Evolution of acoustic and visual signals in Asian barbets

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Abstract

The study of animal communication systems is an important step towards gaining greater understanding of the processes influencing diversification because signals often play an important role in mate choice and can lead to reproductive isolation. Signal evolution can be influenced by a diversity of factors such as biophysical constraints on the emitter, the signalling environment, or selection to avoid heterospecific matings. Furthermore, because signals can be costly to produce, trade-offs may exist between different types of signals. Here, we apply phylogenetic comparative analyses to study the evolution of acoustic and visual signals in Asian barbets, a clade of non-Passerine, forest-dependent birds. Our results suggest that evolution of acoustic and visual signals in barbets is influenced by diverse factors, such as morphology and signalling environment, suggesting a potential effect of sensory drive. We found no trade-offs between visual and acoustic signals. Quite to the contrary, more colourful species sing significantly longer songs. Song characteristics presented distinct patterns of evolution. Song frequency diverged early on and the rate of evolution of this trait appears to be constrained by body size. On the other hand, characteristics associated with length of the song presented evidence for more recent divergence. Finally, our results indicate that there is a spatial component to the evolution of visual signals, and that visual signals are more divergent between closely related taxa than acoustic signals. Hence, visual signals in these species could play a role in speciation or reinforcement of reproductive isolation following secondary contacts.

Introduction

Animals transmit a diverse array of information through a suite of visual, acoustic, olfactory, electric and chemo-sensory signals. Evolutionary changes in such signals may result from intraspecific selection such as sexual selection, the abiotic environment, through the combination of intraspecific and environmental conditions termed sensory drive, or interspecific selection such as competition or reinforcement (Endler, 1992; Maynard Smith & Harper, 2003). Because signals can play an important role in mate choice, such different selection pressures may lead to reproductive isolation and speciation (Boughman, 2002; Slabbekoorn & Smith, 2002).

There are two basic kinds of signals, indices and handicaps. In indices, the information being transmitted is causally related to the intensity of the signal and hence dishonesty is precluded by this causal relationship. For example, in red deer (*Cervus elaphus*), body size determines the pitch of the roar and therefore this index signal reliably transmits information on the size of the emitter (see Maynard Smith & Harper, 2003). In handicaps, reliability is ensured by the elevated cost either of producing the signal itself or because emitting the signal might incur costly consequences, which preclude low-quality individuals from producing dishonest signals (see Zahavi & Zahavi, 1997; Maynard Smith &

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Harper, 2003). Handicap signals are generally assumed to transmit information about the quality of the emitter without any causal relationship between trait and signal; hence the need for elevated costs to ensure reliability. Because handicap signals represent a major energetic investment that is traded off against investment in life-history traits, allocation conflicts are predicted to be strong enough to cause negative phenotypic correlations between two handicap signals (Iwasa & Pomiankowski, 1994; Johnstone, 1996; Andersson *et al.*, 2002). This expected trade-off is at the heart of the paradox of evolution of multiple, apparently costly signals found in many species (Candolin, 2003), which led to the suggestion that such multiple signals are unlikely to be handicaps. Indeed, condition-dependent signals are rare in multi- compared to single-ornamented species (Møller & Pomiankowski, 1993). Although several studies have tested for evidence of interspecific trade-offs in investment between signals, results are mixed (see Shutler, 2011).

The environment in which the signal is produced and through which it must travel to reach the recipient may also have an important influence on signal evolution (Richards & Wiley, 1980; West-Eberhard, 1983; Endler, 1992). Physical particularities of the signalling environment can attenuate signal transmission: high temperatures might accelerate volatility of pheromones, reducing durability of the signal, and the light environment may influence reflectance or contrast of specific coloration. For example, habitat influences bird song; forest-dwelling species generally produce lower average frequency songs than open-habitat species because lower frequencies transmit better in dense vegetation (see Brown & Handford, 2000; Slabbekoorn & Smith, 2002).

A combination of environmental conditions during emission and signaller and/ or receiver morphology may also influence signal evolution, termed sensory drive (Endler, 1992). Evolution of signals in particular directions may be constrained or facilitated by characteristics of the species (Endler, 1992). For example, adaptation to divergent ecological niches can lead to signal divergence if there is selection for changes in phenotypic traits that are functionally related to signal production (Derryberry, 2009). Body size has a strong negative relationship with song frequency in many bird species (Badyaev & Leaf, 1997; Tubaro & Mahler, 1998; Bertelli & Tubaro, 2002) and as such, body size may constrain the evolution of song frequency. Beak morphology can affect the patterning of songs as demonstrated by the negative relationship found between beak size and trill rate in Darwin's finches, *Geospiza* spp. (Podos, 2001). Because beak size is under strong selection for maximizing foraging efficiency, the relationship between morphology and song structure has been proposed as evidence of ecological adaptation shaping song evolution (Podos, 1997, 2001).

Finally, interspecific interactions may also influence signal evolution (Endler, 1992; Farina *et al.*, 2011). In the presence of congeneric species, selection may favour divergence in the signals, signalling behaviour, or signal processing of one or both species (Endler, 1992). A nice example of signal divergence caused by presence of a congener is that of the song of two species of Darwin's finches (*Geospiza fortis* and *G. scandens*), which diverged following the colonization of the island by a larger, competing finch (*G. magnirostris*). Interestingly, in both species, changes were in the direction away from the acoustical space of *G. magnirostris* (Grant & Grant, 2010). Furthermore, if selection directly acts on signals to avoid hybridization, signals would be expected to diverge when closely related species occur in sympatry. This was found to be the case in antbirds, where songs were more divergent among sympatric than allopatric species in both temporal and frequency characteristics (Seddon, 2005).

Asian barbets are a monophyletic clade of non-passerine birds (Moyle, 2004) distributed from lowland forests to high mountain ranges (over 3000 m above sea level (asl)) with different degrees of sympatry among species. Barbets present visual signals in the form of conspicuous and highly variable plumage coloration on the head, face and neck, and acoustic signals, in the form of song (Short & Horne, 2001; Horne & Short, 2002). They are relatively long lived and establish long-term pair bonds; even nonsocial species are usually paired and maintain contact with a mate most of the year. All barbets are frugivorous, primary cavity nesters. They are territorial and pugnacious, often involved in aggressive interactions with members of the same species, other barbets, and other frugivorous or cavity-nesting birds (Short & Horne, 2001). Acoustic displays play an important role in territory establishment and maintenance. The dominance hierarchy among barbets is usually based on size, suggesting an important role for body size in contests for limited nesting or roosting cavities, even among species (Short & Horne, 2001). Counter singing occurs in some Asian barbets, sometimes involving several individuals with the effect of a chorus. Barbets sing both during the mating season, when the frequency of singing increases considerably, as well as outside it. The moustached barbet (*Megalaima incognita*), for example, sings for up to 9 months a year (Short & Horne, 2001). Barbets also sing a lot, and songs may be heard continuously for long periods, sometimes even through much of a moonlit night (Short & Horne, 2001). Songs comprise elements repeated numerous times in very brief intervals, for example, the brown-headed barbet (*M. zeylanica*) utters double notes with a frequency of 60 to up to 200 per minute, the blue-eared barbet's (*M. australis*) song can last more than 4 min, with 96 to 132 notes per minute. Finally, the fire-tufted barbet (*Psilopogon pyrolophus*) has a fast cicada-like trill, 3–8 s in duration

with 78–80 notes per second (Short & Horne, 2001). In a few species, females also sing and duets are incorporated into the acoustic signal repertoire, possibly playing a role in pair-bond maintenance or communication. Songs are also employed in a social context, for example, to signal dominance status in social species (Short & Horne, 2001). Acoustic displays are often intricately bound with visual displays, which involve showing off particularly conspicuous areas of the bird's head, neck or beak. Short & Horne (2001) note that patterns and markings conspicuous to human observers are those used in visual displays of barbets.

Here, we apply this theoretical framework to the study of acoustic and visual signal evolution in Asian barbets using recently developed phylogenetic comparative analyses. Little is known of the selective factors influencing signal evolution in barbets, thus our interest lay in determining whether signals functioned as handicaps or as indexes, whether signalling environment influenced signal evolution and finally, whether shifts in signals among closely related taxa might play a role in reproductive isolation. First, if signals function as indexes, we predicted there would be a significant association between body size and both acoustic and visual signals, because size is important in dominance and signals are used in territory establishment and maintenance. Alternatively, because elevated costs to emitters determine the reliability of handicap signals, if signals function as handicaps, we predicted there would be trade-offs between acoustic and visual signal characteristics. Second, if signalling environment influences signal evolution, we predicted a significant association between altitude and acoustic or visual signal characteristics, as barbets present notable variation in their altitudinal distribution. Third, if species recognition influences signal evolution, we predicted degraded phylogenetic signal of these traits, as phenotypic evolution will be influenced more by the geographical distribution of the species relative to congeners than phylogenetic relatedness. As a result, signals influencing species recognition were predicted to present more differences between closely related species than signals not involved in species recognition. In addition, we predicted that such signals will present nonnegligible spatial signal, demonstrating the influence of spatial distance among distribution ranges on trait evolution (Freckleton & Jetz, 2009). Finally, because songs are composite entities, like other complex behavioural phenotypes, they can be regarded as composites of discrete traits, arranged at different levels of organization and which vary across time (Tinbergen, 1951; Podos *et al.*, 2004b). Vocal evolution is hence best viewed as the product of evolutionary change, or stasis, in multiple, potentially independent traits (Podos *et al.*, 2004a). Therefore, we also tested whether different components of acoustic signals presented distinct patterns of evolution.

Methods

Morphology

Morphological data such as modal wing length, tarsus length and beak length; signal data such as coloration of the head, face and neck; song characteristics (see below for details); and ecological data such as minimal, modal and maximal altitude of the distribution were obtained from the primary literature (Short & Horne, 2001; Horne & Short, 2002; Collar, 2006). Geographical ranges were digitized using ArcMap (ESRI, 2009) from where data on sympatry as well as longitude and latitude of the centroid of the distribution were obtained.

Preliminary analyses showed that wing length was significantly associated with tarsus length, which has been found to be an adequate proxy for body size (Senar & Pascual, 1997), even when controlling for phylogenetic relatedness among species (PGLS: $\lambda = 1.0$, $\beta \pm SE = 1.14 \pm 0.09$, $t = 12.31$, $P < 0.0001$). Hence, given that wing length was available for a larger number of species, we used this measure as a proxy for body size in the analyses.

Song

Asian barbets have stereotyped, conspicuous songs that seem to be species specific. Because barbets are non-Passeriformes, it is assumed that they do not learn their song. Barbet songs differ among species in the degree of complexity: some species merely repeat single notes a certain number of times, whereas other species group notes into clearly distinguishable elements (henceforth referred to as syllables) that are separated by a silence – notably longer than that separating different notes – and which may be repeated. Thus, simple songs are composed of the same or few notes repeated several times, whereas more complex songs may be composed of several syllables, which may in turn be composed of different notes. For each species, we collected data on minimum, maximum and dominant frequency, note length (in seconds), syllable length and bout length as well as number of notes in a bout and number of syllables per bout. Song characteristics were measured and analysed with on-screen cursors using the program Praat v5.1.31 (Boersma & Weenink, 2010; http://www.praat.org/). Frequency and number of notes per bout were natural logarithm transformed for normality. These measures were chosen because they could be objectively measured, facilitating repeatability of the study and enabled us to capture variation in length and complexity of the songs. In total, 112 sound recordings of 29 species were downloaded from the Xeno-canto website (http://www.xeno-canto.org/asia). The only missing species was the Chinese barbet (*Megalaima faber*). The analyses are based on 42 operational taxonomic units (OTU), each of which has a different plumage

(either species, or morphologically distinct subspecies, following Horne & Short, 2002 and found to be genetically distinct den Tex & Leonard in review). Songs were missing for nine OTU and for the remaining 33 taxa, the average number of recordings was 3.39 ± 1.98. The missing taxa include the subspecies of the blue-eared barbet (*M. australis australis*) from Java whose vocalizations are well within the range of variation of the mainland and Bornean forms (Short & Horne, 2001, p 274). Also, no vocalizations of the Philippine population of the coppersmith barbet (*M. haemacephala*) were available, but there are no obvious geographical differences in song characteristics across this species (Short & Horne, 2001). Therefore, the song characteristics from other populations of these species were used in analyses.

Coloration

Most Asian barbets show striking coloration and patterning of their head, neck and face, whereas the rest of the body is mainly of greenish coloration. This green feather coloration has been shown to be of structural origin (in *M. chrysopogon*; Hill & McGraw, 2006). The striking red and yellow colours are assumed to be carotenoids, and the black, brownish and sandy colorations melanins. The blue colour is assumed to be structural as well. We assume that by scoring the head, face and neck patterns of these barbets, we capture the most important signals in interspecific communication. However, we do acknowledge that other body parts, like the red undertail feathers in the great barbet (*M. virens*) and the red-vented barbet (*M. lagrandieri*), the streaked patterning of the underparts in the coppersmith barbet and great barbet and differences in beak colouration, could also be important in signalling.

Quantification of the coloration in barbets is particularly challenging due to the great variety of colours and patterns observed across the clade. To get as unbiased of estimates as possible, three volunteers with birdwatching experience, but not involved with barbet research, were selected to perform the scoring. Each person was shown illustrations of barbet heads taken from the Handbook of the Birds of the World, volume 7 (Horne & Short, 2002) and asked to count the number of different patches and the number of different colours of each barbet head, with minimal further instructions. In total, 43 different heads were scored per volunteer. All data used in this study are available as Online Supplementary Material and in the Dryad repository (http://dx.doi.org/10.5061/dryad.kd73k).

Distribution and GIS analyses

Distribution maps of all Asian taxa were created using ArcMap (ESRI, 2009) based on species-distribution maps from the literature (Horne & Short, 2002).

The distribution map of *M. faber sini* and altitudinal information on *M. faber faber* was taken from Collar (2006). Range overlap between two taxa was calculated (in km²) from the digitalized distribution maps. The amount of sympatry between two taxa was then calculated as the area of overlap divided by the smallest total distribution area of either taxon (Fitzpatrick & Turelli, 2006). For taxa that were identified as being sympatric based on range overlap, altitudinal information of both taxa was checked to determine if they were altitudinally sympatric or segregated.

Phylogenetic comparative analyses

We used the most recent molecular phylogeny of Asian barbets available (Den Tex & Leonard in review). Den Tex and Leonard's (in review) phylogeny includes all recognized species and identifies some genetically divergent lineages, which we use as taxa (OTU) in our analyses. This phylogeny is based both on mitochondrial and nuclear sequence data, and was dated using a variety of methods.

First, we used phylogenetic principal components analyses (PPCA) (Revell, 2009) in R (R Core Development Team, 2009), using code provided by L. J. Revell, to combine correlated song measurements into a single component incorporating all the information. Because data obtained from multiple species often violate the assumption of independence of data points, ignoring phylogenetic relationships in preliminary data transformation may result in elevated variance. PPCA incorporates the expected covariance among trait values resulting from shared ancestry into the principal component analysis (Revell, 2009). The following variables were created from the combined song measures: (1) frequency, is a combination of natural log-transformed maximum, minimum and dominant frequencies. All variables loaded positively on the first component (loadings range = 0.93–0.98) and the lambda value (0.93) indicated that there was strong phylogenetic signal. (2) Length, is a combination of length of the syllable and the bout, number of notes per bout (ln transformed) and number of syllables per bout. These variables were combined into a single component, which should capture information on song length, and all variables loaded negatively on the first component (loadings range = 0.66–0.90) and the lambda value (0.99) also indicated that there was strong phylogenetic signal. One song measure, note length, was not included in the PPCAs as it was not strongly correlated with other variables. Results of all PPCA analyses are available in the Supplementary material available online.

Correlations between traits were analysed using phylogenetic generalized least squares analyses (PGLS) (Martins & Hansen, 1997). PGLS models have the advantage of incorporating different evolutionary

models (e.g. Brownian motion, Ornstein–Uhlenbeck), combining categorical and continuous traits in a single analysis and estimating an evolutionary parameter (in this case λ) simultaneously with model fit that adjusts the variance–covariance matrix to adequately fit the model of evolution, in our case, a Brownian motion model (Freckleton *et al.*, 2002; Revell, 2010). A high value of lambda (i.e. $\lambda = 1$) indicates that the covariance between the traits follows that predicted under a Brownian model, whereas values of $\lambda < 1$ indicate that the actual covariance between the traits is lower than would be expected under a Brownian model (Freckleton *et al.*, 2002). In other words, the maximum likelihood estimate of λ provides a measure of the importance of phylogenetic relationships on correlated evolution of traits. All comparative analyses were undertaken including branch lengths calculated for the molecular phylogeny. For each analysis, we systematically visually verified the phylogenetically corrected residuals to ensure that they fit model assumptions (Freckleton, 2009). We note, to avoid any confusion, that although a regression method is used for the analyses, results can only be interpreted as correlations because relationships between traits do not address the issue of evolutionary origin, and correlated changes between extant traits do not necessarily mean that one trait is responsible for changes in the other (Martins, 2000).

We also estimated phylogenetic signal for the morphological, acoustic and visual traits. Estimates of phylogenetic signal indicated whether trait evolution followed a Brownian process, where change accumulates gradually through time, or whether other processes also influenced trait evolution. Departure from a Brownian process can result from a diversity of factors (Revell *et al.*, 2008); one of these involves environmental effects (Freckleton & Jetz, 2009). Indeed, species traits are the result of a combination of phylogenetic and environmental effects. Phenotypes may be similar because they were inherited from a common ancestor or because species have been under similar selective pressures resulting from similar environmental conditions (Freckleton & Jetz, 2009). In our particular case, if species recognition plays an important role in signal evolution, we would expect to find a geographical signal in trait evolution because sympatric species, whose geographical ranges would present some degree of overlap, are expected to present more divergent signals than allopatric species pairs. Hence, signal evolution should present a nonnegligible geographical component. Using R code provided by R. Freckleton, we simultaneously estimated the phylogenetic (λ) and geographical (ϕ) components of trait evolution for visual and acoustic signals and body size (Freckleton & Jetz, 2009). We used the latitudinal and longitudinal coordinates of the centroid of species geographical range maps digitized using ArcView (ESRI, 2009) to

estimate the spatial distance of species data points to each other (Freckleton & Jetz, 2009). As an estimate of the temporal dynamic of phenotypic evolution, we calculated the value of the disparity index (DI) using the package GEIGER (Harmon *et al.*, 2008) in R (R Core Development Team, 2009). Disparity was calculated from average pairwise Euclidean distances between species. Disparity through time was calculated as the average relative disparity of each subclade by dividing the average disparity of all subclades whose ancestral lineages were present at that time by the average disparity of the clade as a whole, and repeating this at each divergence event (i.e. each node) moving up the phylogeny from root to tip. A null hypothesis was constructed by simulating phenotypic divergence of each trait along the phylogeny under an unconstrained Brownian motion model and estimating disparity through time of the simulated trait. The DI was calculated as the sum of the areas between the curve describing the phenotypic disparity of the trait and the curve describing the disparity under the null hypothesis of Brownian motion. Areas in which observed values were above expected were assigned positive values, whereas those below expected were assigned negative values (Harmon *et al.*, 2003). The DI thus describes how phenotypic disparity is partitioned along the phylogeny: values above 0 indicate that most phenotypic disparity is distributed within clades, suggesting that subclades include a large proportion of the overall disparity in the clade, negative values suggest that disparity is distributed among clades, suggesting early divergence, and values near 0 indicate that evolution has followed Brownian motion (Harmon *et al.*, 2003; Gonzalez-Voyer *et al.*, 2009; Gonzalez-Voyer & Kolm, 2011). Because we compare among traits within the same group of species, a trait with a high DI compared with another with a low DI suggests that closely related species differ more with respect to the former trait than with respect to the latter (Gonzalez-Voyer *et al.*, 2009; Gonzalez-Voyer & Kolm, 2011). We present the median value of the DI estimates obtained from comparison of the evolution of the trait of interest with 1000 simulations of trait evolution under Brownian motion and present 95% confidence intervals (CI) calculated using nonparametric bootstrapping (Gonzalez-Voyer & Kolm, 2011). Methods to calculate 95% CI generally assume that the sample statistic follows a normal distribution, and when the distribution of the sample statistic is unknown or nonnormal, it is preferable to use resampling methods to generate standard errors (Quinn & Keough, 2002). Because the distributions of the simulated parameters were generally nonnormal, we used nonparametric bootstrapping, which makes no assumptions about the distribution of the data, and a sampling frequency of 10 000 (Manly, 1997; Quinn & Keough, 2002). Finally, we estimated the maximum likelihood value of the parameter delta (δ), which indicates

whether phenotypic evolution is concentrated early in the diversification of the clade (towards the root of the phylogeny; $\delta < 1$) or whether phenotypic evolution is recent (concentrated at the tips of the phylogeny; $\delta > 1$) (Pagel, 1999). Maximum likelihood estimates of δ were obtained using the GEIGER package (Harmon *et al.*, 2008) in R.

Results

Morphology and signal evolution

Body size was significantly associated with certain song characteristics (Table 1). Specifically, body size was positively related with note length and negatively related with frequency, indicating that larger birds sing songs with longer notes and at lower frequency than smaller sized birds. Interestingly, beak length was not correlated with any song characteristic when controlling for the effects of body size ($\lambda = 0.95$, frequency: $\beta \pm SE = -0.003 \pm 0.004$, $t = -0.89$, $P = 0.38$; song length: $\beta \pm SE = -0.004 \pm 0.004$, $t = -0.88$, $P = 0.39$; note length: $\beta \pm SE = -0.020 \pm 0.243$, $t = -0.08$, $P = 0.94$; wing: $\beta \pm SE = 1.526 \pm 0.184$, $t = 8.28$, $P = 1.72 \times 10^{-08}$, $n = 29$ species). Controlling for body size in the analyses was important because beak length was significantly associated with wing length (PGLS: $\lambda = 0.91$, $\beta \pm SE = 1.45 \pm 0.16$, $t = 9.13$, $P = 1.14 \times 10^{-10}$, $n = 36$). These results suggest that the size of the beak does not influence evolution of song in Asian barbets.

The number of colours found on a bird's head, face and neck was not significantly related with body size (PGLS: $\lambda = 0.78$, $\beta \pm SE = -1.97 \pm 1.60$, $t = -1.23$, $P = 0.23$, $n = 42$). On the other hand, the number of patches on a bird's head, face and neck was significantly negatively related with body size ($\lambda = 0.92$, $\beta \pm SE = -7.10 \pm 3.17$, $t = -2.24$, $P = 0.03$, $n = 42$), indicating that small Asian barbets have more patches than larger ones, although not necessarily more colours.

Trade-offs between acoustic and visual signals

We did not find any indication of possible trade-offs between acoustic and visual signals. On the contrary,

Table 1 Phylogenetic generalized least squares linear model of the relationship between wing length and song characteristics (see Methods for details). The minimum model is shown, $n = 29$ species.

	$\beta \pm SE$	t value	P value
Lambda value = 1.0			
Intercept	4.40 ± 0.11	39.67	< 0.0001
Frequency	-0.01 ± 0.004	−2.39	0.02
Note length	0.50 ± 0.24	2.11	0.04

Table 2 Phylogenetic generalized least squares linear models of the relationship between number of patches and number of colours with song characteristics (see Methods for details). Minimum models are shown, $n = 33$ species.

	$\beta \pm SE$	t value	P value
Number of patches			
Lambda value = 0.77			
Intercept	33.65 ± 13.59	2.48	0.02
Song length	-0.20 ± 0.08	−2.36	0.03
Wing length	-6.23 ± 3.01	−2.07	0.05
Number of colours			
Lambda value = 0.63			
Intercept	11.73 ± 6.87	1.71	0.10
Song length	-0.10 ± 0.04	−2.22	0.03
Wing length	-1.81 ± 1.52	−1.19	0.24

number of patches on a bird's head, face and neck was significantly positively related with song length, but not with frequency or note length (see Table 2; note that variables loaded negatively on the length of song PC axis), even when controlling for the effect of body size by including wing length (our proxy for body size) as a covariate. Similarly, we found a significant, positive relationship between number of colours on a bird's head, face and neck and song length, but no relationship with frequency or note length (see Table 2), even when controlling for the effect of body size, although wing length was not significantly associated with number of colours.

Signalling environment and signal evolution

Signalling environment was significantly associated with song characteristics. Modal altitude was significantly positively associated with note length (PGLS: $\lambda = 1.0$, $\beta \pm SE = 3.08 \pm 1.26$, $t = 2.45$, $P = 0.02$, $n = 33$). This result suggests that birds living at higher altitudes sing longer songs than birds found at lower altitudes. Note that we did not include wing length as a covariate because we had previously found no relationship between wing length and altitude; furthermore, wing length was significantly associated with frequency and length of the note possibly leading to multicolinearity if included in the analyses. On the other hand, neither number of colours nor number of patches was significantly associated with altitude, when controlling for body-size effects (results not shown).

Species recognition and signal evolution

All of the song characteristics measured presented high phylogenetic signal. The two visual signals, number of colours and number of patches, however, presented much lower phylogenetic signal (see Table 3). None of the song characteristics showed significant departure from a Brownian motion model of evolution, when comparing the fit of a model with the λ parameter to

Table 3 Maximum likelihood estimates of the lambda (λ) and phi (ϕ) evolutionary parameters, which provide information on the degree of phylogenetic and spatial signal in the data respectively. The disparity index (DI) provides information about the temporal pattern of trait evolution (see Methods for details). DI values higher than 0 indicate that disparity is accumulated within subclades, negative values indicate that disparity is accumulated among subclades, whereas values close to 0 indicate Brownian evolution. Maximum likelihood of the delta (δ) parameter, which describes whether phenotypic evolution is concentrated towards the root ($\delta < 1$) or towards the tips ($\delta > 1$) of the tree. Asterisks highlight models which differed significantly from the basic Brownian model (results shown in the Results section). Estimates of the four parameters were obtained for each of the eight song characteristics we measured (see Methods for details).

	λ	Log Likelihood	λ/ϕ	Log Likelihood	DI	95% CI	δ	Log Likelihood
Wing length	0.99	39.49	0.99/0.0	39.8	−0.14	−0.15 to −0.13	1.34	38.29
Number of patches	0.88*	−90.76	0.87/0.01	−90.77	0.12	0.11 to 0.13	2.99*	−92.30
Number of colours	0.64*	−64.13	0.49/0.17	−63.91	0.17	0.16 to 0.18	2.99*	−73.57
Minimum frequency	1.0	−12.09	1.0/0.01	−12.04	−0.26	−0.27 to −0.25	0.24	−10.54
Maximum frequency	1.0	−1.40	1.0/0.0	−1.47	−0.28	−0.29 to −0.27	0.13*	2.73
Dominant frequency	1.0	−7.68	1.0/0.0	−7.78	−0.25	−0.26 to −0.24	0.16*	−4.40
Note length	1.0	38.75	1.0/0.0	38.67	−0.14	−0.15 to −0.13	0.64	38.88
Syllable length	1.0	−26.06	1.0/0.0	−26.13	0.04	0.03 to 0.05	2.11	−25.69
Bout length	0.95	−51.29	1.0/0.0	−74.07	0.26	0.25 to 0.27	1.70	−74.43
Notes per bout	1.0	−15.49	1.0/0.0	−109.90	0.24	0.23 to 0.25	2.41	−39.70
Syllables per bout	1.0	−41.36	1.0/0.0	−41.40	0.05	0.03 to 0.06	2.64	−40.93

the fit of the basic Brownian model. On the other hand, evolution of both visual signals differed significantly from Brownian motion (Log-likelihood ratio test: $\chi^2 = 9.32$, d.f. = 1, $P = 0.002$; $\chi^2 = 27.18$, d.f. = 1, $P < 0.0001$ respectively for patch and colour). Finally, one of the visual signals, number of colours, presented nonnegligible spatial signal in its evolution (see Table 3), as predicted under the species-recognition hypothesis. Note, however, that the maximum likelihood value of the model does not suggest that it provides a better fit to the data than a model including only the estimate of lambda (see Table 3). None of the acoustic signals presented any spatial signal in trait evolution (Table 3).

As would be expected based on the maximum likelihood estimates of lambda, most song characteristics presented very low (virtually null) DI values (Table 3). Nonetheless, there is evidence for distinct patterns of evolution for the different song characteristics we measured. For instance, frequency (minimum, maximum or dominant) showed very negative DI values, indicating early divergence followed by gradual evolution within subclades. The negative values of DI are supported by low δ values, which indicate that evolution of this trait is concentrated early on the diversification of the clade (Table 3). Note that for maximum and dominant frequency, a model with δ provides a significantly better fit to the data than a Brownian model (Log-likelihood ratio test: Chi-squared = 8.27, d.f. = 1, $P = 0.004$; and Chi-squared = 6.54, d.f. = 1, $P = 0.011$ respectively). On the other hand, length of the bout (DI = 0.29) and notes per bout (DI = 0.25) presented high DI values, possibly suggesting a means by which barbet songs differentiate between species. The maximum likelihood estimates of δ for these two traits are higher than 1, suggesting that trait evolution is

concentrated towards the tips of the tree (see Table 3). However, for these two traits, there is no significant difference between the null Brownian model and a model with δ.

Both visual signals presented relatively high DI values, indicating recent divergence in these traits. DI values for the visual signals were higher than those of most song characteristics, with the exception of length of the bout and notes per bout (see Table 3). Maximum likelihood values of δ for both visual signals (Table 3) are in accord with the high DI values, indicating that phenotypic evolution of the traits is concentrated towards the tips of the tree. Note that for both traits, the model with an estimate of δ provides a significantly better fit to the data than the null Brownian model (Log-likelihood ratio test: Chi-squared = 6.14, d.f. = 1, $P = 0.013$; and Chi-squared = 8.92, d.f. = 1, $P = 0.003$).

The number of species with which there is range overlap (range: 0–23; only five species ranges present no overlap with that of other Asian barbets) is not significantly associated with number of patches, nor number of colours (results not shown). Similarly, number of sympatric species was not significantly associated with any song characteristic (results not shown). These results suggest that increasing the number of sympatric congeners does not influence visual or acoustic signals. Interestingly, we did find a significant, negative association between number of sympatric species and minimal altitude of a species' range (PGLS: $\lambda = 0$, $\beta \pm SE = -0.61 \pm 0.22$, $t = -2.76$, $P = 0.009$, $n = 42$). The association between number of sympatric species and altitude only held for minimal altitude, as it was nonsignificant (although negative nonetheless) when using modal altitude (PGLS: $\lambda = 0.02$, $\beta \pm SE = -1.66 \pm 1.16$, $t = -1.43$, $P = 0.16$, $n = 42$), suggesting

that species specializing in high-altitude ranges, which appear to avoid lowlands, minimize range overlap with other barbet species.

Discussion

Morphology and signal evolution

Body size was significantly negatively related to song frequency, thus larger barbet species sing at a lower frequency than smaller species. Similarly, in antbirds, body size was found to be significantly related with song frequency (Seddon, 2005). Song frequency is negatively related to body size through the allometric relationship of syrinx mass. Because the minimum vocal frequencies that a bird can produce depend on syrinx mass, body size constrains song frequencies and directly influences the evolution of this trait (Ryan & Brenowitz, 1985; Podos *et al.*, 2004a). Body size was also correlated with note length, in this case positively. Note length may be constrained by body size through the effect of lung capacity influencing the amount of air individuals can expel. Price & Lanyon (2004) also found a significant association between body size and note length in oropendolas and caciques. Such morphological and physical constraints on acoustic signal production suggest that song frequency and note length in barbets are index traits, which reliably signal body size (Maynard Smith & Harper, 2003). This observation is consistent with the use of song for territorial defence and agonistic interactions in Asian barbets. Indeed, as mentioned above, larger barbet species are described as being dominant over smaller heterospecifics and within social species, song is used to signal dominant status (Short & Horne, 2001), hence the importance of a signal providing reliable information on the size of the emitter.

On the other hand, there was no significant association between beak size and song characteristics. Long beaks have been associated with low vocal frequencies, because large-volume vocal tracts are appropriate resonance filters for comparatively low source frequencies. A negative correlation between beak length and vocal frequencies was found in dendrocolaptid woodcreepers (Palacios & Tubaro, 2000). However, in corvids, the relationship was in the opposite direction, where higher emphasized frequencies were found for species with larger beaks (Laiolo & Rolando, 2003), although 'rattle-calls' of these species are nontonal and thus may not be expected to fit vocal resonance model (Podos *et al.*, 2004b). In antbirds, beak width was significantly associated with temporal features of the song; birds with broad beaks produced songs with fewer notes of longer duration repeated at low rates than those with narrow beaks (Seddon, 2005). It is possible that the absence of any relationship between beak size and song in barbets is due to limited precision in the measures used, as only

beak length was available for a large number of species. Alternatively, it is possible that there is little variation in beak size beyond allometric effects, given that all species exploit similar dietary niches (Short & Horne, 2001; Horne & Short, 2002). In support of this suggestion, we found no relationship between modal altitude of the distribution and beak length controlling for body size ($\beta \pm$ SE $= -0.02 \pm 0.03$, $t = -0.73$, $P = 0.47$, $n = 36$), suggesting that altitude does not influence beak size.

Body size was also significantly related with visual signals. Our results show that smaller birds have a higher number of patches in the head, face and neck than larger birds. Interestingly, the relationship between number of colours and body size was not significant. We suggest that smaller birds might need to increase the number of patches to increase the strength of the visual signal as more patches possibly result in higher overall contrast. It is particularly interesting that conspicuous coloration in barbets is limited to the head and neck, whereas the rest of the body is generally of a dull, green coloration (Short & Horne, 2001; Horne & Short, 2002). As barbets are hole-nesting species, it is possible that selection has favoured conspicuous coloration to evolve in the part of the body most commonly exposed. Such a reduction of the amount of conspicuous coloration may also reflect potential trade-offs with predation risk.

Trade-offs between signals

Our results suggest that rather than species trading-off investment in acoustic vs. visual signals, increased number of patches and colours on the head, face and neck of species are significantly positively related to song length. In other words, Asian barbets that invest highly in visual signals also invest highly in acoustic signals, assuming increased coloration and longer songs are costly. The colours that barbets present on the head, neck and face probably result from a combination of carotenoids for the yellow and red colours, melanin for black coloration and structural elements for the blue colour (Hill & McGraw, 2006). Melanin and carotenoids are assumed to be costly as investments, and such signals are generally found to trade-off against investments in life-history traits. For example, melanism is related to fecundity and parental care through trade-offs between investment in sexual signals and parental efforts in cardueline finches (Bokony & Liker, 2005), and carotenoids have been found to modulate both immune function and sexual attractiveness in zebra finches (Blount *et al.*, 2003). Although no direct measure of the energetic costs of singing is available for Asian, or indeed any barbet (as far as we know), the observation that these birds sing during long periods of time and often out of the mating season strongly suggest that song may be a costly signal. Indeed, there are

several indirect lines of evidence pointing towards high metabolic costs of song production in birds (Podos *et al.*, 2004a; Berg *et al.*, 2005). It is hard to imagine that uttering 70–80 elements per second, as in the fire-tufted barbet (*P. pyrolophus*), or 60–200 double notes per minute, as in the brown-headed barbet (*M. zeylanica*), is relatively cost free. Such fast trills are expected to be comparatively difficult to produce because of higher demands on vocal performance, including more rapid respiration and vocal tract movements (Podos *et al.*, 2004a). Finally, song characteristics have been found to correlate with reproductive success in the brown skua (*Catharacta antarctica lonnbergi*) a non-passerine species (Janicke *et al.*, 2008).

The presence of multiple signals is difficult to reconcile with handicaps. Theoretical studies suggest that multiple costly criteria for mate choice are unstable (Iwasa & Pomiankowski, 1994; Johnstone, 1996) and the suggestion is supported by an empirical comparative analysis, which found that condition dependence of ornaments was more common in species with single rather than multiple ornaments (Møller & Pomiankowski, 1993). Costly handicap signals are expected to be negatively correlated as a result of potential allocation conflicts stemming from the assumed elevated investment and trade-off with life-history traits (Andersson *et al.*, 2002). However, the 'multiple receiver' hypothesis allows reconciliation between the assumed costs of handicap signals with the presence of multiple costly signals (Andersson *et al.*, 2002). Whereas previous theoretical studies typically involved integrated signals that were simultaneously perceived by a single receiver, the 'multiple receiver' hypothesis proposes that multiple signals may in fact be directed at different kinds of receivers, and as such might reflect different qualities and have distinct associated costs (Andersson *et al.*, 2002). In this particular case, barbets are hole-nesting species and actively defend territories against conspecifics and other barbet species (Short & Horne, 2001; Horne & Short, 2002). It is thus possible that acoustic and visual signals in barbets transmit information intended for different receivers. For example, acoustic signals may offer information about body size, through song frequency, to competitors and serve to attract mates through song duration, which is unrelated to body size, whereas visual signals could be related with mate choice and species recognition (see below). On the other hand, a recent model indicates that multiple costly ornaments can evolve when they provide independent information to the receiver, even if assessment of signal information is costly (van Doorn & Weissing, 2004). Hence, elevated investment in acoustic and visual signals may also be explained if such signals provide independent information to, for example, potential mates.

We must note that assessing trade-offs in a comparative framework has inherent limitations. Such analyses have the underlying assumption that examined species have similar energy budgets, or in other words, that costs to the emitters are similar across species. If this is not the case, absence of interspecific trade-offs may not necessarily rule out the presence of intraspecific trade-offs. Furthermore, a recent study suggests that resource distribution among species may also influence the observed phenotypic correlations among costly signals. Simulations indicate that when resources are normally distributed among species, the predicted trade-off is observed; however, when resources are distributed in a negative-binomial fashion, positive correlations among traits were observed (i.e. no trade-offs) (Shutler, 2011). In the particular case of barbets, resource distribution may vary among species based on body size, because dominance relationships among species are dependent on size (Short & Horne, 2001). Variation in resource distribution among species based on body size may explain the absence of the predicted trade-off between costly signals if such differences in turn affect the costs of signal emission. At present, the lack of empirical distributions of resource distribution among species make it difficult to make generalizations about the expected likelihood of trade-offs (Shutler, 2011).

Signalling environment and signal evolution

We found a significant effect of altitude on acoustic signal characteristics, as predicted. Barbet species living at higher altitudes sang songs with longer notes than species living in lowlands. It is possible that highland and lowland habitats differ in characteristics of the vegetation such as density, humidity or temperature, which in turn influence signal attenuation and thus song evolution in these species (Endler, 1992). Previous studies have found differences in song characteristics between species living in different forest habitats and grasslands (Ryan & Brenowitz, 1985; Tobias *et al.*, 2010), and in different wetland habitats (Ballentine, 2006). Antbirds in the densely vegetated forest understory and canopy sing at lower frequencies than close relatives of the more open midstory (Seddon, 2005), and green hylia (*Hylia prasina*) males sang at lower frequency at higher elevation and at lower tree cover levels (Kirschel *et al.*, 2009). Our results suggest that sensory drive could possibly explain acoustic signal evolution in Asian barbets (Endler, 1992).

Species recognition and signal evolution

The results of the rate of evolution analyses in combination with the spatial signal analyses provide independent lines of evidence suggesting a potential role of visual signals in species recognition and hybridization avoidance. Whereas body size and certain song characteristics presented strong phylogenetic signal, indicating evolution of the traits followed Brownian motion

where divergence accumulates gradually with time, visual signals (number of patches and number of colours) presented low phylogenetic signal. These results suggest that sister species are more divergent in visual signals than acoustic signals, and also that visual signals accumulate more change than acoustic signals for a given amount of time; in other words, they evolve faster. The higher values of DI for the two visual signals in comparison with most of the measured characteristics of the acoustic signal, with the exception of length of the bout and notes per bout, also suggest that there is a higher degree of divergence in visual than acoustic signals among closely related species. This suggestion is supported by the high δ value for both visual traits (see Table 3), suggesting that phenotypic evolution is concentrated towards the tips of the tree, or in other words, recent divergence. Note that we cannot rule out that the lower phylogenetic signal and higher DI values for both visual signals are influenced by how variation in the traits was quantified. Both are continuous traits. The song component 'syllables per bout' is a continuous trait with discrete variation that nonetheless presents a high lambda and low DI value. Only visual signals, namely the number of colours, presented nonnegligible spatial signal, indicating that distance among distribution ranges of the species have an influence on trait evolution (also see, Freckleton & Jetz, 2009). Note, however, that the likelihood value of the model including the spatial evolutionary component does not suggest that the model provides a better fit for the data than a model including only the phylogenetic component (see Table 3). Taken together, the evidence from these results does follow our initial predictions: phylogenetic signal was predicted to erode in traits that play an important role in species recognition because, under such circumstances, the spatial distribution of species would be expected to have a stronger influence in evolution of these traits than phylogenetic relatedness. This prediction has the underlying assumption that allopatric speciation in the study group is more common than sympatric speciation, which is the general case in birds (Phillimore *et al.*, 2008). We also predicted that spatial signal should be detected in traits which play a role in species recognition, because the species recognition hypothesis predicts divergence of signals when species ranges overlap (i.e. are in sympatry) to avoid hybridization. In antbirds, loud songs are more divergent when compared among sympatric species than when compared among allopatric ones (Seddon, 2005).

Rate of evolution analyses suggest that different song components present distinct patterns of evolution. Strong phylogenetic signal in song characteristics might have been expected given that barbets are not thought to learn their songs, which possibly reduces the potential for evolutionary lability. However, our results paint a more complex picture, suggesting that while certain components are constrained in their rate of evolution by other phenotypic traits, as is the case for song frequency, others may be more labile, as is the case for bout length and notes per bout. The three measures of song frequency (minimum, dominant and maximum) presented the most negative DI values of all traits, indicating early divergence. Body size, which correlated negatively with frequency, also showed a negative DI value, as did note length, the other song trait significantly correlated with body size. Together, these results suggest that body size constrains the evolution of these traits and hence the temporal pattern of evolution follows that of body size (see Figure S1 a, d, e, f, g in Online Supplementary Material).

On the other hand, the high disparity indices for bout length and notes per bout suggest that these are possible avenues by which songs are modified more easily and rapidly. These are the only song characteristics that presented disparity indices comparable to those of the visual traits (see Table 3). Recently, Weir & Wheatcroft (2011) found that rates of evolution of song varied with latitude across species. In accord with our results, they found that while rates of evolution of syllable diversity varied with latitude in oscines only, the rate of evolution of song length varied with latitude in oscines and suboscines. The high disparity indexes for bout length and notes per bout apparently result from a peak in relative disparity occurring roughly halfway up the phylogeny (see disparity through time plots available as Supplementary Online Material). Indeed, most notable differences between species in both traits are concentrated in two clades sharing a common ancestor at this point. Interestingly, denTex and Leonard (in review) found signal for a potential shift in the rate of diversification (due to a decrease in the rate of extinction) in their reconstructed phylogeny that coincides with the location of the increase in relative disparity found here. The subclade stemming from the inferred rate shift differs from the rest of the tree in that it has a more widespread and continental distribution, notably the species from this subclade have colonized the entire mainland distribution of the genus, from India, through the Himalayas to China and throughout Indochina (denTex and Leonard in review). The high δ values for these traits also support the suggested recent divergence, however, there was no statistical difference between the basic Brownian model and a model with δ. The apparent discordance between the models used to analyse rate of phenotypic evolution probably results from the way the models function. Models with an evolutionary parameter such as λ or δ, estimate the maximum likelihood value of the parameter that best describes evolution of the trait throughout the tree (Pagel, 1999). On the other hand, the DI analyses the temporal (from root to tip of the phylogeny) distribution of differences among species trait values (Harmon *et al.*, 2003). As such, if a particular clade of the

tree presents a distinct rate of phenotypic evolution, models with λ or δ will provide an average rate fitting the entire tree best, while the DI value may be more sensitive to changes limited to a specific subclade. Recent developments in phylogenetic comparative methods allow more detailed analyses of rates of evolution among traits, including detection of changes in the rate of phenotypic evolution, and present an exciting avenue for future research into signal evolution (e. g. O'Meara *et al.*, 2006; Thomas *et al.*, 2006; Revell *et al.*, 2011). However, the limited number of Asian barbet species for which information was available did not allow us to apply such methods in this study.

There was no significant correlation between number of sympatric species and number of patches, number of colours or any of the song characteristics we measured. This result, however, does not undermine our suggestion that visual signals possibly play an important role in species recognition as we did not expect the number of patches or colours to increase with number of sympatric species, rather we had predicted that sympatry would favour divergence of signals. Interestingly, we did find that species apparently specializing in highland habitats present a decrease in the number of species with which the range overlaps. In other words, it appears as though in Asian barbets, one way to avoid range overlap and potentially competition from congeners is to specialize on living in highlands.

In conclusion, our results show that evolution of signals in barbets is influenced by diverse factors, such as morphology, signalling environment and species recognition, as has been found for other species (reviewed in Endler, 1992). Interestingly, acoustic and visual signals did not present evidence for phenotypic trade-offs; on the contrary, species with more patches and more colours on the head, face and neck also sang longer songs, suggesting that high investment in visual signals is accompanied by elevated investment in acoustic signals. It remains to be investigated whether this results from signals aimed at different types of receivers (multiple receiver hypothesis; Andersson *et al.*, 2002) or whether the two types of signal provide independent information to a single receiver (van Doorn & Weissing, 2004). Our results also suggest that song characteristics present different patterns of evolution, in accord with the view that vocal evolution is best viewed as the product of evolutionary change in multiple, potentially independent traits (Podos *et al.*, 2004a). Finally, our results provide different lines of evidence suggesting that visual signals may play a role in species recognition. An interesting avenue for future research would be to investigate the extent to which mate choice is influenced by visual signals and the acuity of discrimination among different species with similar colouration. Our study demonstrates that phylogenetic comparative analyses combined with a solid theoreti-cal framework can be used to gain valuable insight into the selective factors having influenced signal evolution.

Acknowledgements

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Morphological and ecological data for the 42 species included in the analyses.

Table S2 Song data, and longitude and latitude of the centroid of the geographical distribution for the 42 species included in the analyses.

Table S3 Song data for the 42 species included in the analyses.

Figure S1 Disparity through time plots for wing length (our proxy for body size), visual and acoustic signals.