Systematics and Biodiversity

The biogeographic history of Phoenicurus redstarts reveals an allopatric mode of speciation and an out-of-Himalayas colonization pattern

Gary Voelker[a], Georgy Semenov[b], Igor V. Fadeev[c], Anna Blick[a] & Sergei V. Drovetski[d]

[a] Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843, USA

[b] Institute of Systematics and Ecology of Animals of Siberian Branch of Russian Academy of Sciences, Frunze St. 11, 630091 Novosobirsk, Russia

[c] State Darwin Museum, Vavilova St. 57, 117292 Moscow, Russia

[d] Department of Natural History, Tromsø University Museum, University of Tromsø – The Arctic University of Norway, PO Box 6050 Langnes, NO-9037 Tromsø, Norway
Published online: 21 Jan 2015.

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis
Taylor & Francis Group

The biogeographic history of *Phoenicurus* redstarts reveals an allopatric mode of speciation and an out-of-Himalayas colonization pattern

GARY VOELKER[1], GEORGY SEMENOV[2], IGOR V. FADEEV[3], ANNA BLICK[1] & SERGEI V. DROVETSKI[4]

[1]Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843, USA
[2]Institute of Systematics and Ecology of Animals of Siberian Branch of Russian Academy of Sciences, Frunze St. 11, 630091 Novosobirsk, Russia
[3]State Darwin Museum, Vavilova St. 57, 117292 Moscow, Russia
[4]Department of Natural History, Tromsø University Museum, University of Tromsø − The Arctic University of Norway, PO Box 6050 Langnes, NO-9037 Tromsø, Norway

Montane areas host high levels of diversity and endemism, and these features are tied to habitat stratification along an elevational gradient. As such, montane areas are often thought of as model systems in which sympatric speciation can occur. To test this idea, we selected *Phoenicurus* redstarts, an avian genus with an extensive distribution across Eurasia, as well as Northwest Africa; nine of the 14 species in the genus have distributions which include the Himalayas. We used sequences of the mtDNA ND2 and cytochrome-*b* genes and intron 9 of the Z chromosome specific ACO1 gene to reconstruct a phylogeny of the genus. The resulting trees were used to reconstruct a biogeographic history of *Phoenicurus*, and to date diversification events. We also analysed the relationship between node age and sympatry to determine the geographic mode of speciation in the genus. Our data suggest a very late Miocene, Himalayan origin for *Phoenicurus*. Diversification and colonization of other parts of Eurasia, as well as Northwest Africa, continued through the Pleistocene, with a rapid pulse of speciation in the late Pliocene. Allopatric speciation was the dominant mode of speciation in *Phoenicurus*, despite extensive distributional overlaps in the Himalayas where ecological conditions are amenable to speciation in sympatry. Our results, along with several other studies, suggest an emerging pattern where the Himalayas served as a source area for montane specialist avian lineages that subsequently colonized other Palaearctic regions.

Key words: allopatric speciation, biogeography, Himalayas, Palaearctic, Phoenicurus, phylogeny

Introduction

Montane regions are often characterized by sharp elevational habitat gradients and as a function of this, many mountain systems are known hotspots of avian diversity and endemism (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000; Orme et al., 2005). This link between elevational gradients and high diversity is thought to provide an opportunity for sympatric speciation (speciation across ecotones; Fjeldså & Bowie, 2008). However, systematic and biogeographic studies of birds distributed in montane regions of Central and South America as well as Africa, have shown that closely related lineages, to include sister taxa, are not found in adjacent habitats along elevational gradients. Instead, sister taxa are found in generally similar environments on different mountain ranges, or when found in the same mountain range, they tend to be on different mountains (Barrera-Guzmán, Milá, Sánchez-González, & Navarro-Sigüenza, 2012; Chaves &

Smith, 2011; Gutiérrez-Pinto et al., 2012). Such patterns suggest an allopatric mode of speciation and indeed allopatric speciation appears to be the dominant mode of speciation in birds (Barraclough & Vogler, 2000; Chesser & Zink, 1994; Coyne & Price, 2000; Drovetski, 2003; Drovetski, Raković, Semenov, Fadeev, & Red'kin, 2014; Drovetski et al., 2004a., 2013; Fitzpatrick & Turelli, 2006; Friesen & Anderson, 1997; Mayr, 1942; Phillimore et al., 2008; Price, 2008).

Although evidence for sympatric speciation in birds remains elusive (Phillimore et al., 2008), avian lineages with species distributed in different montane habitats may still provide the most realistic opportunity for detecting ecologically driven lineage divergence that would be consistent with speciation in sympatry. The (almost entirely) Palaearctic genus *Phoenicurus* (redstarts) is one such lineage. The 14 species in the genus *Phoenicurus*, which includes species formerly placed in *Rhyacornis* and *Chaimarrornis* (Voelker, 2010), are distributed in the Atlas Mountains in northwest Africa and broadly across the

Correspondence to: Gary Voelker. E-mail: gvoelker@tamu.edu
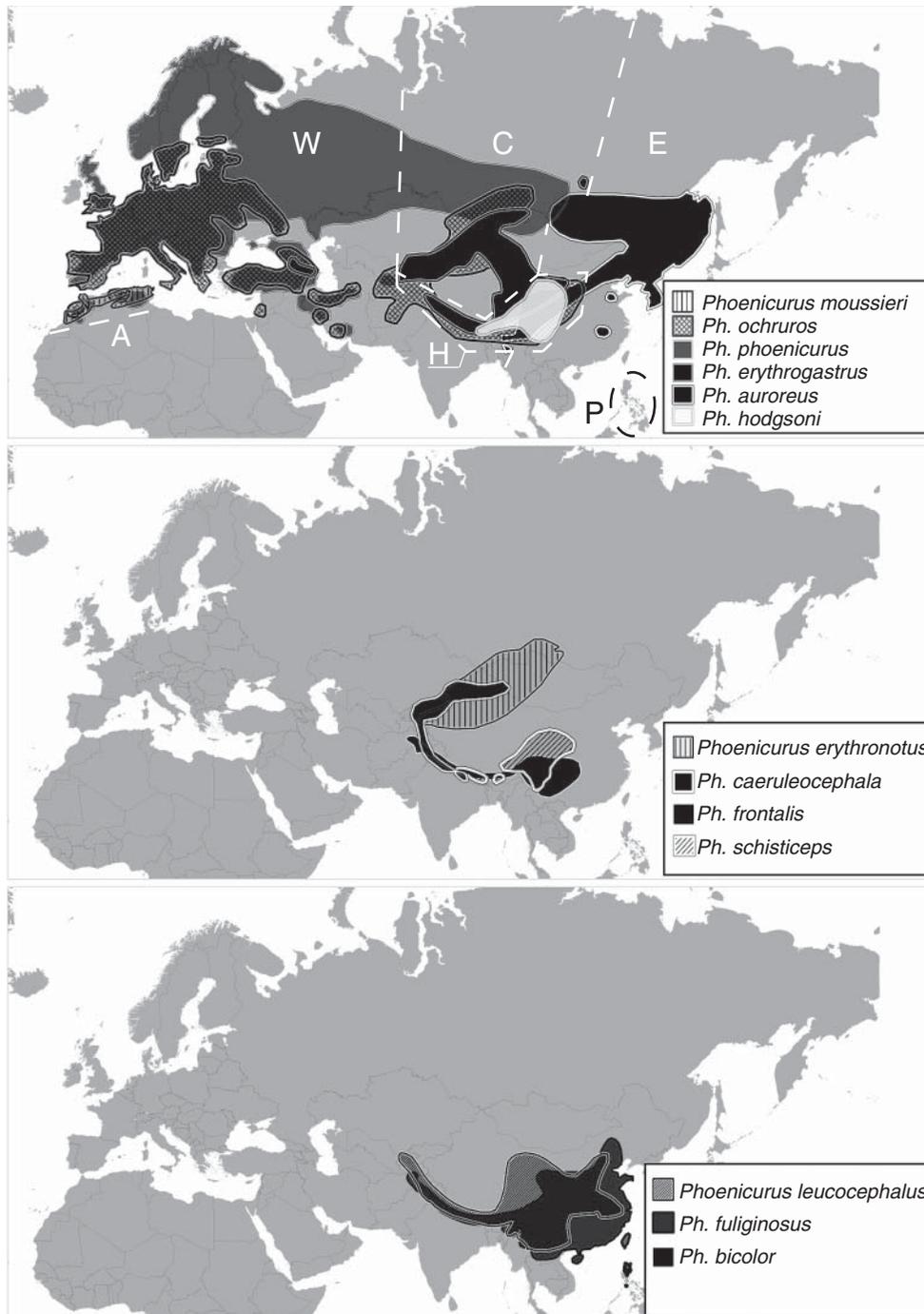
Fig. 1. Breeding species ranges of *Phoenicurus* redstarts; wintering ranges are generally adjacent to breeding ranges and in the same biogeographic regions. The top panel delineates the biogeographic regions used in ancestral area reconstructions.

Palaearctic, with limited distributions in Southeast Asia and the Philippines. Importantly, nine of these 14 species have ranges that include the Himalayas (Fig. 1; Collar, 2005).

The underlying question then is whether the high level of sympatry amongst *Phoenicurus* species in the Himalayas can be attributed to sympatric speciation. Such a result would be, as suggested above, novel for birds

particularly given that previous studies of Palaearctic birds have generally shown that co-distributed Palaearctic species tend to result from multiple colonizations of a given region, or from range expansion after divergence in allopatry (Drovetski, 2003; Johansson et al., 2007; Voelker, 1999, 2002, 2010) An additional but related question is whether the Himalayas have served as the

ancestral source area for *Phoenicurus*; this pattern is evident in two other montane specialist genera (*Leucosticte* finches; Drovetski, Zink, & Mode, 2009 and *Prunella* accentors; Drovetski et al., 2013). Subsequent diversification in these genera is related to the colonization of other Palaearctic areas, and at least in the case of *Prunella* is related to glacial retreat near the Plio-Pleistocene boundary (Drovetski et al., 2013). Furthermore, although there is a high number of sympatric species in the Himalayas, results clearly show that *Prunella* species diverged in allopatry.

In this study we seek to address questions regarding the geographic mode of speciation and biogeographic history in the genus *Phoenicurus*, which has a high number of montane specialist species. To accomplish this we used sequences of the mitochondrial ND2 and cytochrome-b genes and intron 9 of the Z chromosome specific Aconitase 1 gene (ACO1I9) to reconstruct a phylogeny of all but one currently recognized species in the genus. Using this phylogeny, we reconstructed a biogeographic history of *Phoenicurus* both spatially and temporally, and tested whether this mountain specialist genus diverged in allopatry or sympatry.

Materials and methods

Taxon sampling

We included tissue samples from 12 of 14 currently recognized species of *Phoenicurus* (Clements et al., 2014), and acquired cytochrome *b* data for *Ph. schisticeps* from GenBank. Where possible we included multiple individuals for each species, particularly for those with comparatively broad distributions (*Ph. phoenicurus*, *auroreus*, *ochruros*). We were unable to acquire samples of or data from *Ph.* [*erythronotus*] *alaschanicus*. We used *Tarsiger indicus*, *T. cyanurus*, *Ficedula mugimaki*, *Luscinia luscinia* and *Monticola rupestris* as outgroups, based on their relatively close relationship to *Phoenicurus* (Zuccon & Ericson, 2010).

Molecular methods

For tissue samples, genomic DNA was extracted using either the JETQUICK Tissue DNA Spin Kit (Genomed, Löhne, Germany) or the DNeasy tissue extraction kit (Qiagen, Valencia, CA), both according to the manufacturer's protocol. We used the polymerase chain reaction (PCR) to amplify the mtDNA cytochrome-*b* (cyt-*b*; 1000 bp) and NADH dehydrogenase subunit 2 (ND2; 1041 bp) genes, as well as intron 9 of the Z chromosome specific Aconitase 1 gene (ACO1I9; 1014 bp). Primers and PCR conditions for amplification and sequencing of cyt-*b* are described in Voelker (2010) and of ND2 and ACO1I9 in Drovetski et al. (2004b), Drovetski, Zink,

Ericson, and Fadeev (2010). Sequence data are available on GenBank (see Table 1).

Automated sequencing was performed either on an ABI 377 sequencer or on an ABI 3730 Genetic Analyser (Applied Biosystems Inc., Foster City, CA, USA) at the University of Florida ICBR facility or the Macrogen Europe (the Netherlands) facility, respectively. The sequences were aligned in Sequencher 5.0.1 (Gene Codes Corporation, Ann Arbour, MI, USA). This process was straightforward for the mitochondrial genes. With respect to ACO1I9, females are hemigametic in their Z-specific (sex-linked) loci and thus have only one allele. In heterogametic males whose ACO1I9 alleles differed in length, the alleles were identified by subtracting the complementary sequence of the allele without the indel from the double peaks in their chromatogram. Although tedious, this procedure allowed us to identify indels and to resolve nucleotide polymorphisms without ambiguity (Drovetski et al., 2013; Hung, Drovetski, & Zink, 2012; Sousa-Santos, Robalo, Collares-Pereira, & Almada, 2005). ACO1I9 alleles of heterogametic males that had the same length but contained multiple nucleotide differences were resolved using PHASE 2.1.1 (Stephens, Smith, & Donnelly, 2001). We conducted two independent PHASE runs. The first 500 interactions were discarded as burn-in. The following 5000 iterations used a thinning interval of 10. Known haplotypes from females, homozygous males and males with a single polymorphic site or indel were set as known alleles.

We used MrModelTest (Nylander, 2004) to determine the appropriate model of nucleotide substitution and best-fit model of sequence evolution for each gene region. The Akaike information criterion (Akaike, 1974) identified the GTR + G + I as the best-fit model for both mtDNA genes, and the GTR + G model for ACO1I9. We also used jModeltest 2.1.5 (Posada, 2008) and the Bayesian Information Criterion for the species tree analysis, with the following models identified: ND2, TrN+I+G; cyt-b, TPM2uf+I+G; ACO1I9, TrN+G.

We incorporated best-fit models for each gene in *BEAST2 (Drummond, Suchard, Xie, & Rambaut, 2012) to reconstruct a species tree using all loci. We incorporated a Yule process speciation prior for all three loci. We also made use of the mean of rates of divergence and associated standard deviations reported by Lerner, Meyer, James, Hofreiter, and Fleischer (2011) for the two mtDNA genes analysed (cyt-*b* 1.4^*10^{-2} [95% CI: $1.2-1.6^*10^{-2}$] per Ma; ND2 2.9^*10^{-2} [95% CI: $2.3-3.3^*10^{-2}$] per Ma). These rates are derived from the sequence of lineage splits in a passerine clade (Hawaiian Honeycreepers: Fringillidae), and calibrated using the well-established dates of sequential uplift of the Hawaiian Archipelago. For ACO1I9 we assigned the rate of 3.2×10^{-3} (95% CI: $2.4-4.0 \times 10^{-3}$) per Ma, based on a previous estimate (Drovetski et al., 2013). This rate was slightly higher than

Table 1. List of included taxa with their collection number (if known), sex, Genbank accession numbers and origin. Dashes (—) indicate missing data.

Species	ID[1]	Sex	cyt*b* 997 bp	nd2 1041 bp	ACO1I9[2] 1014 bp	Origin
Phoenicurus erythronotus	UWBM 66692/SVD 2290	male	GU930799	GU930810	KP172467/8	Russia: Tyva
	UWBM 46289/CDS 4581	male	KP184823	KP173645	KP172446/7	Russia: Gorno-Altay
Phoenicurus caeruleocephala	FMNH 347849	male	GU930798	GU930809	KP172451/2	Pakistan: North-West Frontier
Phoenicurus ochruros	UWBM 64755/SVD 2065	male	GU930803	GU930814	KP172465/6	Russia: Krasnodarskiy krai
	UWBM 66329/NAM 158	male	KP184824	KP173646	—	Russia: Tyva
	UWBM 64739/SVD 2049	female	KP184826	KP173648	KP172464	Russia: Krasnodarskiy krai
	UWBM 66744/SVD 2344	female	KP184825	KP173647	KP172469	Russia: Tyva
	USNM 640702/SVD 3612	female	KP184827	KP173649	KP172471	Switzerland: Canton Vaud
Phoenicurus phoenicurus	UWBM 64720/SVD 2028	female	GU930804	GU930815	KP172463	Russia: Krasnodarskiy krai
	UWBM 49729/BKS 2130	male	KP184829	KP173651	KP172443/4	Russia: Murmanskaya oblast'
	UWBM 56809/CSW 5666	female	KP184830	KP173652	—	Russia: Tyumenskaya oblast'
	UWBM 58091/DAB2378	male	KP184828	KP173650	KP172449/50	Mongolia: Töv Aymag
	BMUM BRB476	female	KP184831	KP173653	KP172445	Russia: Arkhangel'skaya oblast'
	BMUM IUK0693	male	KP184832	KP173654	—	Russia: Arkhangel'skaya oblast'
Phoenicurus hodgsoni	USNM 585826	male	GU930802	GU930813	—	India: Arunachal Pradesh
Phoenicurus schisticeps		unknown	DQ285427	—	—	China: Qinghai
Phoenicurus auroreus	UWBM 47088/CSW 4847	male	GU930797	GU930808	—	Russia: Khabarovskiy krai
	UWBM 47089/CSW 4848	female	KP184823	KP173655	KP172448	Russia: Khabarovskiy krai
	UWBM 73291/JML 279	female	KP184834	KP173656	—	Russia: Irkutskaya oblast'
	USNM 640539/SVD 3439	male	KP184835	KP173657	—	Russia: Zabaikal'skiy krai
	USNM 640611/SVD 3517	female	KP184836	KP173658	KP172470	Russia: Zabaikal'skiy krai
Phoenicurus moussieri	A285779	male	KP184837	KP173659	KP172436/7	Morocco: Lac Afennourir
	A285780	female	KP184838	KP173660	KP172438	Morocco: Lac Afennourir
	A285782	female	KP184839	KP173661	KP172439	Morocco: Lac Afennourir
Phoenicurus erythrogastrus	UWBM 66361/NAM 190	male	GU930800	GU930811	KP172460/1	Russia: Tyva
	MSUZM 1999/ 220	male	—	—	KP172475/6	Russia: Tyva
Phoenicurus frontalis	AMNH/JGG1022	male	GU930801	GU930812	KP172458/9	Nepal: Malde
Phoenicurus leucocephalus	USNM B06058	male	GU930796	GU930807	KP172440/1	Myanmar: Sagaing
Phoenicurus fuliginosus	FMNH 347850	male	GU930806	GU930816	KP172453/4	Pakistan: North-West Frontier
Phoenicurus bicolor	FMNH 429238	male	GU930805	GU930817	KP172455/6	Philippines: Luzon
Luscinia luscinia	UWBM 49508/BKS1908	female	KP184841	KP173662	KP172442	Russia: Sverdlovskaya oblast'
Ficedula mugimaki	UWBM 51150	female	DQ674495	DQ674468	KP172473/4	Russia: Primorskiy Kray
Monticola rupestris	UWBM 95293	female	EF434527	EF434554	KP172457	South Africa: Western Cape
Tarsiger cyanurus	UWBM 51572/SVD 352	female	AY329475	AY329438	KP172462	Russia: Magadanskaya oblast'
	MSUZM SVD4730	unknown	—	—	KP172472	Russia: Sverdlovskaya oblast'
T. indicus	AMNH/GFB3372	female	KP184840	KP173663	—	China: Taiwan

[1] Museum or collector acronyms, followed by museum number: A, S.V. Drovetski; AMNH, American Museum of Natural History; BMUM, Bell Museum, University of Minnesota; FMNH, Field Museum of Natural History; MSUZM, Moscow State University Museum; USNM, U.S. National Museum; UWBM, University of Washington Burke Museum. Collector initials and number, if known, follow the "/."

[2] Multiple GenBank accession numbers reflect multiple alleles from males, e.g., for UWBM 66692/SVD 2290, KP172467/8 refers to both KP172467 and KP172468.

the evolutionary rates reported for 13 autosomal loci (Lerner et al., 2011). The higher evolutionary rates for Z-specific as compared with autosomal loci are expected due to the former having $^3/_4$ of the effective population size of the latter. We utilized a Relaxed LogNormal clock, as there were no significant differences between that clock and a strict clock for the mitochondrial genes (via maximum likelihood ratio tests; *P* values ranged from 0.6 to 1.0). Gene regions were unlinked to allow independence of assigned clock parameters.

Two independent Markov-chain Monte Carlo (MCMC) analyses were run for 1×10^7 generations, with parameters sampled every 5×10^3 steps and a pre-burn-in of 1×10^3. Independent runs were combined using Log-Combiner v.1.7.4 (Drummond et al., 2012). Tracer v1.5 (http://beast.bio.ed.ac.uk/Tracer) was used to determine the effective sample size of each parameter and calculate the mean and 95% highest posterior density interval (95% HPD) for divergence times. Tree topologies were assessed using TreeAnnotator v.1.7.4 (Drummond et al., 2012) and

visualized in FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/soft ware/figtree/).

We also analysed a concatenated dataset in MrBayes (v. 3.1.2; Huelsenbeck & Ronquist, 2001) which included individuals for which we had all three genes, plus *Phoenicurus schisticeps* (cyt-*b* only). Where the ACO1I9 alleles were different in an individual (males) we appended the different alleles to the same mtDNA genes for that same individual. The combined mitochondrial (mtDNA) sequence data were analysed using two alternate mixed-model strategies under a Bayesian framework. In our first partitioning strategy (three partitions) the ND2, cyt-*b* and ACO1I9 were unlinked and allowed estimation of gene appropriate GTR + I + Γ parameters. In the second strategy (seven partitions), each codon position for the two mtDNA genes were unlinked from one another, while ACO1I9 remained a single partition.

For each analysis, we initiated two runs of four MCMC chains of 5 000 000 generations each from a random starting tree, sampling every 100 generations. Each run resulted in 50 000 trees and converged on the same topology. The first 5000 trees from each analysis were removed as our 'burn-in', and the remaining 90 000 trees were used to generate a majority rule consensus tree. Bayes factors were computed using the harmonic means of the likelihoods calculated from the *sump* command within MRBAYES. A difference of 2 ln Bayes factor > 10 was used as the minimum value to discriminate between

partitioning strategies (Brandley, Schmitz, & Reeder, 2005; Brown & Lemmon, 2007), and the seven partition analysis was identified as the best fit to the data (−ln 11621.47 versus −ln 12487.67 for the three partition strategy).

Historical biogeography and geographic mode of speciation

For historical biogeography reconstruction we used maximum likelihood analysis of geographic range evolution based on the dispersal-extinction cladogenesis model implemented in LaGrange v. 2.0.1 (Ree & Smith, 2008). LaGrange identifies the geographic areas that are included in the most probable ancestral range and which areas were 'inherited' by its descendants, for each node along the phylogenetic tree. Using distribution maps (Collar, 2005), each species (breeding range only) was coded in a first analysis as being present or absent in each of six areas: Western, Central and Eastern Eurasia, the Himalayan region (inclusive of mountains of central China), the Philippines and Northwest Africa (Fig. 1). Due to the somewhat surprising reconstruction of Northwest Africa as an ancestral area for the *ochruros/moussieri/phoenicurus* clade (Fig. 2), we conducted a second analysis in which we eliminated Northwest Africa (merging it with Western Eurasia). Although eliminating Northwest Africa required
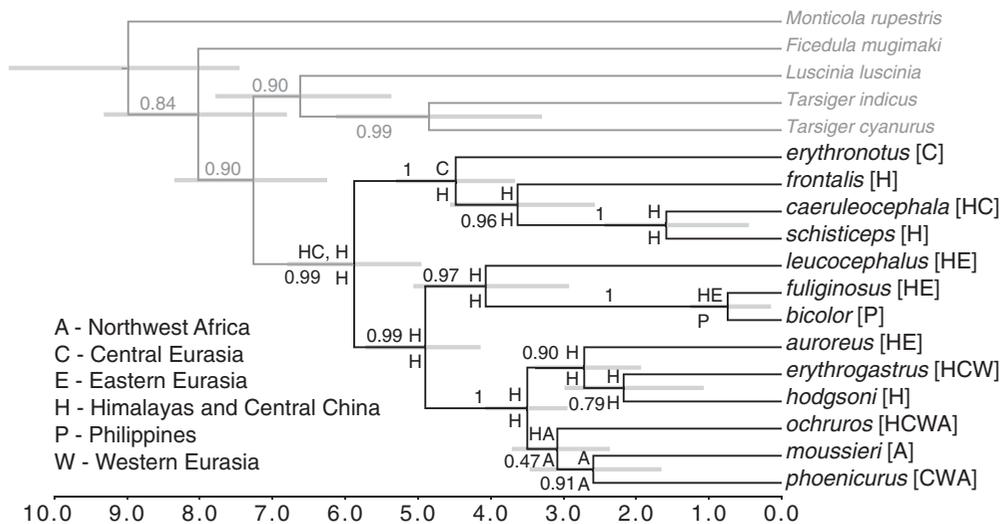

Fig. 2. Species tree with molecular clock dating for *Phoenicurus*. Numbers at nodes indicate posterior probabilities for the species tree; all nodes were supported at ≥0.95 PP in the concatenated Bayesian analysis. Scale bar is time in millions of years before present (Ma), with bars at nodes indicating 95% highest posterior density intervals (HPD) around the mean divergence times at each node. Ancestral area reconstructions are based on LaGrange analysis, therefore two reconstructions are shown at each node. The upper reconstruction is the most likely area reconstruction derived from all node reconstructions from lineages 'above' the node, and the lower is the most likely reconstruction derived from all node reconstructions from lineages 'below' the node. We report the highest relative probability reconstruction at each node, which were at least twice (or in one case very nearly twice) the relative probability of the next most likely reconstruction. The exception is at the basal node of the phylogeny, where nearly equiprobable reconstructions are separated by a comma.

us to code the endemic *moussieri* as Western Eurasian, we primarily eliminated it because the breeding range of both *ochruros* and *phoenicurus* in Northwest Africa (portions of the Atlas Mountains) constitutes a very small fraction of their overall breeding ranges (Fig. 1). As such, including Northwest Africa in the distribution of each may be overweighting its importance as a possible ancestral area. We also eliminated the Philippines in this second analysis (merging it with Eastern Eurasia), leaving four distributional areas. Ancestral areas were reconstructed by performing likelihood optimizations on our species tree. For both of these analyses, default software assumptions were used, i.e. we did not penalize movements between geographically non-adjacent areas.

Geographic range data were obtained from Collar (2005). Species range maps were digitized in MapInfo Professional v. 9.5.1 (Pitney Bowes Inc., Stamford, CT, USA). The digitized species ranges (Fig. 1) were used to calculate their area, the area of clade ranges, and measure range overlap amongst clades and species.

To analyse geographic modes of speciation in *Phoenicurus* we used a node-based approach that compares the ranges of two clades connected by a node on the phylogenetic tree (Barraclough & Vogler, 2000). Clade ranges were calculated as joint ranges of clade-member species. Sympatry index (sympatry) for sister clades was calculated as their range overlap area divided by the area of the smaller of the two ranges. Sympatry values for all nodes were then regressed onto the node ages. If speciation was predominantly allopatric, the most recent divergence events in the phylogeny are expected to have no sympatry (0 sympatry value), with sympatry values increasing with range changes through time (i.e. towards the base of the phylogeny). Clustering of 0 sympatry values at younger nodes, and the presence of non-zero sympatry values at older nodes, results in a positive correlation between the node age and sympatry. The opposite relationship is expected for predominantly sympatric speciation (Barraclough & Vogler, 2000; Drovetski, 2003; Drovetski et al., 2013).

When allopatric speciation appears to be the geographic mode of speciation, the range symmetry index (the smaller clade range area divided by the sum of both clade range areas) can be regressed onto the node age to determine whether the speciation was predominantly peripatric (Barraclough & Vogler, 2000). Peripatric speciation is expected to result in low range symmetry for the recent nodes of the phylogenetic tree but should increase through time because range expansion of a small peripherally isolated clade is more likely to increase with time than decrease. Both sympatry and symmetry indexes are proportions with values bounded by 1 and 0.5 respectively. In order to use them in regression analyses, they were arcsine transformed. The symmetry index values were doubled prior to arcsine transformation.

Results

Phylogeny of *Phoenicurus*

Both our species tree and concatenated (seven partition) Bayesian analyses strongly supported the monophyly of *Phoenicurus* relative to outgroup taxa (1.0 posterior probability (PP)), and both analyses resulted in the same topology of species relationships (Fig. 2). Overall the species tree analysis strongly supported 8 of 12 nodes while the seven partition concatenated analysis supported all 12 nodes at ≥ 0.95 PP (Fig. 2). As has been shown previously (Sangster, Alström, Porsmark, & Olsson, 2010; Voelker, 2010), species previously in the genera *Chaimorrornis* (*leucocephalus*) and *Rhyacornis* (*fuliginosus* and *bicolour*) clearly fall within *Phoenicurus*. Individual gene trees (not shown) are largely concordant with the species tree.

A recent paper by Hogner et al. (2012) found deep mtDNA divergences within *Ph. phoenicurus* samples collected primarily from the western Palaearctic. Although our analyses are focused at the inter-specific level, we note that we also find deep mtDNA divergences between our samples of *Ph. phoenicurus* collected from the western and eastern Palaearctic (Table 1). This suggests further phylogeographic analysis of *Ph. phoenicurus* is warranted to determine the extent of haplotype distributions (see Hogner et al., 2012, where few eastern Palaearctic samples were included), as is phylogeographic analysis of variation within *auroreus* and *ochruros*, both of which show similarly deep divergences across our sampling points (Fig. 2).

Historical biogeography and geographic mode of speciation

The genus *Phoenicurus* appears to split from *Tarsiger*/*Luscinia* clade *c*. 7.3 Ma (95% HPD interval 6.3−8.4 Ma), in the late Miocene. Lineage divergences within *Phoenicurus* began during the Miocene/Pliocene transition (5.9 Ma; 5.0−6.8) which resulted in two primary clades: one comprised of four species restricted to the Himalayas and mountains of the Central Eurasia (the *erythronotus* clade) and a more speciose clade distributed across the Eurasia and Northwest Africa. Diversification within the genus continued through the Middle Pleistocene (*c*. 0.7 Ma, 0.2−1.3 Ma; Fig. 2), with one clade (*auroreus-phoenicurus*) showing a pulse of speciation in the late Pliocene−early Pleistocene between 3.5 Ma (2.9−4.1 Ma) and 2.2 Ma (1.1−3.0 Ma; Fig. 2) resulting in six species.

Ancestral area reconstructions using six distributional areas indicate a primarily Himalayan origin for the genus (Fig. 2), with the phylogenetic position and distribution of *erythronotus* in central Eurasia suggesting a Himalayan + Central Eurasia area for the small clade that *erythronotus* is a member of (Fig. 2). Overall, the ancestral area
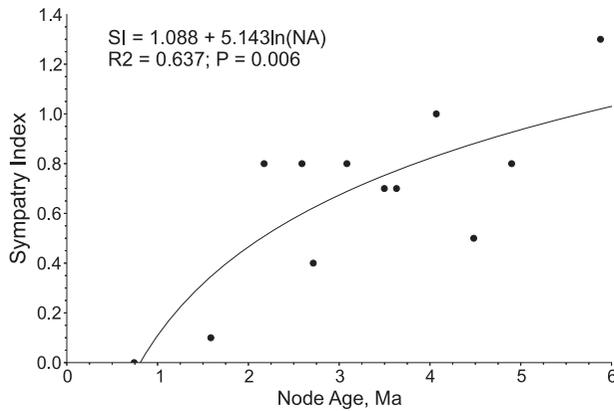
SI = 1.088 + 5.143ln(NA)
R2 = 0.637; P = 0.006

Fig. 3. Sympatry index versus node age. Sympatry index values were arcsine transformed (Barraclough & Vogler, 2000).

reconstructions at the majority of nodes indicate a Himalayan ancestral area, with later movements of species to other generally adjacent areas (Fig. 2). The exception to this is the Northwest African ancestral area inferred for *ochruros*, *moussieri* and *phoenicurus* (Fig. 2), where the colonization of both the Philippines and Northwest Africa are inferred as having been directly from the Himalayas (i.e. not from an adjacent area).

Reducing the number of distributional areas from six to four (see Methods) did little to change ancestral reconstructions. The *fuliginosus* + *bicolour* node became HE/E, the *moussieri* + *phoenicurus* node became W/W, and the *ochruros*/*moussieri*/*phoenicurus* node became HW/W. While this reconstruction did eliminate Northwest Africa as an ancestral area for the latter two nodes, it did not eliminate the need to ultimately explain the distribution of these three taxa in Northwest Africa.

Sympatry index values (Barraclough & Vogler, 2000) were positively correlated with node age (sympatry = $0.019 + 0.195 \times$ Age; $r^2 = 0.603$, d.f. = 10, $P = 0.008$). However, the logarithmic curve provided a more appropriate fit to the data (sympatry = $1.088 + 5.143 \times$ ln(Age); $r^2 = 0.637$, d.f. = 10, $P = 0.006$; Fig. 3). This relationship suggests that speciation in redstarts was predominantly allopatric. The power curve provided the best fit model for the relationship between clade range symmetry and age, however, even this best fit relationship was not statistically significant (symmetry = $0.689 \times$ Age$^{0.260}$; $r^2 = 0.115$, d.f. = 10, $P = 0.337$. The lack of the relationship between the range symmetry and age is inconsistent with peripatric speciation.

Discussion

Phylogeny of *Phoenicurus*

A previous study on *Phoenicurus* systematics (Ertan, 2006) included nine species, relied solely on partial sequence of the cytochrome-*b* gene, and used outgroups

from distantly related avian families. Virtually all interspecific nodes and all more basal nodes in this study collapse to polytomies (Ertan, 2006) thereby providing little insight into *Phoenicurus* systematics.

By comparison, our results are strongly supported (Fig. 2). Our phylogenetic results for *Phoenicurus* differ somewhat from the reasonably well-supported *Phoenicurus* phylogeny of Voelker (2010). This is not surprising given our inclusion here of two additional taxa (*schisticeps* and *moussieri*), and additional data (the sex-linked ACO1I9 gene). Perhaps the most important difference between Voelker (2010) and this study is that the addition of species and data has provided strong posterior probability support for all nodes in the genus (Fig. 2).

Former *Chaimarrornis* and *Rhyacornis fuliginosa* were originally described as members of *Phoenicurus* (Collar, 2005), and our results indicate that the three species in these genera are indeed members of *Phoenicurus* as was previously suggested by Sangster et al. (2010) and Voelker (2010). It is clear from our results that a possible close relationship between *frontalis* and '*Rhyacornis*', based on tail-flicking behaviour, is not supported (Collar, 2005). Also not supported is the sister relationship between *ochruros* and *phoenicurus* despite these two taxa having hybridized in the past (Collar, 2005).

Biogeography

Voelker (2010) had suggested that *Phoenicurus* originated roughly 6 Mya, and further suggested that many geographic diversification patterns in *Phoenicurus* were consistent with sister lineages having undergone allopatric isolation in Western, Eastern or Southern Palaearctic refugia. Isolation in these refugia was driven either by increased aridity peaks or glacial periods (An, Kukla, Porter, & Xiao, 1991; Donghuai, Zhisheng, Shaw, Bloemendal, & Youbin, 1998; Guo et al., 2002; Kukla & An, 1989; Zhiseng, Kutzbach, Prell, & Porter, 2001) that would have rendered Central Asia inhospitable to songbirds (Voelker, 2010). For *Phoenicurus*, aridity peaks at 4.5 and 3.5 Mya and a glacial peak at 2.4 Mya were implicated as driving factors of broad-scale diversification patterns in the genus (Voelker, 2010).

Our results, which are based on the inclusion of two additional *Phoenicurus* species, an additional independent locus and more robust calibrations of gene evolution through time (Lerner et al., 2011) instead suggest that *Phoenicurus* originated *c*. 7.5 Mya, with lineage diversification within the genus beginning *c*. 6 Ma (Fig. 2). Subsequent divergences are generally consistent with those inferred by Voelker (2010), with the exception of the *fuliginosus*−*bicolour* split which appears to be more recent here (Fig. 2). Overall, most *Phoenicurus* diverged within the Pliocene (Fig. 2). As a consequence, it appears that

both aridity and glacial peaks during that period (Voelker, 2010) played an important role in the broad-scale diversification patterns in the genus.

Our study, which included a larger number of distributional areas than did Voelker (2010) is the first to show that *Phoenicurus* originated in the Himalayas (Fig. 2). A Himalayan ancestral area reconstruction is consistent with the results from a study of another montane specialist avian genus (*Prunella*) that is widely distributed across the Palaearctic (Drovetski et al., 2013). Further, lineage dating of the *Prunella* radiation shows a period of rapid radiation near the Plio-Pleistocene boundary that somewhat post-dates the pulse of lineage divergences within *Phoenicurus*. For *Prunella*, diversification was possibly related to glacial retreats (e.g. after the glacial peak at 2.4 MYA) and the opening of additional habitats/areas during that time (Drovetski et al., 2013). For *Phoenicurus*, the earlier divergences could be related to the opening of additional habitats/areas following aridity peaks, or the fragmentation/isolation of habitats leading up to the late Pliocene glacial peak inferred as driving divergence in *Prunella* (Voelker, 2010).

After initial lineage diversifications in the Himalayas, *Phoenicurus* lineages dispersed from there to colonize other Palaearctic areas, the Philippines, and Northwest Africa (Fig. 3). The colonization of the Philippines (occupied by *bicolour*) is clearly the result of overwater dispersal, as the relevant islands (Luzon and Mindoro) are of oceanic origin (Yumul, Dimalanta, Maglambayan, & Marquez, 2008).

Less easy to explain is the colonization of Africa, where colonization scenarios are dependent on whether Africa is or is not included as a possible ancestral area. If Africa is included as a possible area, ancestral area reconstructions suggest a direct Himalayan to Africa dispersal (Fig. 2). Land-based movement between the Himalayas and Northwest Africa (specifically to the Atlas Mountains in Morocco and Algeria) via colonization of intervening mountain ranges in the Middle East and Africa seems unlikely, given that no range in these regions is high enough to suitably accommodate *Phoenicurus* habitats.

Further, the Himalayan to Northwest African colonization is dated to *c*. 3.5 Mya (Fig. 2). This date is far too recent for the distributions of the Northwest African endemic *moussieri* and the partial ranges in Northwest Africa of *ochruros* and *Ph. phoenicurus* to be explained by colonization during the Messinian Salinity Crisis (MSC). During this period (5.96−5.33 Mya; Krijgsman, Hilgen, Raffi, Sierro, & Wilson, 1999) the Mediterranean became desiccated which would facilitate land-based interchange between Europe, the Middle East and Africa. By greatly post-dating the MSC, overwater dispersal seems necessary to explain the colonization of Africa by the ancestor of these taxa. Under the six distributional area model where Africa is allowed to be ancestral, this explanation assumes colonization from the closest

possible suitable montane habitats (European), and would also require extinction(s) of ancestral lineages in Europe.

Such an explanation would further require subsequent colonization of the Western and Central Palaearctic by both *ochruros* and *Ph. phoenicurus*. In other words, both *ochruros* and *Ph. phoenicurus* would have originated in Northwest Africa (Atlas Mountains) from which they later colonized the Palaearctic, again most likely by over-water dispersal. This scenario is plausible, as North Africa (the Maghreb) has been shown to be (1) an important speciation centre for organisms during the Pliocene and Pleistocene, and (2) a source area for colonizations of Europe (Husemann, Schmitt, Zachos, Ulrich, & Habel, 2013).

Alternatively, if Africa is not considered a unique area and is instead coded as part of the Western Palaearctic, then the distributions of *Ph. phoenicurus* and *ochruros* in Northwest Africa must be related to over-water dispersal from Europe, with a subsequent speciation event to account for the Northwest African endemic *moussieri*. This scenario would eliminate the need to invoke ancestral extinctions in Europe (see above). A reasonable scenario for explaining *Ph. phoenicurus* and *ochruros* distributions in Northwest Africa could be migratory drop-offs into suitable breeding habitats. Both of these species have wintering areas that include portions of Africa (Collar, 2005), and an increasing number of avian studies have provided support for southern sedentary populations/species being established from otherwise migratory species (e.g., Voelker, Bowie, & Klicka, 2013 and references therein). Because this scenario would not require multiple extinctions in Europe (see above) it may be a more reasonable explanation of *Phoenicurus* distributions in Northwest Africa.

Testing between these scenarios can potentially be accomplished by conducting population genetic and phylogeographic analyses of *ochruros* and *phoenicurus*. Depending on gene flow and phylogeographic patterns (if any), results could elucidate whether Africa was colonized from Europe, or vice versa. In the case of *phoenicurus* for example, Hogner et al. (2012) conducted population genetic and phylogeographic analyses which revealed two deep lineages. While these lineages occur in sympatry over much of the sampled range, the study detected the possibility of a distinct southern lineage.

Geographic mode of speciation

Despite a reasonable amount of overlapping distributions across species (Fig. 1) and their inferred ancestral areas, speciation in *Phoenicurus* was allopatric. Irrespective of a linear or logarithmic relationship, the positive correlation between sympatry and node age is significant and strong (Fig. 3). In other words, ranges overlap more for old divergences and less for taxa that diverged recently. With respect to range symmetry, there is a trend between clade ranges and age that would suggest the possibility of

peripatric speciation, but the relationship is not significant. This result mirrors that found for another montane specialist avian genus (*Prunella*; Drovetski et al., 2013), and can likely be explained by the same reason: a combination of mountain specialists whose range size is determined by the size of mountain ranges, and lowland taxa that are widespread. Therefore, while peripatric speciation may be considered the most common mode of speciation in animals (Barraclough & Vogler, 2000), montane regions where habitats are not continuous but are instead island-like in distribution, may preclude peripatric speciation as a driver of divergences.

Although allopatric speciation is inferred as the primary means of speciation in *Phoenicurus*, there are three species pairs that share a restricted portion of their distributional range, as we have defined those ranges. In each case however, ecological or elevational differences serve to separate them in these areas of overlap. In the case of *erythrogastrus* and *hodgsoni*, the former occupies the alpine zone (3900−4500 m), while the latter is restricted to habitats between 2400−3600 m (Collar, 2005). Habitat also serves to separate *moussieri* and *phoenicurus* in Northwest Africa, where *moussieri* occupies xerophytic, open habitat, while *phoenicurus* occupies old oak and conifer forests (Collar, 2005). Finally, *caeruleocephala* tends to be restricted to lower stories of coniferous forests where it geographically overlaps with *schisticeps* (Collar, 2005). Therefore, ecological differences facilitate secondary range overlap of sister taxa rather than ecological speciation.

To conclude, the ancestral area reconstructions presented here for *Phoenicurus*, along with reconstructions for *Leucosticte* finches and *Prunella* accentors (Drovetski et al., 2009, 2013), suggest an emerging pattern where the Himalayas are a source area for montane specialist lineages, which subsequently colonize other Palaearctic regions.

Acknowledgements

Funding

References

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control, 19*, 716−723.

An, Z., Kukla, G. J., Porter, S. C., & Xiao, J. (1991). Magnetic susceptibility evidence of monsoon variation on the Loess Plateau of central China during the last 130,000 years. *Quaternary Research, 36*, 29−36.

Barraclough, T. G., & Vogler, A. P. (2000). Detecting the geographic pattern of speciation from species level phylogenies. *American Naturalist, 155*, 419−434.

Barrera-Guzmán, A. O., Milá, B., Sánchez-González, L. A., & Navarro-Sigüenza, A. G. (2012). Speciation in an avian complex endemic to the mountains of Middle America (*Ergaticus*, Aves: Parulidae). *Molecular Phylogenetics and Evolution, 62*, 907−920.

Brandley, M. C., Schmitz, A., & Reeder, T. W. (2005). Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Systematic Biology, 54*, 373−390.

Brown, J. M., & Lemmon, A. R. (2007). The importance of data partitioning and the utility of bayes factors in Bayesian phylogenetics. *Systematic Biology, 56*, 643−655.

Chaves, J. A., & Smith, T. B. (2011). Evolutionary patterns of diversification in the Andean hummingbird genus *Adelomyia*. *Molecular Phylogenetics and Evolution, 60*, 207−218.

Chesser, R. T., & Zink, R. M. (1994). Modes of speciation in birds: a test of Lynch's method. *Evolution, 48*, 490−497.

Clements, J. F., Schulenberg, T. S., Iliff, M. J., Roberson, D., Fredericks, T. A., Sullivan, B. L., & Wood, C. L. (2014). *The eBird/Clements checklist of birds of the world: Version 6.9*. Ithaca, New York: Cornell University.

Collar, N. J. (2005). Family Turdidae (Thrushes). In J. del Hoyo, A. Elliott, & D. A. Christie (Eds.), *Handbook of Birds of the World, Vol. 10* (pp. 514−807). Barcelona, Spain: Lynx Edicions.

Coyne, J. A., & Price, T. D. (2000). Little evidence for sympatric speciation in island birds. *Evolution, 54*, 2166−2171.

Donghuai, S., Zhisheng, A., Shaw, J., Bloemendal, J., & Youbin, S. (1998). Magnetostratigraphy and palaeoclimatic significance of Late Tertiary Aeolian sequences in the Chinese Loess Plateau. *Geophysical Journal International, 34*, 207−212.

Drovetski, S. V. (2003). Plio-Pleistocene climatic oscillations, Holarctic biogeography and speciation in an avian subfamily. *Journal of Biogeography, 30*, 1173−1181.

Drovetski, S. V., Raković, M., Semenov, G., Fadeev, I. V., & Red'kin, Ya.A. (2014). Limited phylogeographic signal in sex-linked and autosomal loci despite geographically, ecologically, and phenotypically concordant structure of mtDNA variation in the Holarctic avian genus *Eremophila*. *Public Library of Science ONE, 9*, e87570.

Drovetski, S. V., Semenov, G., Drovetskaya, S. S., Fadeev, I. V., Red'kin, Y. A., & Voelker, G. (2013). Geographic mode of speciation in a mountain specialist avian family endemic to the Palearctic. *Ecology and Evolution, 3*, 1518−1528.

Drovetski, S. V., Zink, R. M., Ericson, P. G. P., & Fadeev, I. V. (2010). A multi-locus study of pine grosbeak phylogeography supports the pattern of greater intercontinental divergence in Holarctic boreal forest birds compared to birds inhabiting other high-latitude habitats. *Journal of Biogeography, 37*, 696−706.

Drovetski, S. V., Zink, R. M., Fadeev, I. V., Nesterov, E. V., Koblik, E. A., Red'kin, Y. A., & Rohwer, S. A. (2004b). Mitochondrial phylogeny of *Locustella* and related genera. *Journal of Avian Biology, 35*, 105−110.

Drovetski, S. V., Zink, R. M., & Mode N. A. (2009). Patchy distributions belie morphological and genetic homogeneity in rosy-finches. *Molecular Phylogenetics and Evolution, 50*, 437−445.

Drovetski, S. V., Zink, R. M., Rohwer, S., Fadeev, I. V., Nesterov, E. V., Karagodin, I. Y., . . . Red'kin, Y. A. (2004a). Complex biogeographic history of a Holarctic passerine. *Proceedings of the Royal Society of London B, 21*, 545−551.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution, 29*, 1969−1973.

Ertan, K. T. (2006). The evolutionary history of Eurasian redstarts, *Phoenicurus. Acta Zoologica Sinica, 52*, 310−313.

Fitzpatrick, B. M., & Turelli, M. (2006). The geography of mammalian speciation: mixed signals from phylogenies and range maps. *Evolution, 60*, 601−615.

Fjeldså, J., & Bowie, R. C. K. (2008). New perspectives on the origin and diversification of Africa's forest avifauna. *African Journal of Ecology, 46*, 235−247.

Friesen, D. L., & Anderson, D. L. (1997). Phylogeny and evolution of the Sulidae (Aves: Pelecaniformes): a test of alternative modes of speciation. *Molecular Phylogenetics and Evolution, 7*, 252−260.

Guo, Z. T., Ruddiman, W. F., Hao, Q. Z., Wu, H. B., Qiao, Y. S., Zhu, R. X., . . . Liu, T. S. (2002). Onset of Asian desertification by 22 Myr ago inferred from loess deposits in China. *Nature, 416*, 159−163.

Gutiérrez-Pinto, N., Cuervo, A. M., Miranda, J., Pérez-Emán, J. L., Brumfield, R. T., & Cadena, C. D. (2012). Non-monophyly and deep genetic differentiation across low-elevation barriers in a Neotropical montane bird (*Basileuterus tristriatus*; Aves: Parulidae). *Molecular Phylogenetics and Evolution, 64*, 156−165.

Hogner, S., Laskemoen, T., Lifjeld, J. T., Porkert, J., Kleven, O., Albayrak, T., . . . Johnsen, A. (2012). Deep sympatric mitochondrial divergence without reproductive isolation in the common redstart *Phoenicurus phoenicurus. Ecology and Evolution, 2*, 2974−2988.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics, 17*, 754−755.

Hung, C.-M., Drovetski, S. V., & Zink, R. M. (2012). Multilocus coalescence analyses support a mtDNA-based phylogeographic history for a widespread Palearctic passerine bird, *Sitta europaea. Evolution, 66*, 2850−2864.

Husemann, M., Schmitt, T., Zachos F. E., Ulrich, W., & Habel, J. C. (2013). Palaearctic biogeography revisited: evidence for the existence of a North African refugium for Western Palaearctic biota. *Journal of Biogeography, 41*, 81−94.

Johansson, U., Alström, P., Olsson, U., Ericson, P. G. P., Sundberg, P., & Price, T. D. (2007). Build-up of the Himalayan avifauna through immigration: a biogeographical analysis of the *Phylloscopus* and *Seicercus* warblers. *Evolution, 61*, 324−333.

Krijgsman, W., Hilgen, F. J., Raffi, I., Sierro, F. J., & Wilson, D. S. (1999). Chronology, causes and progression of the Messinian salinity crisis. *Nature, 400*, 652−655.

Kukla, G. C., & An, Z. (1989). Loess stratigraphy in central China. *Palaeogeography, Palaeoclimatology, Palaeoecology, 72*, 203−225.

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. *Current Biology, 21*, 1838−1844.

Mayr, E. (1942). *Systematics and the origin of species*. New York: Columbia University Press.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. M & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature, 403*, 853−858.

Nylander, J. A. A. (2004). *MrModeltest v2. Program distributed by the author*. Evolutionary Biology Centre: Uppsala University.

Orme, C. D. L., Davies, R. G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V. A., . . . Owens, I. P. F. (2005). Global hotspots of species richness are not congruent with endemism or threat. *Nature, 436*, 1016−1019.

Phillimore, A. B., Orme, C. D. L., Thomas, G. H., Blackburn, T. M., Bennett, P. M., Gaston, K. J., & Owens, I. P. F. (2008). Sympatric speciation in birds is rare: insights from range data and simulations. *American Naturalist, 171*, 646−657.

Posada, D. (2008). jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution, 25*, 1253−1256.

Price, T. (2008). *Speciation in Birds*. Greenwood Village, Colorado: Roberts and Company.

Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology, 57*, 4−14.

Sangster, G. L., Alström, P., Porsmark, E., & Olsson, U. (2010). Multi-locus phylogenetic analysis of Old World chats and flycatchers reveals extensive paraphyly at family, subfamily and genus leves (Aves: Muscicapidae). *Molecular Phylogenetics and Evolution, 57*, 380−392.

Sousa-Santos, C., Robalo, J. I., Collares-Pereira, M.-J., & Almada, V. C. (2005). Heterozygous indels as useful tools in the reconstruction of DNA sequences and in the assessment of ploidy level and genomic constitution of hybrid organisms. *DNA Sequence, 16*, 462−467

Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *The American Journal of Human Genetics, 68*, 978−989.

Voelker, G. (1999). Dispersal, vicariance, and clocks: historical biogeography and speciation in a cosmopolitan passerine genus (Anthus: Motacillidae). *Evolution, 53*, 1536−1552.

Voelker, G. (2002). Systematics and historicalbiogeography of wagtails: dispersal versus vicariance revisited. *Condor, 104*, 725−739.

Voelker, G. (2010). Repeated vicariance of Eurasian songbird lineages since the late Miocene. *Journal of Biogeography, 37*, 1251−1261.

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. *Molecular Ecology, 22*, 3333−3344.

Yumul Jr., G. P., Dimalanta, C. B., Maglambayan, V. B., & Marquez, E. J. (2008). Tectonic setting of a composite terrane: a review of the Philippine island arc system. *Geosciences Journal, 12*, 7−17.

Zhisheng, A., Kutzbach, J. E., Prell, W. L., & Porter, S. C. (2001). Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. *Nature, 411*, 62−66.

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

Associate Editor: Elliot Shubert