown and Handford, 2000) have mainly shown that reverberation effects in environments with scattering surfaces, such as forests, severely degrade the transmission of sound with rapid amplitude and frequency modulations (particularly of higher frequencies). On the other hand, biologists have looked directly at bird vocalizations, either (1) within one species (Nottebohm, 1975; Hunter and Krebs, 1979; Wasserman, 1979; Gish and Morton, 1981; Handford, 1981; Anderson and Conner, 1985; Douglas and Connor, 1999; Doutrelant *et al.*, 1999), (2) within a set of closely related species (Jilka and Leisler, 1974; Bowman, 1979, 1983; Lemon *et al.*, 1981; Shy, 1983; Sorjonen, 1986a; Van Buskirk, 1997), or (3) across the members of whole communities or avifaunal regions (Chappuis, 1971; Morton, 1975; Ryan and Brenowitz, 1985; Sorjonen, 1986b; Wiley, 1991). Most of the studies that looked directly at bird vocalizations corroborated the results of the acoustic experiments by showing that populations or species living in closed vegetation tend to have lower-pitched songs with less pronounced amplitude modulations, while their open-habitat counterparts tend to have songs that are characterized by greater amplitude modulations and a less constrained frequency range. These findings imply that the sound transmission properties of habitats with differing vegetation structure have a noticeable impact on the direction of song evolution. In particular, closed environments appear to have selected for songs with lower frequencies and less amplitude modulation, as these are less prone to degradation by reverberation effects.

However, many of the earlier interspecific studies failed to acknowledge two major confounding variables that may play an important role in cross-species comparisons. One is the size of the syrinx and, by inference, body size or body mass, all of which are strongly correlated to song properties involving frequency (Wallschager, 1980). The other is phylogeny. It was not until a few decades ago that biologists began to recognize that evolutionary studies incorporating several species may be confounded if phylogenetic relationships are not taken into account (Harvey and Pagel, 1991). In numerous instances, it has been shown that higher Type I error rates and inaccurate parameter estimates may be obtained from performing comparative studies in the absence of phylogenetic correction (Harvey and Pagel, 1991; Martins and Garland, 1991). Ryan and Brenowitz (1985) were the first to attempt an analysis of the relationship between bird song features and habitat in which corrections for both body mass and phylogeny were made. They applied a simplistic method of incorporating phylogeny into their analysis by separating Morton's (1975) uncorrected data set into three taxonomic groupings. They showed that Morton's (1975) hypothesis of selection for lower frequencies in forest habitat is supported after correcting for body mass and phylogeny.

With recent advances in the study of comparative methods (Harvey and Pagel, 1991; Martins and Garland, 1991; Garland *et al.*, 1992), Wiley (1991) was able to account for phylogeny more accurately in his Nearctic oscine data set by applying Grafen's (1989) 'phylogenetic regression' (in addition to corrective measures for body weight). Concentrating on both temporal and spectral parameters of bird song, he found only one of his frequency parameters (highest frequency) to display significantly lower values in the forest habitat, whereas song properties unrelated to frequency, such as the presence of buzzes or the minimal repetition period, were largely different among the habitats investigated.

Meanwhile, progress in comparative evolutionary studies has been made in two ways: First, as for continuous characters, methods have been devised that incorporate existing branch length information of the phylogenetic tree in question as well as particular models of evolutionary change (Harvey and Pagel, 1991; Garland *et al.*, 1992; Martins *et al.*, 2002). The most prominent and widely used among these is Felsenstein's (1985) method of independent contrasts. Independent contrasts have been shown in simulation studies to be a statistically robust method (Diaz-Uriarte and Garland, 1998). Though these approaches may be of limited use in most organisms because of our ignorance of their exact evolutionary history (Losos, 1994; Abouheif, 1998), they are expected to yield far more accurate results in birds, for which extensive phylogenetic information has become available in the last two decades (see Sibley and Ahlquist, 1990; Sibley and Monroe, 1991). Secondly, evolutionists have started to recognize that measuring the amount of phylogenetic signal in comparative data is important in interpreting and understanding evolutionary patterns (Gittleman *et al.*, 1996; Abouheif, 1999; Blomberg and Garland, 2002; Freckleton *et al.*, 2002; Blomberg *et al.*, 2003). Objections to the indiscriminate application of phylogenetically based comparative methods have been raised (Hansen and Martins, 1996; Price, 1997; Martins, 2000), and it has been argued that some traits may not be correlated with their phylogenetic history or that their phylogenetic signal is not detectable for a variety of reasons, such as rapid evolutionary speed (Gittleman *et al.*, 1996; Rosenzweig, 1996). For these traits, then, the application of a phylogenetically based comparative method may not be necessary, and in some cases may even create problems of statistical non-independence when none initially existed, because of errors in assuming the correct branch lengths and models of evolutionary change (Gittleman and Luh, 1994). Abouheif (1999) has suggested that these problems can be circumvented by empirically testing the assumption of phylogenetic independence before and after applying a phylogenetically based comparative method. He presented a statistical diagnostic, called the test for serial independence for continuous data and the RUNS test for discrete data, that can test for phylogenetic independence both before and after the application of a phylogenetically based comparative method. One can use this diagnostic to assess the amount of phylogenetic signal in comparative data, and determine whether one's data set needs phylogenetic correction, and whether one has adequately corrected for phylogenetic history after the application of a phylogenetically based comparative method. This method is generally applicable and has adequate power and Type I error rate (E. Abouheif and J. Reeve, in prep.). Furthermore, the method performs as expected under a range of different models of evolutionary change (E. Abouheif and J. Reeve, in prep.) and has been used widely in the literature to assess phylogenetic independence in a number of different studies (e.g. Diniz and Torres, 2002; Tieleman *et al.*, 2003). Abouheif's method does not explicitly assume that the branch lengths and model of evolutionary change are known. This is an advantage of the method in cases where the branch lengths and evolutionary model are unknown to the researcher and must be arbitrarily assumed (which is currently the case in most comparative studies; see also Price, 1997).

The high incidence of fairly closely related bird species that differ strikingly in their song properties (see, for example, Shy, 1983; Helbig *et al.*, 1996; Isler *et al.*, 1998) hints at a generally rapid rate of song evolution. Thus, considerations of whether or not to take phylogeny into account are highly relevant to the analysis of song evolution. If the evolution of bird song could proceed much more rapidly than cladogenesis, song parameters would consequently be unlikely to reflect their phylogenetic trajectory. In this case, the application of phylogenetically based comparative methods may be unnecessary and erroneous, as song parameters – over time – lose more and more of their association to phylogenetic history, especially if environmental factors like habitat promote the rapid differentiation of song properties through directional selection.

Here we analyse two spatially adjacent bird communities in Côte d'Ivoire, one of savanna habitat and the other of gallery forest. Before exploring the relationship between habitat and presumably rapidly evolving song features, we examine whether or not the song parameters of the members of these communities display phylogenetic independence. We contend that, in rapidly evolving traits, this step is crucial for deciding whether phylogeny has to be taken into account. We go on to derive conclusions by comparing results of traditional analyses with those of phylogenetically based comparative methods in the context of the presence or absence of phylogenetic autocorrelation displayed by each trait.

METHODS

Field methods and study area

During a 2-month period from May to July 2000, recordings of the songs of 62 species (mostly passerines) were obtained from an area of approximately 7 km^2 in the southwestern corner of the Comoé National Park in northeast Côte d'Ivoire (West Africa). All recordings were made with a Marantz recorder (PMD 221) and a directional microphone (Vidmike EM-2800), with about an equal amount of time spent in savanna and in forests. The study area is characterized by a mosaic of forest islands and river-lining gallery forests within savanna habitat. Though some habitat heterogeneity can be found within the savanna and the forests of this area, the dominant feature of the landscape is a strong dichotomy between these two vegetation types that is reflected in a local avifauna consisting of two very different communities that have hardly any species in common (present study). This strict ecological division may be partly due to the non-gradual nature of habitat boundaries. The local savannas are maintained by annual anthropogenically induced fires that prevent the recruitment of forest plants in the savanna, but that only enter marginal parts of the forest, resulting in very abrupt forest edges that immediately make way to savanna habitat (Hovestadt *et al.*, 1999).

Our recordings encompass almost 30% of the total number of bird species presumed to live in this part of the national park (Salewski, 2000; not counting a great proportion of non-passerine families and northern migrants). We suspect that most species missed were either generally rare, seasonally absent or vocally inactive, so that only a very small part of all vocally dominant species of that season had been omitted in the analysis. We opted for confining our analysis to our own recordings rather than boost the sample size by recourse to commercial recordings, mainly because (a) the songs of many West African birds are still imperfectly known, and (b) the songs and habitat requirements of many species vary significantly over geographical areas, so that the only way to confidently assign many of the songs to a particular habitat is to make one's own recordings. We see no reason to believe that there is a bias in the composition of species recorded and consider them to adequately represent the local singing avifauna of that season.

We tried to record only songs, not call notes (which are generally more simple-structured and have no or little function in mate attraction or the maintenance of territories), though often the boundary between calls and songs is blurred. After the field observations, each recorded species was assigned to one of five habitat categories (see Appendix), of which the lowest represents open savanna and the highest closed gallery forest. The few generalist species were classified in the intermediate category, along with species that were confined to savanna thickets and comparable habitats of intermediate character. The second and fourth categories were used for those species typical of one or the other habitat but occasionally found outside of it.

Acoustic analysis

SIGNAL sound analysis software (Engineering Design, Belmont, MA) was used to measure three continuous frequency parameters of bird song: highest frequency (HF), lowest frequency (LF) and dominant frequency (DF) (see Appendix). To obtain highest and lowest frequency, a sonagram of the song was created with the grey shade settings constant at −25 to −30 dB (Fig. 1a) to ascertain that sounds of the same amplitude are given consideration in the measurement of these parameters in each species. Dominant frequency was computed by the program over the whole length of the song (Fig. 1b).

In addition to these three frequency parameters, we recorded the absence or presence of harmonics. Harmonics are evenly spaced frequency components representing integer multiples of a fundamental, which are not necessarily advantageous to long-range communication and should therefore not be expected to be more prominent in any one habitat (Wiley, 1991). Also, we examined the sonagrams (e.g. Fig. 1) with regard to the presence or absence of ‘buzzes’, which were defined as entire song elements of strong amplitude modulation or qualitatively comparable periods of more than 100 ms within a longer song element. Note that as a result of this definition, many sounds classified as buzzes were not actually recognizable as such (sounding more like chatters, clicks or babbles).

Statistical and phylogenetic analysis

Standardized independent contrasts (Felsenstein, 1985) of continuous characters were computed with the program PDTREE (Garland *et al.*, 1993; freely distributed by T. Garland). Standardized independent contrasts were positivized and the adequacy of the branch lengths for statistical purposes was verified as illustrated by Garland *et al.* (1992). Log transformations were conducted wherever required for proper standardization (Garland *et al.*, 1992). The two discrete characters were investigated using Maddison’s (1990) concentrated changes test, which was applied with the computer program MacClade 4.0 (Sinauer Associates, Sunderland, MA), using the option of permitting either character state to be ancestral. The test for serial independence and the RUNS test (both with 2000 replicates) were conducted with the computer program ‘Phylogenetic Independence’ (Reeve and Abouheif, 2003, version 2.0; http://www.mcgill.ca/biology/faculty/abouheif/programs.html). Simple linear regressions (Sokal and Rohlf, 1981) were carried out with S-Plus (Lucent Technologies, Inc.). Body masses were obtained from Dunning (1993) and,

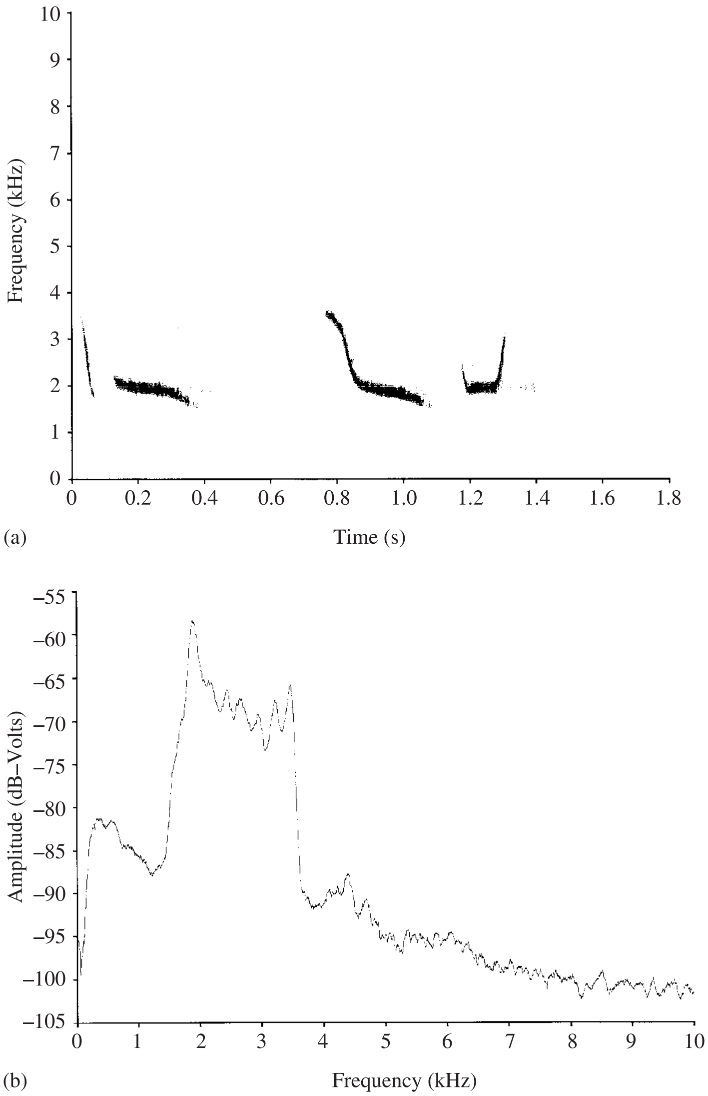

Fig. 1. A sonagram (a) and a frequency spectrum (b) of the song of the emerald cuckoo (*Chrysococcyx cupreus*). The highest and lowest frequency can easily be read off the sonagram (note that the grey scale of each sonagram was held constant). The frequency spectrum reveals the dominant frequency as computed by SIGNAL. As can be seen on the sonagram, this song is a series of smooth whistles without harmonics or strong amplitude fluctuations.

whenever missing therein, from Keith *et al.* (1992), Urban *et al.* (1997) or Fry and Keith (2000).

The topology and branch lengths of the working phylogeny of the species in question (Fig. 2) were mostly obtained from Sibley and Ahlquist's (1990) DNA–DNA hybridization data, which – frequently termed the 'tapestry' – still constitute the only comprehensive bird

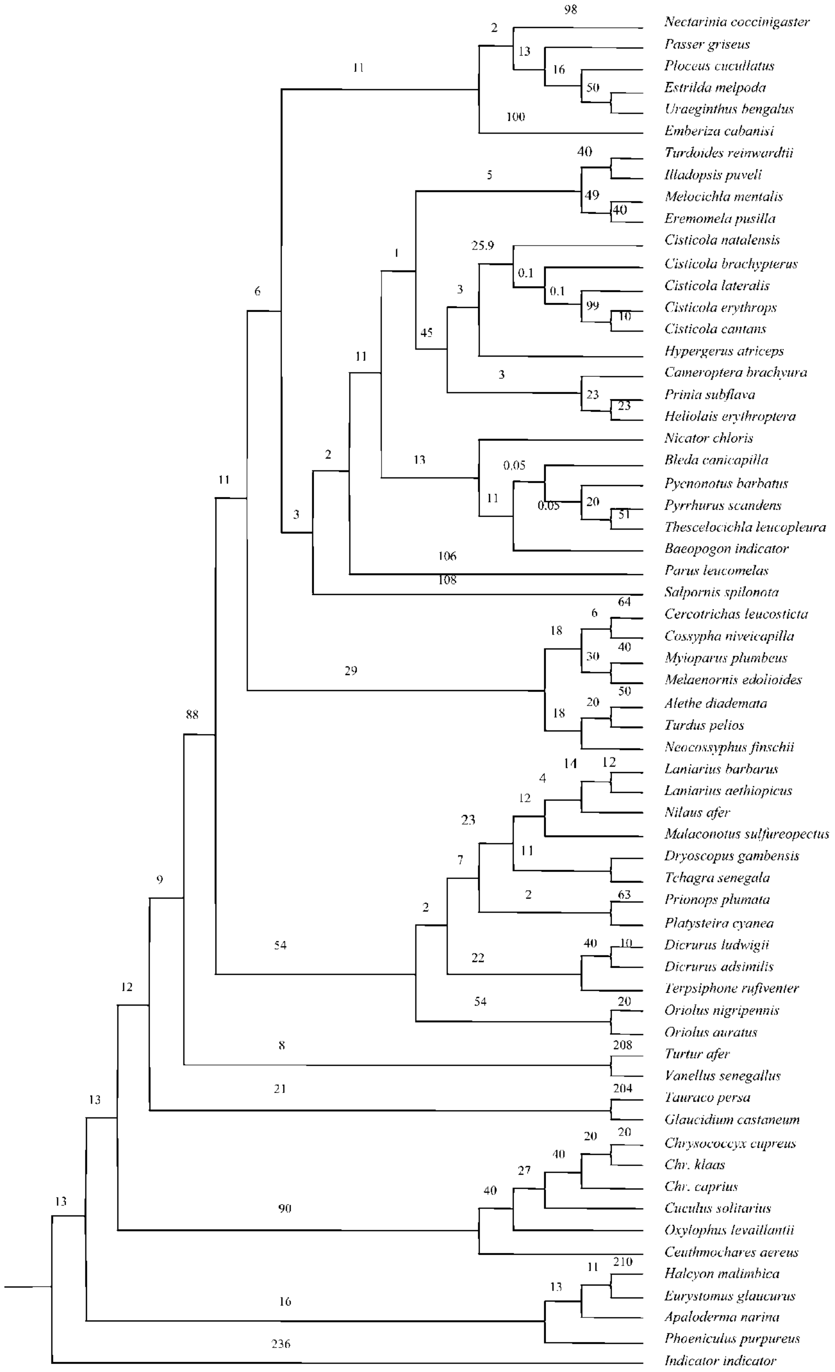

Fig. 2. Phylogenetic tree of the species included; most of the topology and the great majority of branch lengths were obtained from Sibley and Ahlquist (1990). Where they lacked information on branch lengths or topology, recent literature was consulted (see Methods). Branch lengths refer to nodes below number.

classification providing branch length information. Sibley and Ahlquist's (1990) work has been criticized in an extensive body of literature over the years (see O'Hara, 1991, for a detailed review), especially with respect to their failure to provide measures of uncertainty for their classification. In view of the necessity to have at one's hands a working phylogeny incorporating all the species investigated, we used Sibley and Ahlquist's (1990) data as a backbone for our working phylogeny, but sought to incorporate data from more recent and more universally accepted studies in particular corners of the tree topology (see below).

There were a few cases (in some of the Cuculidae, Muscicapidae, Estrildidae, Pycnonotidae, Corvidae, Cisticolidae and Sylviidae) for which Sibley and Ahlquist (1990) did not provide full branch length information or collapsed the clades involved into polytomies for want of unambiguous molecular evidence. For some of these unresolved groupings, recent studies of molecular systematics are available (Aragón *et al.*, 1999; Cibois *et al.*, 1999; Pasquet *et al.*, 1999; Johnson *et al.*, 2000). The few remaining ones were resolved with morphological and life-history information mostly from Keith *et al.* (1992), Urban *et al.* (1997) or Fry and Keith (2000). Wherever possible, branch lengths (if missing) were roughly estimated on grounds of the sequence divergence provided in molecular studies (Aragón *et al.*, 1999; Cibois *et al.*, 1999). In a handful of remaining cases, branch length information was estimated to the best of our knowledge. Although we are fairly confident about the correctness of the topology of our tree, we recognize that around 30% of the branch length information in our working phylogeny (Fig. 2) has been derived by a different means than that of Sibley and Ahlquist (1990). Therefore, when a significant relationship between song parameters and habitat was found, we additionally analysed the data using randomized branch lengths computed with Grafen's (1989) arbitrary method to assess the confounding effect of branch length information.

Whenever only one of two characters displayed phylogenetic autocorrelation in a comparative investigation, we additionally analysed the data using standardized independent contrasts derived from the best tree available with regard to the phylogenetically autocorrelated trait, but using contrasts derived from an evolutionarily neutral star phylogeny with regard to the phylogenetically independent trait. We then assessed whether the results of this type of analysis differ from those using independent contrasts derived from the same topology.

RESULTS

Continuous parameters

As expected, all three continuous frequency parameters were significantly associated with body mass (HF: $r^2 = 0.073$, $P = 0.033$; LF: $r^2 = 0.136$, $P = 0.003$; DF: $r^2 = 0.123$, $P = 0.005$; linear regression) and were therefore corrected for body mass by computing residuals. All three mass-corrected continuous parameters were phylogenetically independent (LF, $P > 0.5$; HF, $P = 0.06$; DF, $P = 0.168$; see Table 1 for C-statistics) according to the test for serial independence (TFSI), while habitat exhibited a significant phylogenetic autocorrelation ($P = 0.0045$; Table 1). This implies that phylogenetic correction with a phylogenetically based comparative method such as Felsenstein's (1985) independent contrasts may be unnecessary in the case of frequency parameters, whereas its omission would be inappropriate with respect to trait correlations involving habitat.

Table 1. *C*-statistics for phylogenetic autocorrelation and their *P*-values in parentheses

	Raw data	Standardized independent contrasts (best available tree)	Standardized independent contrasts (arbitrary branch lengths)
Lowest frequency	−0.075 ($P > 0.5$)	0.126 ($P = 0.372$)[a]	—
Highest frequency	0.147 ($P = 0.06$)	0.173 ($P > 0.5$)	0.737 ($P > 0.5$)
Dominant frequency	0.095 ($P = 0.168$)	−0.076 ($P = 0.469$)	−0.203 ($P > 0.5$)
Habitat index	0.231 ($P = 0.0045$)*	−0.017 ($P > 0.5$)[a]	—
Presence of buzzes	0.082 ($P = 0.249$)	—	—
Presence of harmonics	−0.073 ($P = 0.295$)	—	—

Note: The three frequency parameters (mass-corrected) and habitat index were subjected to the test for serial independence (TFSI), while the data for the two discrete parameters (presence of buzzes and harmonics) were computed with the RUNS test. Significant *P*-values indicate phylogenetic non-independence. The continuous traits were analysed before ('raw data') and after applying Felsenstein's (1985) independent contrasts. For highest and dominant frequency, the TFSI was also carried out on a tree with Grafen's (1989) arbitrary branch lengths (see text).

[a] Branch lengths were log-transformed to avoid overstandardization of long branches (see Garland *et al.*, 1992).

* Significant phylogenetic autocorrelation.

Regardless of whether phylogeny was corrected for in both traits of a comparison (habitat *vs* frequency) or whether it was left entirely unaccounted for, lowest frequency showed no relationship with habitat, while highest and dominant frequency did so significantly (Table 2). To ascertain that this significant relationship was no artifact brought about by the derivation of branch lengths from different sources, we additionally randomized branch lengths using Grafen's (1989) method. This procedure did not change the significance of the relationship markedly in either instance (Table 2), indicating that in our data set, branch length information has little effect when accounting for phylogeny (Diaz-Uriarte and Garland, 1998).

Interestingly, all the comparisons conducted comprised one trait that is phylogenetically autocorrelated and one that is not. The best way to take this difference in phylogenetic signal among two traits into consideration is by computing Felsenstein's (1985) independent contrasts for the phylogenetically autocorrelated trait (i.e. habitat) on the best tree available, and to derive a set of contrasts for the other trait (i.e. frequency) from an evolutionarily neutral star phylogeny. Essentially, this method enables one to statistically compare phylogenetically corrected contrasts with the raw data, since deriving contrasts from star phylogenies does not yield results different from those of traditional analyses (Blomberg *et al.*, 2003). Using this method, none of the three frequency parameters displayed a significant relationship with habitat (Table 2).

Applying the test for serial independence (TFSI) to phylogenetically corrected independent contrasts of habitat yielded strongly non-significant results (see Table 1 for *C*-statistics and *P*-values), which demonstrates that the application of independent contrasts was successful in accounting for phylogeny. Not surprisingly, the TFSI revealed that independent contrasts of lowest, highest and dominant frequency – the raw data of which had not been significantly phylogenetically autocorrelated – invariably exhibit phylogenetic independence.

Table 2. P-values and coefficients of determination (r^2) of linear regression of the three mass-corrected frequency parameters (dependent variable) on habitat index (independent variable)

		Regression of standardized independent contrasts		
Dependent variable (mass-corrected)	Regression of raw data	Best tree available	Tree with arbitrary branch lengths	Contrasts of frequency derived from star topology; contrasts of habitat index derived from best tree available
Lowest frequency	0.730 ($r^2 = 0.002$)	0.212[a] ($r^2 = 0.026$)	—	0.316[b] ($r^2 = 0.017$)
Highest frequency	<0.001** ($r^2 = 0.292$)	0.018*[b] ($r^2 = 0.092$)	<0.001**[a] ($r^2 = 0.213$)	0.886[b] ($r^2 = 0.0004$)
Dominant frequency	<0.001** ($r^2 = 0.191$)	0.012*[b] ($r^2 = 0.103$)	0.003**[a] ($r^2 = 0.141$)	0.397[b] ($r^2 = 0.012$)

Note: For highest and dominant frequency, independent contrasts were additionally computed using a tree with Grafen's (1989) arbitrary branch lengths (see text).

[a] In the computation of independent contrasts of both habitat and frequency, all branch lengths were log-transformed to avoid overstandardization of long branches (see Garland *et al.*, 1992).

[b] In the computation of independent contrasts of habitat, all branch lengths were log-transformed to avoid overstandardization of long branches (see Garland *et al.*, 1992).

* Significant ($P < 0.05$). ** Highly significant ($P < 0.01$).

Discrete parameters

Wiley (1991) showed that body mass need not be corrected for in the two discrete song properties investigated in this study, since the mass of a vibrating structure (i.e. the syrinx) only influences frequency characteristics. Both the presence of harmonics (PH) and buzzes (PB) are not phylogenetically correlated according to the RUNS test (Table 1). To examine the relationship between habitat and the presence of buzzes or harmonics, species were classified as open-habitat inhabitants (first three habitat categories) or as forest inhabitants (last two categories). We discarded three species of either precisely intermediate or generalist habitat preference. It was found that the two traits are not unequally distributed among open- and closed-habitat inhabitants if no phylogenetic correction is applied (two-tailed two-sample t-test; PH: $t = 1.374$, $P = 0.222$; PB: $t = 0.173$, $P = 0.393$).

Given a tree topology, phylogenetic correction in discrete data can be accomplished using the concentrated changes test (Maddison, 1990), which explores whether gains or losses of two or more traits are concentrated on any particular branch of a tree. The results of this test strongly depend on the ancestral states that the concentrated changes test reconstructs using parsimony. All ancestral character states were unequivocally reconstructed with respect to the presence of harmonics (PH), and the pattern observed (seven out of nine gains and zero losses of PH in savanna habitat) gave no hint at an association between the presence of harmonics and savanna habitat ($P = 0.188$). For buzzes, ancestral character states could not always be unequivocally assigned, and the estimates of probability of an association between habitat index and the presence of buzzes varied with the number of gains and losses of buzziness assumed, with a tendency to obtain a higher probability of an association between savanna habitat and the presence of buzzes with an increasing number of gains reconstructed (Table 3).

Table 3. Four models of reconstructing ancestral states with regard to the presence of buzzes

Model for the total number of gains (g) and losses (l) reconstructed: (g, l)	The observed number of gains and losses in savanna habitat (g, l) under the specified model and their *P*-values	The observed number of gains and losses in forest habitat (g, l) under the specified model and their *P*-values
(13, 4)	(10, 1) $P = 0.0097$**	(4, 3) $P = 0.0284$*
(12, 5)	(9, 2) $P = 0.0302$*	(4/3, 3) $P = 0.0877$ and 0.0350*
(11, 6)	(8, 3) $P = 0.0815$	(4/3, 3) $P = 0.1912$ and 0.0918
(10, 7)	(7, 4) $P = 0.1719$	(3, 3) $P = 0.1896$

Note: *P*-values refer to the probabilities of the observed patterns of gains and losses in savanna (left) and forest (right) habitat according to the concentrated changes test and looking at all four possible ways of parsimoniously reconstructing ancestral states.
* Significant ($P < 0.05$). ** Highly significant ($P < 0.01$).

DISCUSSION

Does habitat influence song frequency?

After correction for body mass, two of the three frequency parameters investigated, namely the highest frequency (HF) and the dominant frequency (DF) of a species' song (Table 2), showed a relationship with habitat if, and only if, both traits were phylogenetically corrected or if both traits were analysed by traditional statistical analyses. However, regression analyses invariably involved one trait (i.e. habitat) that was shown to be phylogenetically autocorrelated by the TFSI and another one (i.e. frequency) that was not (Table 1). To take into account these differences in phylogenetic signal among traits, we also conducted regression analyses in which the best tree available was used to compute Felsenstein's (1985) independent contrasts for habitat, and an evolutionarily neutral star phylogeny was used to derive contrasts for each frequency parameter. However, when using this approach, no association between habitat and frequency was detected (Table 2).

These results are not in agreement with previous studies that have reported either higher HFs (Morton, 1975; Hunter and Krebs, 1979; Shy, 1983; Anderson and Conner, 1985; Wiley, 1991) or both higher HFs and DFs in open habitats than in forests (Ryan and Brenowitz, 1985). As an explanation for the association of only some frequency parameters with habitat, Wiley (1991) suggested that the lowest and dominant (as opposed to the highest) frequencies in his data set were outside the range of frequencies that suffer great attenuation in sound-scattering environments such as forests. This seems to apply to all three of our frequency parameters. Note, however, that no previous authors have assessed the level of phylogenetic independence of their song traits in order to ascertain whether or not phylogenetic correction should be conducted.

Alternatively, Price (1997) suggested that differences in the statistical outcome of phylogenetically based comparative methods versus traditional analyses may have a biological significance. Under his adaptive radiation model, Felsenstein's (1985) contrasts themselves are a poor guide to the significance of allometric relationships across species. But the comparison of traditional analyses and independent contrast analyses may provide insights into the history of the evolution of the traits under investigation. Thus, failing to produce significantly correlated contrasts in the presence of significantly correlated raw data may hint at a period of correlated evolution during a burst of adaptive radiation early in the history of a group, but not later. In our study, the regression between habitat and two frequency parameters was significant, but this association vanished when we corrected for phylogeny in the one trait that exhibited phylogenetic autocorrelation. Under the adaptive radiation model, this different outcome may hint at the correlated evolution of highest/dominant frequency and habitat only during an early period, possibly at a time when most present-day bird families began to radiate. Later, after all the major present-day genera had been well established, this association between song frequency and habitat may have become weaker. If this model appropriately describes avian evolution, the fact that Wiley (1991) or other authors working with Holarctic birds did not find a similar pattern may have to do with the lack of recurring glaciations in Africa. The selective pressure of habitat on song may have been higher in the Holarctic, where newly exposed continental areas and newly emerging vegetation zones continually had to be re-colonized from outside. Birds were more often confronted with new types of habitat during allopatric speciation events here than in tropical Africa, where forests have been long-established and savanna made its first appearance at the end of the Miocene (Axelrod and Raven, 1978). This intriguing problem will have to be addressed in future research.

Does habitat influence the presence of buzzes and harmonics?

The RUNS test indicates that both the presence of buzzes and harmonics are independent of phylogeny (Table 1) and therefore provides a key as to whether or not phylogeny should be taken into account. Wiley (1991) presented strong evidence that open-country birds are free to incorporate into their songs (as communication signals) elements that are subject to amplitude modulation, while forest birds avoid such sound signals because of the strong level of degradation that reverberation effects have on them. Therefore, song features such as the presence of buzzes should be expected more frequently in savanna birds. Harmonics, in contrast, are generally unfavourable to concentrating maximal energy into a single frequency and should thus be avoided by birds of any habitat (Wiley, 1991). Our analysis showed that, as expected, the presence of harmonics was independent of habitat.

The presence of buzzes was found to be strongly associated with open habitat by previous authors (Wiley, 1991), but its correlation with habitat in this study is equivocal. The presence of buzzes showed no phylogenetic signal according to the RUNS test. It was independent of habitat – *contra* Wiley (1991) – when phylogeny was neglected, as suggested by the RUNS test. Applying a phylogenetically based comparative method such as the concentrated changes test (Maddison, 1990) – *contra* the premises of the RUNS test – yielded equivocal results (Table 3): their interpretation depends on the number of gains and losses of ‘buzziness’ one is prepared to allow for in the ancestral state reconstruction, where a high number of gains of ‘buzziness’ supports an association between the presence of buzzes and habitat, while a low number of gains of that trait repudiates such an association. Lorch and Eadie (1999) showed that the concentrated changes test is fairly robust in its diagnosis of evolutionary correlations, although it does have a tendency to commit Type II errors (fail to find correlations where they exist). However, the test computes ancestral state reconstructions by means of parsimony, which has been questioned by several authors (Cunningham, 1999; Omland, 1999), especially when the traits are only loosely correlated to their phylogeny, and the reconstruction of ancestral character states may be ambiguous.

Even though these results are equivocal, we believe that the best course may well be to reject an association between the presence of buzzes and habitat on grounds of the demonstration that the presence of buzzes is phylogenetically independent and phylogenetic correction is possibly unnecessary. Wiley (1991) has not only presented data, but also good acoustical reasoning to argue that buzzes should be associated with open habitats. In light of the RUNS test, our data indicate otherwise, but there may be a number of reasons why. The 'open end' of the habitat spectrum we investigated constitutes 'guinée savanna' typical of West Africa south of the Sahel Zone, with a tree ground cover of approximately 40% and a maximal tree height of 7 m (Hovestadt *et al.*, 1999). This habitat may be too closed and forest-like to match the sound transmission properties of Wiley's (1991) grasslands and marshes.

The quantification of bird song

There are many bird lineages that are characterized by common themes in song structure, but it has so far proved difficult to analyse this acoustical 'likeness'. The development of methods to measure song similarity is still in its infancy (see, for example, Tchernichovski *et al.*, 1999). More importantly, these methods have not yet been utilized by biologists interested in the effect of habitat on bird song. Instead, previous authors (including ourselves) have concentrated on a small and limited number of song properties (involving frequency, and rarely one to three other aspects) in their examination of the association of song with habitat. However relevant this handful of parameters may be in the characterization of bird song, they span but a tiny proportion of the different ways in which songs can be described. A song need not be similar to another song that precisely matches it in all of these sound properties. Conversely, two songs can be qualitatively very similar even though they differ dramatically in these parameters. As long as we resort to a few simple measurements, such as highest frequency, because we do not feel the need to apply (or do not have) methods that successfully quantify the general quality of a bird's song, we need to be aware that we are only dealing with one little and possibly volatile component among a large number of elements that constitute the song as an evolutionary entity.

Rapidly evolving traits

Generations of ornithologists have appreciated the spectacular vocal differences that enable them to identify otherwise indistinguishable avian sister species. This simple observation is strong evidence for the possibility that bird song in its entirety, not just a few select parameters, can be subject to great structural modifications within a short evolutionary time (Van Buskirk, 1997). If this is generally the case, song evolution may frequently be 'saturated' from an evolutionary point of view, meaning that song parameters change so drastically between speciation events that they leave no trace of phylogenetic correlation.

The song parameters we selected for this study were shown to be independent of phylogeny (though it could be argued that one of them – highest frequency – was within the range of near-significant phylogenetic autocorrelation; see Table 1). Most plausibly, this is so because their ties to phylogenetic history are weak and they are free to evolve rapidly. Many previous authors (Morton, 1975; Hunter and Krebs, 1979; Shy, 1983; Anderson and Conner, 1985; Ryan and Brenowitz, 1985; Wiley, 1991) showed habitat to be a major factor in the shaping of bird song, but ecological relationships among syntopic bird species (competition, feeding associations, etc.) have thus far received little attention and may be of similar importance (Van Buskirk, 1997). We hypothesize that all these environmental variables are probably responsible for fast directional selection that can strip bird song of its phylogenetic signal within short periods of time.

Should phylogeny be taken into account when analysing rapidly evolving traits?

The last decade has seen a broad consensus in the scientific community about the need to incorporate phylogenetic information into cross-species comparisons of evolutionary traits. However, it has been correctly pointed out that phylogenetic correction should not be blindly adopted for all characters (Gittleman *et al.*, 1996; Abouheif, 1999), as it may generate problems of statistical non-independence in those cases where phylogenetic autocorrelation of the trait in question is non-existent (Gittleman and Luh, 1994). In the present study, we applied Abouheif's (1999) test for serial independence and RUNS test to empirically verify whether the traits investigated display phylogenetic independence. As expected, all the song parameters investigated were found to be phylogenetically independent, suggesting that their evolutionary rate outruns that of speciation events, such that the similarity of these traits among species leaves no trace of phylogenetic affinity. Therefore, when no phylogenetic autocorrelation is initially present, the application of Felsenstein's (1985) independent contrasts to these parameters may potentially introduce statistical error or bias. Habitat was the only trait investigated that was significantly correlated to phylogeny and that clearly required phylogenetic correction.

Abouheif's (1999) method has been criticized because it fails to incorporate branch lengths, which may provide important information about expected species' similarity that cannot be gained from the topology alone. Alternative methods have been proposed that transform branch lengths to optimally fit the tip data in phylogenies where the tree at hand does not exhibit optimal phylogenetic signal (Freckleton *et al.*, 2002; Blomberg *et al.*, 2003). However, branch length information is often not available in comparative studies, and where it is, it is prone to bias and error. In our study, for instance, several sources that had derived branch lengths by different methods had to be consulted to provide complete branch length information. However, in our study it is also evident that contrast regressions using a tree with branch lengths mostly from DNA–DNA hybridization do not arrive at conclusions different from those using random branch lengths. Diaz-Uriarte and Garland (1998) also showed that Felsenstein's (1985) contrasts are insensitive to branch length variation.

Abouheif's (1999) test was originally designed in a non-phylogenetic context and is not based on any specific model of evolution. As an alternative, tests have been devised that envisage evolutionary models that make specific assumptions (Freckleton *et al.*, 2002; Blomberg *et al.*, 2003). However, in rapidly evolving traits, making specific assumptions about the mode of evolution could potentially be very misleading (Price, 1997; also see below).

Virtually all traits associated with a species should in one way or another be reflected in its phylogenetic history, either through direct descent or because of the fact that closely related species have a tendency to resemble each other in all facets of life, including those life-history traits that are not usually viewed as inherited (Price, 1997). Therefore, some evolutionists may advise against the abandonment of phylogenetically based comparative methods in the absence of a phylogenetic signal. We agree, in that researchers must not neglect the evolutionary processes that underlie the pattern studied. However, the application of the TFSI on rapidly evolving traits challenges not the process of evolution, but whether this process is conservative enough to constitute a confounding factor. Phylogenetically based comparative methods automatically assume that traits have to display some phylogenetic signal, however frail. If this were the case, a test like the TFSI should recover it most of the time.

Variation in bird song parameters is subject to certain restrictions, such as syrinx size, sound transmission, and so on. These restrictions allow for parameter evolution not beyond a well-defined range, such as frequencies between about 0.2 and 8 kHz (in our study). Within this range, however, song frequencies can be widely different among closely related taxa (see Appendix for some of the frequencies within the genus *Cisticola* or among the shrike species of the genera *Laniarius*, *Dryoscopus* and *Nilaus*). Given this rapid rate of evolution of a narrowly confined continuous trait, it is not surprising that the trait values retain no trace of their phylogenetic affinity. In scenarios such as the present one, in which phenotypic traits can make big leaps at rapid speed but within a confined parameter space, there is a good possibility that similarities in trait values are erroneously interpreted as phylogenetic signal, when they are homoplasies. The opposite would apply to differences in trait values by analogy.

What if traditional and contrast analyses yield different results?

Numerous authors agree that habitat seems to exert a selective pressure on some frequency parameters (Morton, 1975; Hunter and Krebs, 1979; Shy, 1983; Anderson and Conner, 1985; Ryan and Brenowitz, 1985; Wiley, 1991). However, none of these authors tested for phylogenetic signal in their data set to determine which traits, if any, require phylogenetic correction, and all of these authors treated both traits in the same way – that is, either both were directly analysed or both were subjected to contrast analysis. In our own data, all regressions involved one phylogenetically autocorrelated and one independent trait. This situation may not be uncommon in comparative studies that involve one rapidly evolving trait. Interestingly, applying phylogenetic correction if, and only if, a trait exhibits phylogenetic signal seemed to be crucial to the outcome. In contrast, traditional analyses (which left the phylogenetic bias of one trait unaccounted for) yielded a different result, as did contrast analyses in which we applied phylogenetic correction although the trait involved was phylogenetically independent. All previous studies on the subject are therefore in conflict with our results from analyses in which traits have been tested for phylogenetic signal and corrected accordingly, but are in agreement with analyses in which we 'over-corrected' or ignored phylogeny. It will be interesting to see if future studies using the same methodology will corroborate our findings that habitat and bird song are not subject to correlated evolution. If so, conflicting results from previous research using different approaches may be due to a failure to account for phylogenetic bias in some studies coupled with erroneous phylogenetic corrections in others.

Thus the question of whether habitat exerts selective pressures on song frequency is not yet settled. In this context, Price's (1997) adaptive radiation model provides another intriguing interpretation of the discrepancies between our results and those of others. As outlined above, Price (1997) advocates that traits which are correlated but whose contrasts are not may hint at a correlated trait evolution only during an adaptive radiation early in the history of a clade, but not later. If his model is valid for West African birds, the selective pressure of habitat on frequency may have relaxed in more recent times, while it may have remained at a high level in North America, where species have constantly had to adapt to habitat shifts in the wake of glaciations, and where Wiley (1991) found the same results in phylogenetically corrected and uncorrected analyses. Thus, our results open up the possibility that, in West African bird communities, habitat may have played a role in the evolution of song frequency at earlier times, but does not continue to do so. Whether this assumption is true, and whether this presumed pattern of change in trait correlation differs in temperate biomes, requires much further research that – unlike Wiley (1991), but like the present study – tests for phylogenetic autocorrelation before applying contrast regressions.

As for continuous traits, applying the concentrated changes test (Maddison, 1990) to account for phylogeny potentially did make a difference to the outcome of the analysis of habitat and the presence of buzzes, but this is difficult to assess given the ambiguities in reconstructing ancestral character states in this particular parameter.

ACKNOWLEDGEMENTS

We extend our gratitude to Karl-Eduard Linsenmair for providing the facilities for field research at the field camp of Würzburg University in Comoé National Park, Côte d'Ivoire. Steve Nowicki is acknowledged for his invaluable advice on the acoustical analysis and for providing his laboratory facilities.

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APPENDIX

The sound parameters, body masses and habitat assignments of all 62 species that were sound-recorded during the study; habitat index ranges from 1 (open savanna) through 3 (closed forest)

Species	Habitat	Body mass (g)	Highest frequency (Hz)	Lowest frequency (Hz)	Dominant frequency (Hz)	Presence of harmonics	Presence of buzzes
1. *Vanellus senegallus*	1	237	7278	550	4102	yes	no
2. *Glaucidium castaneum*	3	120	1206	502	893	no	no
3. *Apaloderma narina*	3	61.0	1057	251	507	no	no
4. *Phoeniculus purpureus*	1.5	86.6	6942	143	4145	yes	no
5. *Indicator indicator*	1.5	52.2	7951	245	3770	no	yes
6. *Tauraco persa*	3	268	1070	365	813	no	yes
7. *Oxylophus levaillantii*	2	122	2446	795	1566	no	no
8. *Cuculus solitarius*	2	71.7	2362	1106	1705	yes	no
9. *Chrysococcyx klaas*	2	24.0	3166	2362	2817	no	no
10. *Chrysococcyx caprius*	2	32.0	3618	2265	2750	no	no
11. *Chrysococcyx cupreus*	3	37.7	3719	1357	1888	no	no
12. *Ceuthmochares aereus*	3	63.8	2915	1357	2045	no	yes
13. *Turtur afer*	2.5	65.6	854	151	489	no	no
14. *Halcyon malimbica*	3	91.8	3467	1859	2280	no	no
15. *Eurystomus glaucurus*	1.5	110	5427	804	2670	yes	no
16. *Dicrurus adsimilis*	2	45.7	6583	251	3661	yes	yes
17. *Dicrurus ludwigii*	3	26.7	6332	1558	2856	no	yes
18. *Nicator chloris*	3	38.2	2905	459	2180	no	yes
19. *Pyrrhurus scandens*	3	44.0	2905	703	1547	no	yes
20. *Bleda canicapilla*	3	45.4	4020	1206	2100	no	yes
21. *Baeopogon indicator*	3	45.9	3668	1558	2919	no	no
22. *Thescelocichla leucopleura*	3	62.0	3792	2202	2995	no	no
23. *Pycnonotus barbatus*	1	35.9	4343	1193	2489	no	yes
24. *Turdoides reinwardtii*	1.5	82.0	5427	653	1647	no	yes

Appendix—*continued*

Species	Habitat	Body mass (g)	Highest frequency (Hz)	Lowest frequency (Hz)	Dominant frequency (Hz)	Presence of harmonics	Presence of buzzes
25. *Illadopsis puveli*	3	42.2	3700	2018	2547	no	no
26. *Turdus pelios*	2.5	55.4	3250	1608	2716	no	no
27. *Alethe diademata*	3	33.6	2538	1223	2300	no	no
28. *Neocossyphus finschii*	3	37.1	2080	1193	1667	no	no
29. *Cercotrichas leucosticta*	3	24.0	2462	3769	3142	no	no
30. *Cossypha niveicapilla*	2.5	33.8	3015	905	2074	no	no
31. *Terpsiphone rufiventer*	2.5	15.1	3715	1457	3136	no	no
32. *Melaenornis edolioides*	1.5	30.8	7676	2477	3703	no	yes
33. *Myioparus plumbeus*	1.5	12.2	4020	2915	3409	no	no
34. *Platysteira cyanea*	3	13.4	5413	1835	3263	yes	no
35. *Melocichla mentalis*	1	33.5	5312	1873	2541	no	yes
36. *Camaroptera brachyura*	2.5	9.3	6482	201	3976	no	yes
37. *Prinia subflava*	1	9.2	6147	2569	4231	no	no
38. *Cisticola brachypterus*	1	9.0	5528	2864	3535	no	no
39. *Cisticola lateralis*	1.5	16.9	3819	1407	2408	no	no
40. *Cisticola natalensis*	1	15.4	5879	905	3438	no	yes
41. *Cisticola erythrops*	1.5	13.6	6030	1508	3208	no	yes
42. *Cisticola cantans*	1	11.9	6080	259	2523	no	no
43. *Eremomela pusilla*	1	6.1	5930	2111	3316	no	yes
44. *Heliolais erythroptera*	1.5	12.7	6231	1910	3316	no	no
45. *Hypergerus atriceps*	1.5	28.0	4893	4193	3940	no	no
46. *Oriolus nigripennis*	3	58.0	2171	751	1609	yes	no
47. *Oriolus auratus*	1.5	79.4	3547	1223	1967	yes	no
48. *Parus leucomelas*	1.5	16.1	7217	153	2979	no	yes
49. *Salpornis spilonota*	1.5	14.0	6575	2783	4033	no	no
50. *Tchagra senegala*	1	49.2	3719	1132	1942	no	no
51. *Prionops plumata*	2	33.0	6269	1193	1810	no	yes
52. *Laniarius barbarus*	1.5	47.0	4107	627	2219	no	yes
53. *Laniarius aethiopicus*	1.5	50.5	1101	459	873	yes	no
54. *Dryoscopus gambensis*	2	33.5	5578	704	3323	yes	yes
55. *Malaconotus sulfureopectus*	1.5	29.8	3058	1590	1809	no	no
56. *Nilaus afer*	1.5	18.7	2549	1407	2143	no	yes
57. *Nectarinia coccinigaster*	1	14.3	4648	1774	3866	no	no
58. *Emberiza cabanisi*	1	24.0	6231	2462	4239	no	no
59. *Uraeginthus bengalus*	1	10.3	7236	1206	3333	no	yes
60. *Estrilda melpoda*	1	7.6	6633	1005	5704	no	yes
61. *Ploceus cucullatus*	1.5	40.8	6758	1896	3540	no	yes
62. *Passer griseus*	1	23.9	5749	1651	3223	no	no