Interspecific competition affects evolutionary links between cavity nesting, migration and clutch size in Old World flycatchers (Muscicapdae)

SAHAS BARVE[1,2,*] & NICHOLAS A. MASON[1,2]

[1]*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853-2701, USA*

[2]*Fuller Evolutionary Biology Program, Cornell Lab. of Ornithology, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA*

The ecology of cavity nesting in passerine birds has been studied extensively, yet there are no phylogenetic comparative studies that quantify differences in life history traits between cavity- and open-nesting birds within a passerine family. We test existing hypotheses regarding the evolutionary significance of cavity nesting in the Old World flycatchers (Muscicapidae). We used a multi-locus phylogeny of 252 species to reconstruct the evolutionary history of cavity nesting and to quantify correlations between nest types and life history traits. Within a phylogenetic generalized linear model framework, we found that cavity-nesting species are larger than open-nesting species and that maximum clutch sizes are larger in cavity-nesting lineages. In addition to differences in life history traits between nest types, species that breed at higher latitudes have larger average and maximum clutch sizes and begin to breed later in the year. Gains and losses of migratory behaviour have occurred far more often in cavity-nesting lineages than in open-nesting taxa, suggesting that cavity nesting may have played a crucial role in the evolution of migratory behaviour. These findings identify important macro-evolutionary links between the evolution of cavity nesting, clutch size, interspecific competition and migratory behaviour in a large clade of Old World songbirds.

Keywords: body size, cavity nesting, clutch size, life history traits, migration.

Passerine birds show remarkable variation in the structure and location of their nests. Nests provide protection from predators and shield young from inclement weather, thereby allowing for significant periods of ontogenetic development. Nesting biology is under multiple selective pressures, and the relative benefit of different strategies is closely linked with other life history traits (Martin 1995). Here, we investigate associations between the evolution of cavity nesting, clutch size, body size, developmental periods and migratory behaviour in a large family of passerine birds.

Avian nests can be divided into two broad categories: open nests and cavity nests (Collias 1997). Over half of all passerine families contain cavity-nesting species (Collias 1964), yet very few species excavate nest cavities; instead, most species are cavity adopters that nest in natural cavities or cavities excavated by other species (Collias 1964). Cavity nests seem to confer certain advantages over open nests, such as lower predation risk (Nice 1957, Alerstam & Hogstedt 1981) and a more stable and favourable microclimate (Martin & Ghalambor 1999, Rhodes *et al.* 2009, Coombs *et al.* 2010). However, suitable nesting cavities can be a limiting resource in temperate (von Haartman 1957) and tropical regions (Cockle *et al.* 2010), leading to intraspecific and interspecific competition among cavity adopters. Together, these selective pressures may result in predictable differences in other life history traits between cavity- and open-nesting lineages.

One consistent life history difference between nest types is that cavity adopters generally have

*Corresponding author.
Email: ssb235@cornell.edu
The two authors provided equal contributions to this paper.

larger clutches of eggs than open-nesting birds (Lack 1947). There are two non-mutually exclusive hypotheses to explain this pattern. First, cavity nests are exposed to lower predation pressure, favouring a larger clutch size (Martin 1993) and longer developmental periods (Martin & Li 1992). Secondly, competition for cavities leads to larger clutch sizes in cavity adopters, as individuals try to maximize their reproductive output at every breeding opportunity (Martin 1993). In other cavity-nesting passerines such as tits (Paridae) and nuthatches (Sittidae), cavity excavation seems to be a derived trait among smaller species, as they are outcompeted by larger, sympatric species for nesting cavities (Dhondt 2007). Hence, if nesting cavities are a limiting factor and species do not have the necessary morphology to extract cavities, cavity-nesting species should be larger than open-nesting species.

Life history traits and reproductive strategies are influenced by multiple selective pressures simultaneously. For example, an increase in clutch size is seen not only in cavity-nesting birds, but also in birds breeding at high latitudes and in migratory birds (Lack 1947, Jetz et al. 2008). In extremely seasonal high-latitude habitats, the breeding season is short, so the opportunity to re-nest is limited. If cavity nests provide the advantage of a favourable microclimate and lower predation risk, then cavity nesting should be more common in migratory lineages and in taxa breeding at higher latitudes (Roper et al. 2010). Studying the evolutionary interplay between these life history traits is most effective within lineages that exhibit variation in these traits among species. A lineage that demonstrates remarkable diversity in migratory behaviour, nest types and other life history traits is the Old World flycatchers (Muscicapidae), a large family of small- to medium-sized insectivorous birds. The group includes both cavity-adopting and open-nesting taxa that breed in diverse habitats throughout Europe, Asia and Africa. Recent advances in comparative phylogenetic methods have produced a robust statistical framework to test for correlated evolution between traits among taxa (Freckleton et al. 2002). When combined with known variation in muscicapid nesting behaviour, these methodological advances allow us to address long-standing questions about links between nesting behaviour and life history evolution. Using the most taxonomically comprehensive family-level muscicapid phylogeny to date,

we test the following predictions: (1) if cavity nesting affords lower predation risk, then cavity-nesting lineages should have larger clutch sizes and longer developmental periods; (2) if competition for cavities is a limiting factor, then cavity-nesting lineages should have larger clutches and should be larger in size than open-nesting species; and (3) cavity nesting should be more common in species breeding at high latitudes and in migratory species with associations between cavity nesting, latitude and bioclimatic variables of the breeding range of the species or migratory behaviour.

METHODS

Study system

A substantial amount of natural history information is documented for many species in Muscicapidae (Collar 2005, Taylor & Peter 2006). Sangster et al. (2010) and Zuccon and Ericson (2010) provide numerous taxonomic revisions to resolve multiple paraphyletic groupings and added many species to this family from its sister taxon, the thrushes (Turdidae). However, these publications used a non-overlapping set of genes and taxa for their analyses. Moreover, genetic data for more species have become available and molecular sampling for many taxa has also improved (e.g. Aliabadian et al. 2012, Oliveros et al. 2012). Incorporating these recent advances into a single phylogeny takes us closer to a more complete and accurate understanding of the evolutionary relationships within this family.

Phylogenetic inference

Our ability to study the evolution of life history traits in Old World flycatchers relies upon a robust phylogeny for the taxa at hand. We searched GenBank for nucleotide sequence data for all muscicapids by following the taxonomy of Clements et al. (2013) and downloaded sequences from all available taxa for 11 molecular markers (Supporting Information Table S1); the resulting concatenated data set included 252 taxa, approximately 82% of the 307 species currently recognized by Clements et al. (2013). Alignments were performed with MAFFT v7.029b (Katoh et al. 2002, Katoh & Standley 2013) and were subsequently checked by eye.

We performed phylogenetic inference within a Bayesian framework; we ran three independent

BEAST v1.7.5 (Drummond & Rambaut 2007, Drummond *et al.* 2012) runs for 4×10^7 generations each and then combined runs to generate the posterior distribution of post burn-in phylogenies and a maximum clade credibility (MCC) tree. To obtain information about the timing of branching events, we used an uncorrelated lognormal relaxed clock that was unlinked across all markers (Drummond *et al.* 2006). To calibrate our molecular clock, we used evolutionary rates and standard errors reported by Lerner *et al.* (2011), which are based on estimations of molecular divergence between species in the Hawaiian honeycreeper radiation and known ages of the Hawaiian islands (Table S1). Although Hawaiian honeycreepers are only distantly related to Old World flycatchers and rates of molecular evolution can vary substantially among lineages, these calibrations have provided reasonable estimates of divergence times in other distantly related taxa (Voelker *et al.* 2013, Mason *et al.* 2014).

To examine whether enforcing a molecular clock influenced topological relationships, we also used MRBAYES v3.2.1 (Ronquist & Huelsenbeck 2003) to infer a posterior distribution of phylogenies. We partitioned each locus separately and applied the best fit model of evolution via JMODELTEST v0.1.1 (Posada 2008). We compared Robinson–Fould distances (Steel & Penny 1993) among trees from the posterior distributions of the MRBAYES and BEAST inferences to assess topological congruence among phylogenetic methods (see Supporting Information Method S1 for additional methodological details).

Life history data

For each taxon in the Muscicapidae, we used the *Handbook of the Birds of the World* (Collar 2005, Taylor & Peter 2006) to obtain information on the following characters: body length (cm), body mass (g), migratory status, nest type, average clutch size, maximum clutch size, incubation period, nestling period and total nesting period. Here, we follow Zyskowski and Prum (1999) in defining cavity nests as 'nests placed in an adopted or self-excavated cavity, such as a subterranean burrow, rock crevice, termite mound, tree cavity, bamboo internode, cavity in a man-made structure'. Many muscicapids, such as species in the genera *Phoenicurus*, *Oenanthe* and *Monticola*, are highly variable in their nest type. In natural habitats, many use

crevices and niches in rocks. These taxa are also well-known for using unusual nest-sites such as mail-boxes, old tyres and unused pipes. Most importantly, they readily take up nestboxes when available, suggesting that these groups are facultative cavity nesters (Collar 2005, Taylor & Peter 2006). Because of their high propensity to use cavities, we classified these species as cavity nesting in our comparative analyses. We also repeated the same analyses with these species categorized as open nesting (for the entire data matrix and sensitivity analyses to how data were coded, see Supporting Information). Similarly, migratory behaviour can also vary within species. For the purposes of our macroevolutionary study, we considered taxa as cavity nesters or migratory if any population exhibits these behaviours. However, as above, we also evaluated the robustness of our inferences to these classifications by repeating each comparative analysis with variable or ambiguous taxa classified as open nesting and non-migratory (Supporting Information).

Comparative analyses

Using the MCC tree, we implemented stochastic character mapping (Bollback 2006) to reconstruct the evolutionary history of nest type and migratory behaviour across 210 species within a Bayesian framework. We omitted 42 taxa because we had no information on migratory status or nesting type. This method calculates the conditional likelihood of each character state at each node, and then samples the posterior distribution of states to simulate transitions between character states along each branch. Therefore, character state transitions can occur at any point along a branch and are not restricted to nodes, reflecting gains and losses of a given character without invoking speciation events. We used a symmetrical model of transition rates and summarized the results of 100 simulated reconstructions via the package phytools (Revell 2011) within the R v3.0.2 programming environment (R Core Development Team 2013).

To assess whether life history traits are correlated with cavity-nesting behaviour, we ran phylogenetic generalized least squares (PGLS; Grafen 1989, Hansen & Martins 1996, Martins & Hansen 1997) analyses with cavity nesting as the independent variable and eight different life history traits as dependent variables: average clutch size, maximum clutch size, incubation period, nestling

period, total nesting period, body length, body mass and month of first breeding. Because many of the life history traits considered here are correlated with distance from the equator (Cardillo 2002), we also included latitude as both a main and an interaction effect in each model. We assigned a single latitude value to each species by taking the midpoint of occurrence records downloaded from GBIF (http://data.gbif.org/) with the R package dismo (Hijmans *et al.* 2005). For each PGLS analysis, we considered two different models of character evolution: Brownian motion (BM; Harvey & Pagel 1991) and Pagel's lambda (PG; Pagel 1999). We tested both models of character evolution across a posterior distribution of 100 randomly sampled post burn-in phylogenies to account for uncertainty in topology and branch-length estimations. We used corrected Akaike information criterion (AIC_C) scores to identify the favoured model of character evolution and averaged PGLS parameters across phylogenies from the posterior distribution. To make model residuals conform to a normal distribution under the best-fit model of evolution, we log transformed body size and maximum clutch size.

To test whether abiotic conditions are associated with different nest types in Old World flycatchers, we first downloaded distributional shapefiles from BirdLife International, which we cleaned to include only the breeding range. Then we downloaded occurrence records for each species from GBIF with the R package dismo (Hijmans *et al.* 2005). For each species, we searched for all possible taxonomic synonyms identified by the Encyclopedia of Life (http://www.eol.org). We extracted 10 bioclimatic variables that describe thermal conditions (Bioclim 1–10) associated with the occurrence records of each species and calculated mean values across occurrence records. Using these climatic data, we performed a phylogenetic principal component analysis (PPCA; Revell 2009). We then used the first principal component axis, which accounted for 99% of the total variation (Supporting Information Fig. S1), in subsequent comparative analyses. The loadings on this axis indicate that higher scores correspond to colder, more seasonal environments. We tested for an association between PC1 and the evolution of cavity nesting using the MCC tree in a phylogenetic logistic regression (PLR) as described in Ives and Garland (2010). We ran a PLR with cavity nesting as the dependent variable and bioclimatic PC1 scores as

the independent variable across 188 species for which these data exist. To obtain confidence intervals in our parameter estimates, we performed 2000 iterations of parametric bootstrapping.

To assess whether the evolution of cavity nesting and migration are correlated, we implemented BAYESTRAITS, a reversible-jump Markov Chain Monte Carlo Bayesian analysis described in Pagel and Meade (2006), across 210 species of Old World flycatchers that have known migratory/non-migratory and cavity/open-nesting behaviours. In brief, this method implements a Bayesian chain that visits dependent and independent models of binary character evolution in proportion to their posterior probability. Using the MCC tree, we ran separate MCMC analyses for independent and dependent models of character evolution with 3×10^7 generations and discarded the first 1×10^6 generations as burn-in. We ran four independent and dependent Markov chains each, which converged upon similar log-likelihood values. We calculated mean transition rates between each character state and compared the relative performance of the dependent and independent models via Bayes factors.

RESULTS

Phylogenetic inference

The topologies inferred by BEAST (Fig. 1) and MRBAYES (Supporting Information Fig. S3) were largely congruent and Robinson–Fould distance indices overlapped between the two posterior distributions of trees (AIC_c, Supporting Information Fig. S4). For simplicity, we restrict our discussion to the posterior distribution of BEAST phylogenies. We inferred a monophyletic Muscicapidae with strong support (Fig. 1; posterior probability (PP) = 0.99). Our molecular clock estimates that the most recent common ancestor of all Muscicapidae occurred 20.78 Mya (95% highest posterior density: 19.69–21.78 Mya). Within Muscicapidae, we recovered the same four subfamilies identified by Sangster *et al.* (2010): (1) Muscicapinae (PP = 0.99); (2) Niltavinae (PP = 0.97); (3) Erithacinae (PP = 0.91); (4) Saxicolinae (PP = 0.97). Evolutionary relationships within these subfamilies were largely congruent with recently published molecular phylogenies of various groups within Muscicapidae (Beresford & Cracraft 1999, Roy 2001,
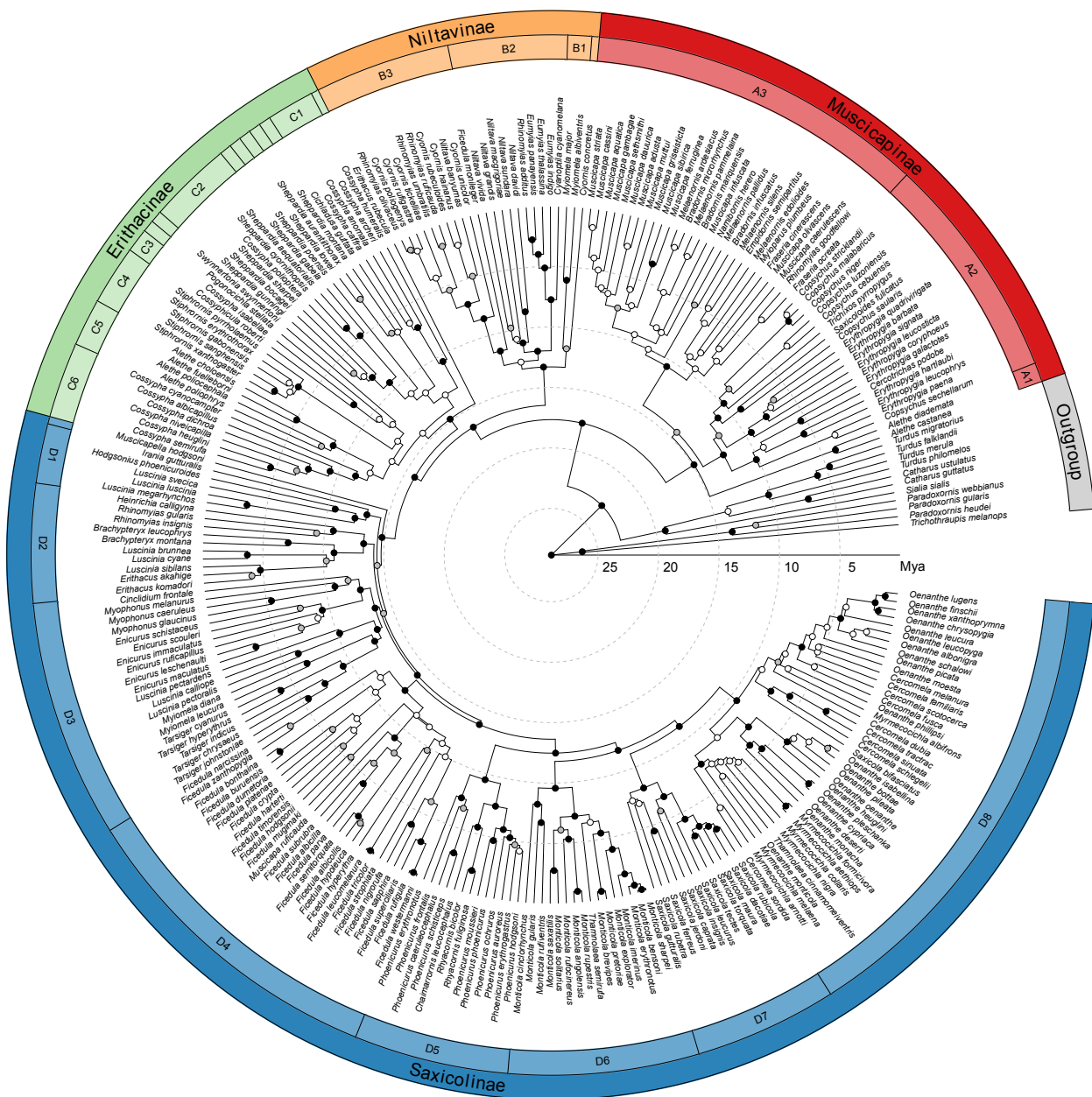
Figure 1. Maximum clade credibility tree of 252 ingroup taxa and 11 outgroup taxa inferred using BEAST. Node support is indicated by colour, wherein black, grey and white nodes reflect strong (PP ≥ 0.95), moderate (0.70 ≤ PP < 0.95) and weak (PP < 70) support, respectively. The outer ring indicates subfamilies and the inner ring designates clades that are discussed in the Supporting Information. Dotted concentric rings correspond to 5-Myr year intervals in divergence timing estimates, which are shown on the horizontal axis.

Dietzen *et al*. 2003, Cibois & Cracraft 2004, Voelker & Spellman 2004, Ertan 2006, Qiao-Wa *et al*. 2006, Seki 2006, Outlaw *et al*. 2007, 2010, Xin *et al*. 2007, Illera *et al*. 2008, Schmidt *et al*. 2008, Treplin *et al*. 2008, Sheldon *et al*. 2009, Zink *et al*. 2009, Sangster *et al*. 2010, Voelker 2010, Zuccon & Ericson 2010, Aliabadian *et al*.

2012, Oliveros *et al*. 2012). A comprehensive analysis of the topology inferred here is outside the scope of the current study; however, we provide a brief discussion of confirmed relationships and novel insights that we have generated from our increased taxonomic and molecular sampling (Supporting Information).

(a) □ Cavity nest
■ Open nest

(b) □ Migratory
■ Non−migratory

Comparative analyses

Stochastic character mapping indicated that the ancestor of all muscicapids was likely to have been non-migratory ($P = 1$) and nested in cavities ($P = 0.85$; Fig. 2). Thus, open nests and migration are both derived characters that had been gained and lost multiple times throughout the evolutionary history of Muscicapidae. Moreover, the evolution of cavity nesting generally preceded changes in migratory status. The reconstruction of migratory behaviour at the root of Muscicapidae was robust to different classifications of ambiguous or geographically variable taxa (Supporting Information Fig. S5). However, it is important to note that the inferred root character state of nesting type in muscicapids is dependent upon how ambiguous or variable taxa are classified. If taxa that are variable or ambiguous were classified as open nesters rather than cavity nesters, then the ancestral state of the common ancestor of all muscicapids was likely to have been an open-nesting species ($P = 0.98$; Supporting Information Fig. S6).

Cavity nesting was correlated with an increase in maximum clutch size ($\lambda = 0.54$, $\beta = 0.13 \pm 0.05$, $P = 0.02$; Table 1). We also uncovered a significant main effect of cavity nesting on body size; cavity nesters tended to be larger than open nesters ($\lambda = 0.95$, $\beta = 0.0813 \pm 0.03$, $P = 0.01$; Table 1). Incubation period, nestling period, total nesting period, body size and month of first breeding did not differ between cavity- and non-cavity-nesting species (Table 1). Latitude was correlated with multiple life history traits. Average clutch sizes were larger at higher latitudes ($\lambda = 0.55$, $\beta = 0.028 \pm 0.005$, $P < 0.001$; Table 1) and a similar trend was observed for maximum clutch sizes ($\lambda = 0.54$, $\beta = 0.009 \pm 0.002$, $P < 0.001$; Table 1). Finally, latitude was correlated with the month of first breeding, such that species at higher latitudes bred later in the season ($\lambda = 0.37$, $\beta = 0.0212 \pm 0.008$, $P = 0.01$; Table 1). These results were sensitive to how ambiguous or geographically variable taxa were coded for nesting type. Specifically, if taxa that were variable or ambiguous were treated as open nesters rather than cavity nesters, then cavity nesters no longer had larger maximum clutch sizes or larger bodies (Supporting Information Table S3). However, the effects of latitude on the aforementioned life history traits were robust to different classifications of ambiguous or variable taxa with respect to cavity nesting (Table S3).

We found no correlation between abiotic conditions and the evolution of cavity nesting via phylogenetic logistic regression (Table 2). In other words, lineages that breed in colder and more seasonally variable environments were no more likely to evolve cavity nesting compared with those in warmer, less seasonal habitat. This result was robust to parametric bootstrapping.

Our BAYESTRAITS analysis demonstrated that the rate at which muscicapids gain or lose migratory behaviour was strongly dependent on the presence or absence of cavity nesting. More specifically, changes in migratory behaviour were far more common in cavity-nesting than open-nesting lineages (Fig. 3). Moreover, the evolution of a migratory, open-nesting state was primarily the result of a loss of cavity nesting among migratory lineages, rather than the gain of migratory behaviour among open-nesting lineages. This was evidenced by support for a dependent evolutionary model of binary character state transitions between migratory status and cavity nesting, which performed better than an independent model (Bayes factor = 7.88, Kass & Raftery 1995). The interdependency of state changes between migratory behaviour and nesting type was robust to different classifications of taxa that were ambiguous or variable in their migration or nesting type. If taxa that were ambiguous or variable were given an alternative classification for migratory behaviour, a dependent model was still preferred over an independent model (Bayes factor = 5.51). Similarly, with the alternative classification for cavity nesting, a dependent model was also preferred over an

---

Figure 2. Stochastic character mapping of (a) cavity nesting and (b) migratory behaviour across 210 species of Old World flycatchers using the maximum clade credibiity tree. Character state transitions are allowed to occur along branches and pie charts at each node represent the likelihood of each character state (black = open nesting, non-migratory; grey = cavity nesting, migratory) from the ancestral state reconstruction across 100 trees taken from the posterior distribution of possible phylogenies using a symmetrical model of character state transitions.

Table 1. Effects of cavity nesting on various life history traits as inferred through phylogenetic generalized least squares (PGLS) analyses. The number of species included in each model is indicated by n, while $dAIC_c$ indicates the how Pagel's λ model performs compared with a Brownian motion model of character evolution. Effect sizes and directionality (β) are with respect to cavity nesting. In other words, positive β-values indicate an increase in a given life history trait value among cavity nesting lineages.

Character	n	$dAIC_c$	λ	Effect	$\beta \pm se$	P
Clutch size avg.	181	21.95	0.55	Cavity	0.2007 ± 0.1371	0.147
				Latitude	0.0288 ± 0.0045	<0.001*
				Cavity : latitude	0.0033 ± 0.005	0.508
ln(Clutch size max.)	181	29.32	0.54	Cavity	0.1261 ± 0.0543	0.022*
				Latitude	0.0087 ± 0.0018	<0.001*
				Cavity : latitude	$3e{-}04 \pm 0.002$	0.859
Incubation period	90	10.45	0.66	Cavity	0.2542 ± 0.4184	0.552
				Latitude	-0.0222 ± 0.0113	0.058
				Cavity : latitude	0.0196 ± 0.0128	0.135
Nestling period	81	3.34	0.82	Cavity	0.1748 ± 0.5897	0.756
				Latitude	0.0058 ± 0.0191	0.764
				Cavity : latitude	-0.0281 ± 0.0204	0.176
Total nesting period	75	0.48	1	Cavity	1.0061 ± 0.9259	0.500
				Latitude	0.0114 ± 0.0303	0.725
				Cavity : latitude	-0.0277 ± 0.0318	0.564
ln(Size)	183	5.58	0.95	Cavity	0.0813 ± 0.0294	0.010*
				Latitude	$1e{-}04 \pm 0.001$	0.761
				Cavity : latitude	$-4e{-}04 \pm 0.0011$	0.689
ln(Mass)	152	3.58	0.97	Cavity	0.104 ± 0.0669	0.194
				Latitude	$-9e{-}04 \pm 0.0022$	0.602
				Cavity : latitude	$-1e{-}04 \pm 0.0023$	0.648
Month	181	28.86	0.37	Cavity	0.5024 ± 0.2579	0.053
				Latitude	0.0212 ± 0.0084	0.012*
				Cavity : latitude	-0.0136 ± 0.0094	0.151

Significant P values ($\alpha = 0.05$) are indicated with an asterisk.

Table 2. Results of phylogenetic logistic regression estimates with Firth correction for the effect of abiotic conditions (lower PC1 values indicate warmer, less seasonal habitat) on the evolution of cavity nesting (0 = non-cavity, 1 = cavity) in Old World flycatchers ($n = 188$). Thus, positive estimates of b_1 would suggest that the evolution of cavity nesting is associated with colder, more seasonal environments.

| Parameter	Estimate	se	t score	Bootstrapped mean	Bootstrapped confidence interval	Bootstrapped P-value
a	−0.62	–	–	−0.71	(−3.28, 0.69)	0.02
b_0 (intercept)	−1.20	0.48	−2.50	−1.08	(−1.92, −0.15)	0.01
b_1 (abiotic conditions)	0.18	0.18	1.00	0.18	(−0.18, 0.57)	0.30

independent model (Bayes factor = 17.75). However, certain transitions were consistently less frequent among different classification schemes. In particular, character state changes in migratory behaviour among open-nesting lineages were consistently less frequent than changes in migratory behaviour among cavity-nesting lineages (Supporting Information Figs S7 and S8).

DISCUSSION

The evolution of cavity nesting is correlated with multiple life history traits in Old World flycatchers. Our phylogenetic comparative study suggests a role for interspecific competition in the evolution of cavity nesting and demonstrates that cavity-nesting lineages have experienced more gains and losses of migratory behaviour during their evolutionary history than open-nesting lineages. Old World flycatchers showed exceptional evolutionary lability in nest type. This lability might have been crucial in acquiring the ability to migrate and colonize new areas, which may have led to further diversification. Previous studies concerning the effects of cavity nesting on life history traits have largely focused
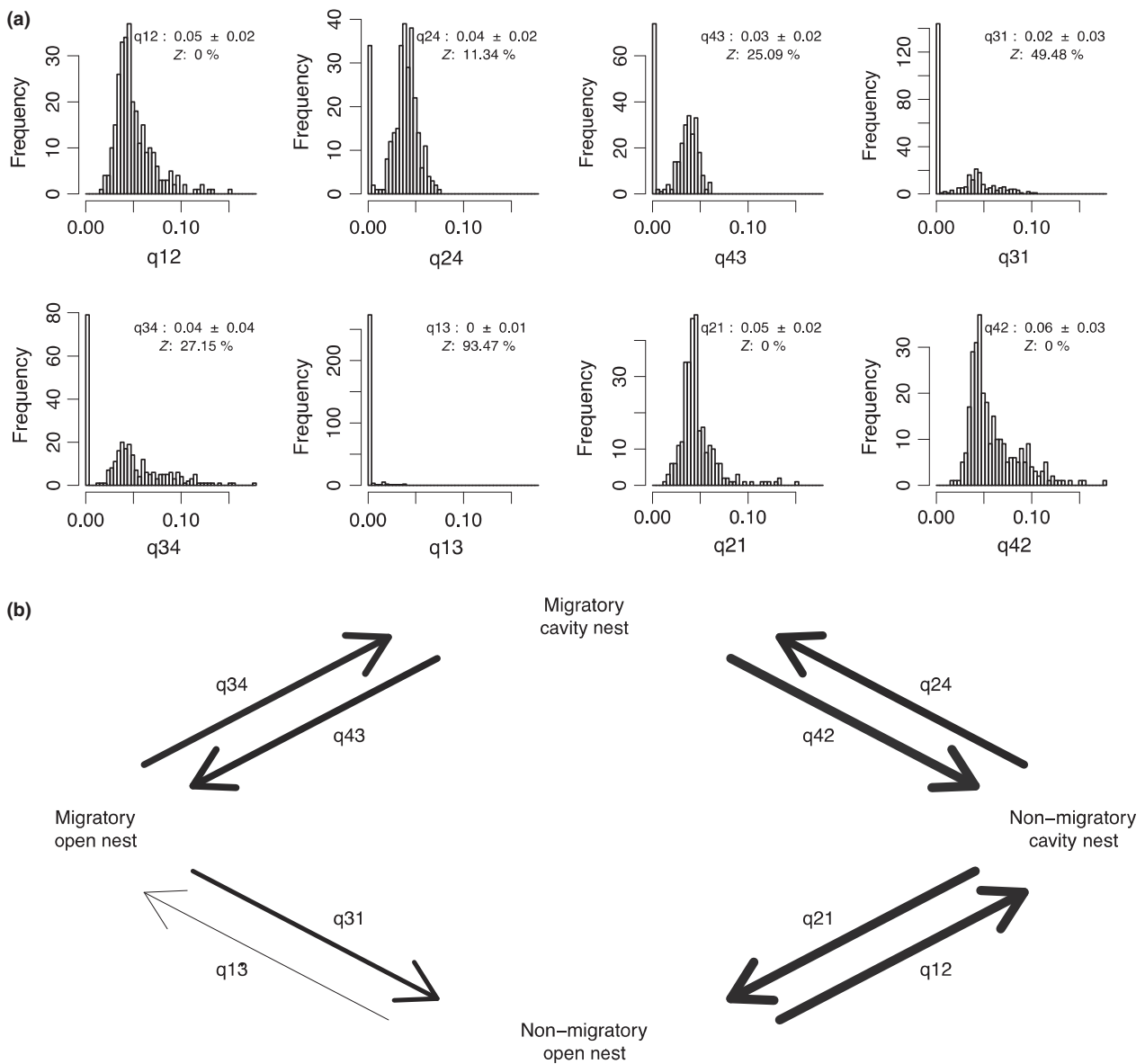
Figure 3. (a) Histograms of estimated transition rates between discrete character states of migratory and cavity nesting behaviour across 210 species of Old World flycatchers taken from the posterior distribution of BAYESTRAITS. Mean and standard errors are shown, while *Z* shows the percentage of sampled generations in the MCMC chain wherein that transition is assigned to the zero. If a model of independent evolution were favoured, then each histogram should be similar to the one that appears either above or below, which would indicate that gains or losses of a given character state do not depend on the state of the other character in question. (b) Visualization of differences in character state transition rates (*q*-values) wherein the darkness of an arrow represents the mean transition rate (darker = higher). Arrow thickness represents the percentage of post burn-in generations wherein the given transition rate is not assigned to zero (thicker = more often greater than zero).

on ecological communities by examining differences among species that share breeding grounds (Cockle *et al.* 2011). Our phylogenetic comparative analysis revealed novel links between cavity nesting, clutch size, body size and migratory behaviour from a macroevolutionary perspective.

Cavity nesting was positively correlated with maximum clutch size and body length in muscicapids. Incubation period, nestling period and total developmental period did not show a positive relationship with cavity nesting, which negated our prediction that cavity nesting evolved to counter

predation. However, the capacity to evolve adaptive developmental periods could be phylogenetically constrained among Old World flycatchers (Pienaar *et al.* 2013). Thus, lowered predation risk may not underlie increased maximum clutch sizes among cavity-nesting muscicapids.

Cavity-nesting muscicapids have significantly longer bodies than open-nesting species (Table 2). Thus, the increased maximum clutch sizes of cavity-nesting species may be related to competition for nesting holes. We postulate that larger species are better able to acquire and defend nesting cavities than smaller species in a community; smaller muscicapids may not be able to compete with larger muscicapids or other cavity nesters. Variation in clutch sizes between different nest types is well known in birds (Martin 1993); however, our findings suggested a possible interplay between body size and interspecific competition for nesting cavities, which might underlie variation in clutch sizes among nest types in muscicapids at a macroevolutionary scale. Our analyses also lend further credence to the well-documented pattern of larger clutch sizes at higher latitudes.

We found no association between cavity nesting and the climatic conditions of species' breeding ranges. Many tropical species in the Saxicolinae have subterranean cavity nests, which may have prevented us from finding an association with climatic variables. Additionally, the first month of breeding was significantly related to latitude alone, which suggested a strong role of geography in determining initiation of breeding, as in most bird families, rather than nest type.

Our BAYESTRAITS analyses revealed that transitions from non-migratory to migratory character states have been considerably more common among cavity-nesting than open-nesting lineages (Fig. 3). Most migratory muscicapids breed at high latitudes, which suggests that cavity nesting might be adaptive at high latitudes for reasons other than predation and might have been crucial in the evolution of migratory behaviour. Tropical species typically have lower nest attendance than temperate species (Skutch 1985); as demonstrated by recently introduced starlings (Johnson & Cowan 1974), the stable microclimate of cavities might have led to lower egg losses to low temperatures in ancestral migratory lineages. Hence, although gains in cavity nesting might have facilitated migration and colonization of new areas, intraspecific and interspecific competition for cavities in temperate areas might have subsequently led to the numerous losses of cavity-nesting behaviour (Fig. 3). We found no relationship between seasonality of the breeding range and cavity nesting (Table 2); however, because cavity nesting evolved before migratory behaviour in this clade, cavity nesting may have played an important role in the evolution of migratory behaviour and colonization of new areas in muscicapids.

Important life history traits, such as reproductive strategies and migratory behaviours, are often the product of a complex interaction between multiple selective pressures. We suggest that interspecific competition, a pervasive selective pressure, drives variation in migratory and nesting behaviour among Old World flycatchers. Our study demonstrated the efficacy of combining ecological knowledge, genetic data and comparative methods to examine variation in important life history traits across species.

REFERENCES

Alerstam, T. & Hogstedt, G. 1981. Evolution of hole-nesting in birds. *Ornis Scand.* **12**: 188–193.

Aliabadian, M., Kaboli, M., Förschler, M.I., Nijman, V., Chamani, A., Tillier, A., Prodon, R., Pasquet, E., Ericson, P.G.P. & Zuccon, D. 2012. Convergent evolution of morphological and ecological traits in the open-habitat chat complex (Aves, Muscicapidae: Saxicolinae). *Mol. Phylogenet. Evol.* **65**: 35–45.

Beresford, P. & Cracraft, J. 1999. *Speciation in African Forest Robins (Stiphronis): Species Limits, Phylogenetic Relationships, and Molecular Biology*. American Museum novitates; no. 3270. New York: American Museum of Natural History.

Bollback, J.P. 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* **7**: 88.

Cardillo, M. 2002. The life-history basis of latitudinal diversity gradients: how do species traits vary from the poles to the equator? *J. Anim. Ecol.* **71**: 79–87.

Cibois, A. & Cracraft, J. 2004. Assessing the passerine 'tapestry': phylogenetic relationships of the Muscicapoidea

inferred from nuclear DNA sequences. *Mol. Phylogenet. Evol.* **32**: 264–273.

Clements, J.F., Schulenberg, T.S., Iliff, M.J., Sullivan, B., Wood, C.L. & Roberson, D. 2013. *The Clements Checklist of Birds of the World: Version 6.7*. Ithaca, NY: Cornell University Press.

Cockle, K., Martin, K. & Drever, M. 2010. Supply of tree-holes limits nest density of cavity-nesting birds in primary and logged subtropical Atlantic Forest. *Biol. Conserv.* **143**: 2851–2857.

Cockle, K., Martin, K. & Wiebe, K. 2011. Selection of nest trees by cavity-nesting birds in the Neotropical Atlantic forest. *Biotropica* **43**: 228–236.

Collar, N. 2005. *Family Turdidae (Thrushes) in Handbook of the Birds of the World. Volume 10: Cuckoo-Shrikes to Thrushes*. Barcelona: Lynx Edicions.

Collias, N. 1964. The evolution of nests and nest-building in birds. *Am. Zool.* **4**: 175–190.

Collias, N. 1997. On the origin and evolution of nest building by passerine birds. *Condor* **99**: 253–270.

Coombs, A., Bowman, J. & Garroway, C. 2010. Thermal properties of tree cavities during winter in a northern hardwood forest. *J. Wildl. Manage.* **74**: 1875–1881.

Dhondt, A. 2007. What drives differences between North American and Eurasian Tit studies. Ecology and behavior of chickadees and titmice: an integrated approach. In Otter, K.A. (ed.) *Ecology and Behavior of Chickadees and Titmice: An Integrated Approach*: 299–310. Oxford: Oxford University Press.

Dietzen, C., Witt, H.-H. & Wink, M. 2003. The phylogeographic differentiation of the Robin *Erithacus rubecula*, Linnaeus 1758, on the Canary Islands revealed by mitochondrial DNA sequence data and morphometrics: evidence for a new robin species on Gran Canaria. *Avian Sci.* **3**: 115–131.

Drummond, A.J. & Rambaut, A. 2007. BEAST: bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**: 214.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* **4**: e88.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**: 1969–1973.

Ertan, K.T. 2006. The evolutionary history of Eurasian redstarts, *Phoenicurus. Acta Zool. Sinica* **52**: 310–313.

Freckleton, R., Harvey, P. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**: 712–726.

Grafen, A. 1989. The phylogenetic regression. *Philos. Trans. R. Soc. B* **326**: 119–157.

von Haartman, L. 1957. Adaptation in hole-nesting birds. *Evolution*, **11**: 339–347.

Hansen, T.F. & Martins, E.P. 1996. Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution* **50**: 1404–1417.

Harvey, P. & Pagel, M.D. 1991. *Oxford Series in Ecology and Evolution: The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**: 1965–1978.

Illera, J.C., Richardson, D.S., Helm, B., Atienza, J.C. & Emerson, B.C. 2008. Phylogenetic relationships, biogeography and speciation in the avian genus *Saxicola*. *Mol. Phylogenet. Evol.* **48**: 1145–1154.

Ives, A.R. & Garland, T. 2010. Phylogenetic logistic regression for binary dependent variables. *Syst. Biol.* **59**: 9–26.

Jetz, W., Sekercioglu, C. & Boehning-Gaese, K. 2008. The worldwide variation in avian clutch size across species and space. *PLoS Biol.* **6**: 2650–2657.

Johnson, S. & Cowan, I.M. 1974. Thermal adaptation as a factor affecting colonizing success of introduced Sturnidae (aves) in North-America. *Can. J. Zool.* **52**: 1559–1576.

Kass, R.E. & Raftery, A.E. 1995. Bayes factors. *J. Am. Stat. Assoc.* **90**: 773–795.

Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**: 772–780.

Katoh, K., Misawa, K., Kuma, K.-I. & Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**: 3059–3066.

Lack, D. 1947. The significance of clutch-size. *Ibis* **89**: 302–352.

Lerner, H.R.L., Meyer, M., James, H.F., Hofreiter, M. & Fleischer, R.C. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian Honeycreepers. *Curr. Biol.* **21**: 1–7.

Martin, T. 1993. Evolutionary determinants of clutch size in cavity-nesting birds – nest predation or limited breeding opportunities. *Am. Nat.* **142**: 937–946.

Martin, T. 1995. Avian life-history evolution in relation to nest sites, nest predation, and food. *Ecol. Monogr.* **65**: 101–127.

Martin, T.E. & Ghalambor, C.K. 1999. Males feeding females during incubation. I. Required by microclimate or constrained by nest predation? *Am. Nat.* **153**: 131–139.

Martin, T. & Li, P. 1992. Life-history traits of open-nesting vs cavity-nesting birds. *Ecology* **73**: 579–592.

Martins, E.P. & Hansen, T.F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**: 646–667.

Mason, N.A., Title, P.O., Cicero, C., Burns, K.J. & Bowie, R.C.K. 2014. Genetic variation among western populations of the Horned Lark (*Eremophila alpestris*) indicates recent colonization of the Channel Islands off southern California, mainland-bound dispersal, and postglacial range shifts. *Auk* **131**: 162–174.

Nice, M.M. 1957. Nesting success in altricial birds. *Auk* **74**: 1–17.

Oliveros, C.H., Reddy, S. & Moyle, R.G. 2012. The phylogenetic position of some Philippine 'babblers' spans the muscicapoid and sylvioid bird radiations. *Mol. Phylogenet. Evol.* **65**: 799–804.

Outlaw, R.K., Voelker, G. & Outlaw, D.C. 2007. Molecular systematics and historical biogeography of the rock-thrushes (Muscicapidae: *Monticola*). *Auk* **124**: 561–577.

Outlaw, R.K., Voelker, G. & Bowie, R.C.K. 2010. Shall we chat? Evolutionary relationships in the genus *Cercomela* (Muscicapidae) and its relation to *Oenanthe* reveals

extensive polyphyly among chats distributed in Africa, India and the Palearctic. *Mol. Phylogenet. Evol.* **55**: 284–292.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

Pagel, M. & Meade, A. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am. Nat.* **167**: 808–825.

Pienaar, J., Ilany, A., Geffen, E. & Yom-Tov, Y. 2013. Macroevolution of life-history traits in passerine birds: adaptation and phylogenetic inertia. *Ecol. Lett.* **16**: 571–576.

Posada, D. 2008. jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**: 1253–1256.

Qiao-Wa, P., Fu-Min, L., Shu-Juan, Y., Zhuo-Hua, Y., Yuan, H., Fa-Dao, T. & Kristin, A. 2006. Phylogenetic analysis of some Turdinae birds based on mitochondrial cytochrome b gene sequences. *Acta Zool. Sinica* **52**: 87–98.

R Core Development Team 2013. *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing.

Revell, L.J. 2009. Size-correction and principal components for interspecific comparative studies. *Evolution* **63**: 3258–3268.

Revell, L.J. 2011. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**: 217–223.

Rhodes, B., O'Donnell, C. & Jamieson, I. 2009. Microclimate of natural cavity nests and its implications for a threatened secondary-cavity-nesting passerine of New Zealand, the South Island Saddleback. *Condor* **111**: 462–469.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Roper, J., Sullivan, K. & Ricklefs, R. 2010. Avoid nest predation when predation rates are low, and other lessons: testing the tropical–temperate nest predation paradigm. *Oikos* **119**: 719–729.

Roy, M. 2001. Molecular systematics and evolutionary history of Akalats (Genus *Sheppardia*): a pre-Pleistocene radiation in a group of African forest birds. *Mol. Phylogenet. Evol.* **18**: 74–83.

Sangster, G., Alström, P., Forsmark, E. & Olsson, U. 2010. Multi-locus phylogenetic analysis of Old World chats and flycatchers reveals extensive paraphyly at family, subfamily and genus level (Aves: Muscicapidae). *Mol. Phylogenet. Evol.* **57**: 380–392.

Schmidt, B.K., Foster, J.T., Schmidt, K.B., Angehr, R.G., Durrant, L.K. & Fleischer, R.C. 2008. A new species of African forest robin from Gabon (Passeriformes: Muscicapidae: *Stiphrornis*). *Zootaxa* **1850**: 27–42.

Seki, S.-I. 2006. The origin of the East Asian *Erithacus* robin, *Erithacus komadori*, inferred from cytochrome b sequence data. *Mol. Phylogenet. Evol.* **39**: 899–905.

Sheldon, F.H., Lohman, D.J., Lim, H.C., Zou, F., Goodman, S.M., Prawiradilaga, D.M., Winker, K., Braile, T.M. & Moyle, R.G. 2009. Phylogeography of the magpie-robin species complex (Aves: Turdidae: *Copsychus*) reveals a Philippine species, an interesting isolating barrier and unusual dispersal patterns in the Indian Ocean and Southeast Asia. *J. Biogeogr.* **36**: 1070–1083.

Skutch, A.1985. Clutch size, nesting success, and predation on nests of neotropical birds, reviewed. *Ornithol. Monogr.* **36**: 575–594.

Steel, M.A. & Penny, D. 1993. Distributions of tree comparison metrics – some new results. *Syst. Biol.* **42**: 126–141.

Taylor, B. & Peter, C. 2006. Family Muscicapidae (Old World flycatchers). In del Hoyo, J., Elliott, A. & Christie, D. A. (eds) *Handbook of the Birds of the World. Volume 11: Old World Flycatchers to Old World Warblers.* Barcelona: Lynx Edicions.

Treplin, S., Siegert, R., Bleidorn, C., Thompson, H.S., Fotso, R. & Tiedemann, R. 2008. Molecular phylogeny of songbirds (Aves: Passeriformes) and the relative utility of common nuclear marker loci. *Cladistics* **24**: 328–349.

Voelker, G. 2010. Repeated vicariance of Eurasian songbird lineages since the Late Miocene. *J. Biogeogr.* **37**: 1251–1261.

Voelker, G. & Spellman, G.M. 2004. Nuclear and mitochondrial DNA evidence of polyphyly in the avian superfamily Muscicapoidea. *Mol. Phylogenet. Evol.* **30**: 386–394.

Voelker, G., Bowie, R.C.K. & Klicka, J. 2013. Gene trees, species trees and Earth history combine to shed light on the evolution of migration in a model avian system. *Mol. Ecol.* **22**: 3333–3344.

Xin, L., Zhen-Min, L., Fu-Min, L., Zuo-Hua, Y. & Hong-Feng, Z. 2007. Phylogeny of some Muscicapinae birds based on cyt b mitochondrial gene sequences. *Acta Zool. Sinica* **53**: 95.

Zink, R.M., Pavlova, A., Drovetski, S., Wink, M. & Rohwer, S. 2009. Taxonomic status and evolutionary history of the *Saxicola torquata* complex. *Mol. Phylogenet. Evol.* **52**: 769–773.

Zuccon, D. & Ericson, P.G.P. 2010. A multi-gene phylogeny disentangles the chat–flycatcher complex (Aves: Muscicapidae). *Zoolog. Scr.* **39**: 213–224.

Zyskowski, K. & Prum, R.O. 1999. Phylogenetic analysis of the nest architecture of neotropical ovenbirds (Furnariidae). *Auk* **116**: 891–911.

SUPPORTING INFORMATION

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

Methods S1. Additional methodological details for phylogenetic inference.

Results S1. Additional information about the results of phylogenetic inference in light of previous studies.

Table S1. Additional information regarding 10 molecular markers used for phylogenetic inference.

Table S2. Data matrix of molecular markers used for phylogenetic inference. Sequence identifier numbers (GI numbers) are indicated when available.

Table S3. Phylogenetic generalized least squares (PGLS) results using an alternative classification of

cavity nesting in which species that are variable or ambiguous in their nesting behaviour are assigned to the open-nesting category.

Figure S1. First principal component loadings of Bioclim variables 1–10 for breeding ranges of 188 species of Old World flycatchers.

Figure S2. Histogram of the number of loci sampled for each taxon included in phylogenetic analyses.

Figure S3. MRBAYES phylogeny inferred for 252 species of Old World flycatchers.

Figure S4. Density plot of Robinson–Fould distance comparisons among posterior distributions of MRBAYES and BEAST phylogenies.

Figure S5. Stochastic character mapping of cavity nesting and migratory behaviour of 210 species of Old World flycatchers using an alternative treatment of ambiguous or variable migratory statuses.

Figure S6. Stochastic character mapping of cavity nesting and migratory behaviour of 210 species of Old World flycatchers using an alternative treatment of ambiguous or variable nesting types.

Figure S7. Output of BAYESTRAITS re-analysis with alternative character coding for migratory statuses.

Figure S8. Output of BAYESTRAITS re-analysis with alternative character coding for nesting types.

Appendix S1. Life history data, including categorizations of migratory behaviour and nesting type, climatic data, and latitudinal data.