



Diversification across the New World within the 'blue' cardinalids (Aves: Cardinalidae)

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ABSTRACT

Aim To examine the history of diversification of 'blue' cardinalids (Cardinalidae) across North and South America.

Location North America (including Middle America) and South America.

Methods We collected 163 individuals of the 14 species of blue cardinalids and generated multilocus sequence data (3193 base pairs from one mitochondrial and three nuclear genes) to infer phylogeographical structure and reconstruct time-calibrated species trees. We then estimated the ancestral range at each divergence event and tested for temporal shifts in diversification rate.

Results Twenty-five lineages of blue cardinalids clustered into two major clades: one confined to North America, and a second concentrated in South America. Blue cardinalids probably originated in North America, but reconstructions were influenced by how migrant taxa were assigned to biogeographical regions. Most of the pre-Pleistocene divergences between extant taxa occurred in the North American clade, whereas most divergences in South America and adjacent Middle America occurred during the Pleistocene. Despite these differences, the rate of diversification for both clades has been similar and relatively constant over the past 10 million years, with little geographical exchange between North and South America outside the Panamanian isthmus region.

Main conclusions Our reconstruction of the diversification history of blue cardinalids indicates a role of both Neogene and Quaternary events in generating biotic diversity across North and South America. Although ancestral area reconstruction suggests a possible North American origin for blue cardinalids, the occurrence of seasonal migration in this group and their relatives limits inference. Our study highlights the importance of considering ecological and behavioural characteristics together with palaeogeological events in order to gain an understanding of the diversification history of widespread, mobile taxonomic groups.

Keywords

Ancestral area, birds, *Cyanocompsa*, dispersal, diversification rates, migration, Neotropics, *Passerina*, phylogeography.

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INTRODUCTION

Patterns of diversification within widespread lineages provide insight into the broad-scale processes that generate biotic diversity (Avice, 2000). Studies on the origins of taxa and diversification across the New World provide examples of how geological processes (Bryson *et al.*, 2012), climate

change (Weir & Schluter, 2004), niche conservatism (Smith *et al.*, 2012a) and the colonization of novel regions (Simpson, 1980) influence lineage accumulation. The two continents of the New World, North and South America, have a number of important differences that impacted diversifications, including connections with other landmasses (Webb, 1991), geographical size (Vrba, 1992), climatic stability

(Hawkins *et al.*, 2006) and niche availability (Ricklefs, 2002). Consequently, modes and rates of evolutionary radiations on these continents may have differed (Rabosky & Lovette, 2008; Derryberry *et al.*, 2011).

Much research on avian diversification in North America has focused on the impact of Quaternary climate change (Klicka & Zink, 1997; Avise & Walker, 1998; Johnson & Cicero, 2004). Although divergences between bird species are generally much older than the late Pleistocene (Klicka & Zink, 1997), many species exhibit phylogeographical structure that was probably generated throughout the Pleistocene (e.g. Klicka *et al.*, 2011; Smith *et al.*, 2011; van Els *et al.*, 2012). The overall rate of diversification in North American birds during the Pleistocene is less well understood. Parts of northern North America were periodically covered with glacial ice during the Pleistocene, causing dramatically fluctuating biotic communities (Webb & Bartlein, 1992). Although habitat fragmentation promoted diversification in some birds (Weir & Schluter, 2004), decreasing habitat availability limited ecological opportunities across much of temperate North America, potentially causing a decreasing rate of diversification (Rabosky & Lovette, 2008) or an absence of a rate increase (in part owing to higher extinction rates; Zink & Slowinski, 1995; Zink *et al.*, 2004; Zink & Klicka, 2006). This absence of an increasing diversification rate during the Pleistocene has also been observed in several co-distributed, wide-ranging, non-avian taxa (snakes: Burbrink & Pyron, 2010; lizards: Harmon *et al.*, 2003; and bats: Barber & Jensen, 2012). In Middle America (herein considered a part of the North American continent; Winker, 2011), the impact of glaciers displacing entire biotas was less extreme (Metcalf *et al.*, 2000). In this region, cyclical habitat fragmentation driven by Pleistocene glacial–interglacial cycles across a more topographically complex area may have triggered diversification in many birds (Miller *et al.*, 2011; Barrera-Guzmán *et al.*, 2012; Smith *et al.*, 2012b).

In South America, avian species diversity reaches its pinnacle (Haffer, 1990), and the processes producing this diversity probably span a temporal continuum (Rull, 2011), from the early Cenozoic (Hawkins *et al.*, 2006) to the end of the Pleistocene (Ribas *et al.*, 2012). A variety of historical processes and the formation of biogeographical barriers, for example Andean uplift, Pleistocene forest fragmentation and riverine barriers, have been linked to this diversification (Hoorn *et al.*, 2010; Ribas *et al.*, 2012). However, the effect of Quaternary processes on the generation of avian species diversity across South America is still debated (Bates *et al.*, 2003; Hoorn *et al.*, 2010; Smith *et al.*, 2012a). Clearly, diversification in many South American birds occurred during the Pleistocene (e.g. Patel *et al.*, 2011; Gutiérrez-Pinto *et al.*, 2012; d’Horta *et al.*, 2012; Smith *et al.*, 2013), but what remains unclear is the relative importance of processes occurring during the past 2.6 Myr compared to events occurring earlier, during the Neogene (the 20 Myr preceding the Pleistocene).

We examined diversification within a widespread group of songbirds in the ‘blue’ clade of Cardinalidae (*sensu* Klicka *et al.*, 2007; hereafter referred to as the blue cardinalids). The 14 species in this group collectively range across the entire New World, from Canada south to Argentina (Figs 1 & 2; Orenstein & Brewer, 2011), making this an attractive species complex for examining diversification patterns at continental scales. Three species of *Passerina* (*P. amoena*, *P. ciris* and *P. cyanea*) breed in temperate North America (Canada south to northern Mexico), and three additional *Passerina* species (*P. leclancherii*, *P. rositae*, *P. versicolor*) and *Cyanocompsa parrellina* are restricted to Middle America (Mexico to Nicaragua). *Amaurospiza carrizalensis*, *Amaurospiza moesta*, *Cyanocompsa brissonii* and *Cyanoloxia glaucocaerulea* are residents of South America. *Passerina caerulea* is distributed widely across both temperate North America and Middle America, and *Amaurospiza concolor* and *Cyanocompsa cyanooides* range across Middle America into South America. Seasonal migration is a life history strategy among many species in this group, simultaneously providing an opportunity to determine whether it might affect diversification rates and a potential problem in determining ancestral areas.

To address the relative impact of Quaternary and pre-Quaternary processes on the tempo and mode of diversification in blue cardinalids, and to assess possible effects of seasonal migration, we combined intraspecific range-wide sampling with multilocus data. We used mitochondrial DNA (mtDNA) and three nuclear loci to infer phylogeographical structure and estimate time-calibrated species trees. We then reconstructed the ancestral range at each divergence event and tested for temporal shifts in diversification rates.

MATERIALS AND METHODS

Taxon sampling and laboratory methods

We collected 163 individuals of blue cardinalids (Klicka *et al.*, 2007) from throughout their distributions (Figs 1 & 2, and see Appendix S1 in the Supporting Information). Our sampling spanned the geographical distributions of all 14 currently recognized species and of 20 of the 30 putative subspecies within this group (Clements, 2007). We included the sister species *Spiza americana*, and used *Pheucticus ludovicianus* and *Habia fuscicauda* as more distant outgroups (Klicka *et al.*, 2007; Barker *et al.*, 2012).

Total genomic DNA was extracted using a DNeasy tissue extraction kit (Qiagen, Valencia, CA, USA). We sequenced the mtDNA gene *ND2* (1038 base pairs, bp) for all 163 individuals and outgroups, and for a subset of specimens ($n = 37$) representing the main lineages inferred from our complete mtDNA data (see below). We also sequenced three nuclear introns, including 951 bp of aconitase 1 intron 9 (*ACO1*), 685 bp of myoglobin intron 2 (*MYC*), and 519 bp of beta-fibrinogen intron 5 (*FGB-15*). Laboratory protocols and primers follow Smith & Klicka (2013).

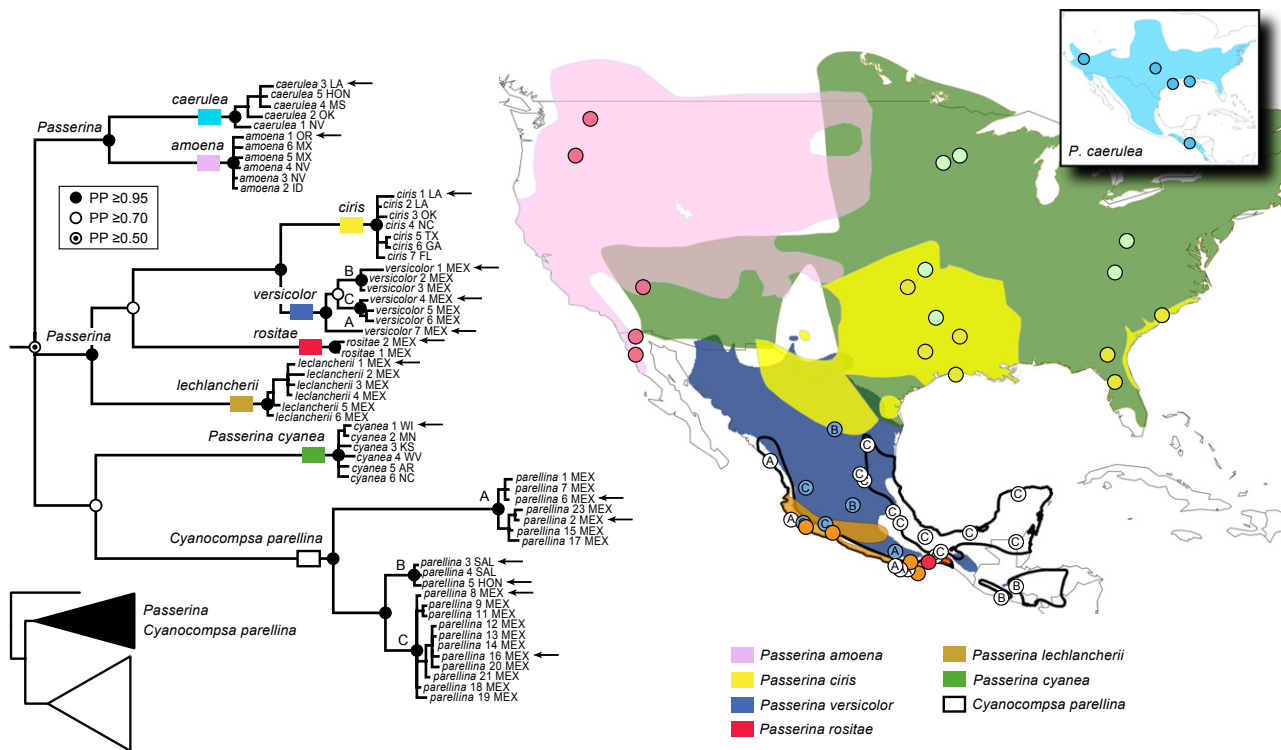


Figure 1 The blue cardinalids of North America. The map on the right shows the breeding distributions of the eight currently recognized species in North America (from Ridgely *et al.*, 2007) and the localities of the specimens sampled. The mitochondrial phylogeny on the left was used to infer phylogeographical structure, and major geographical lineages are noted. Bayesian posterior probability (PP) support for nodes is indicated by coded dots. Multilocus data were generated from specimens marked with an arrow. Additional locality data can be found in Appendix S1.

We edited and manually aligned forward and reverse sequences using SEQUENCHER 4.2 (Gene Codes Corporation, Ann Arbor, MI, USA). INDELIGENT 1.2 (Dmitriev & Rakitov, 2008) was used to resolve indels between homologous nuclear alleles. The gametic phase of heterozygous variants was determined using PHASE 2.1.1 (Stephens & Donnelly, 2003). For each nuclear dataset, separate runs of 400 iterations each were performed, accepting results with probability ≥ 0.7 . All polymorphic sites with probability < 0.7 were coded in both alleles with the appropriate IUPAC ambiguity code. The *ACO1* intron is sex-linked on the Z-chromosome, and so only males contain two alleles. We therefore cross-checked sequences against the sexes of our vouchered specimens (Appendix S1). Male specimens were retained with biallelic states, whereas phased *ACO1* sequences from females and specimens of unknown sex were reduced to only one allele. Sequences were deposited in Dryad (see Data Accessibility below).

Phylogeographical estimation

To assess range-wide genetic structure and delineate geographical lineages, we generated a mtDNA phylogeny of all individuals ($n = 166$ including outgroups) using MRBAYES 3.2.1 (Ronquist *et al.*, 2012). Lineages were defined as genetically distinct geographical clusters with strong support values

(≥ 0.95 Bayesian posterior probability; Huelsenbeck & Rannala, 2004), consistent with the term 'phylogroup' (Avise & Walker, 1998; Avise *et al.*, 1998; Rissler & Apodaca, 2007). Single divergent samples from unique geographical areas were also referred to as lineages for convenience. We implemented two separate models for the first plus second and for the third codon positions. jMODELTEST 0.1.1 (Posada, 2008) was used to select the best-fit model of evolution, based on the Akaike information criterion (AIC), for each partition (1st+2nd: HKY + I + G; 3rd: GTR + I). Four runs were conducted using the 'nrns = 4' command, each with three heated and one cold Markov chain sampling every 100 generations for 10 million generations. Changing the temperature of the heated chain from the default value of 0.1 to 0.03 improved the efficiency of the Metropolis coupling. Output parameters were visualized using TRACER 1.4 (Rambaut & Drummond, 2007) to ascertain stationarity and convergence. We further assessed convergence between runs using AWTY (Nylander *et al.*, 2008). The first 25% of generations were discarded as burn-in.

Species trees and divergence times

We used *BEAST (Heled & Drummond, 2010), part of the BEAST 1.7.4 package (Drummond & Rambaut, 2007), to

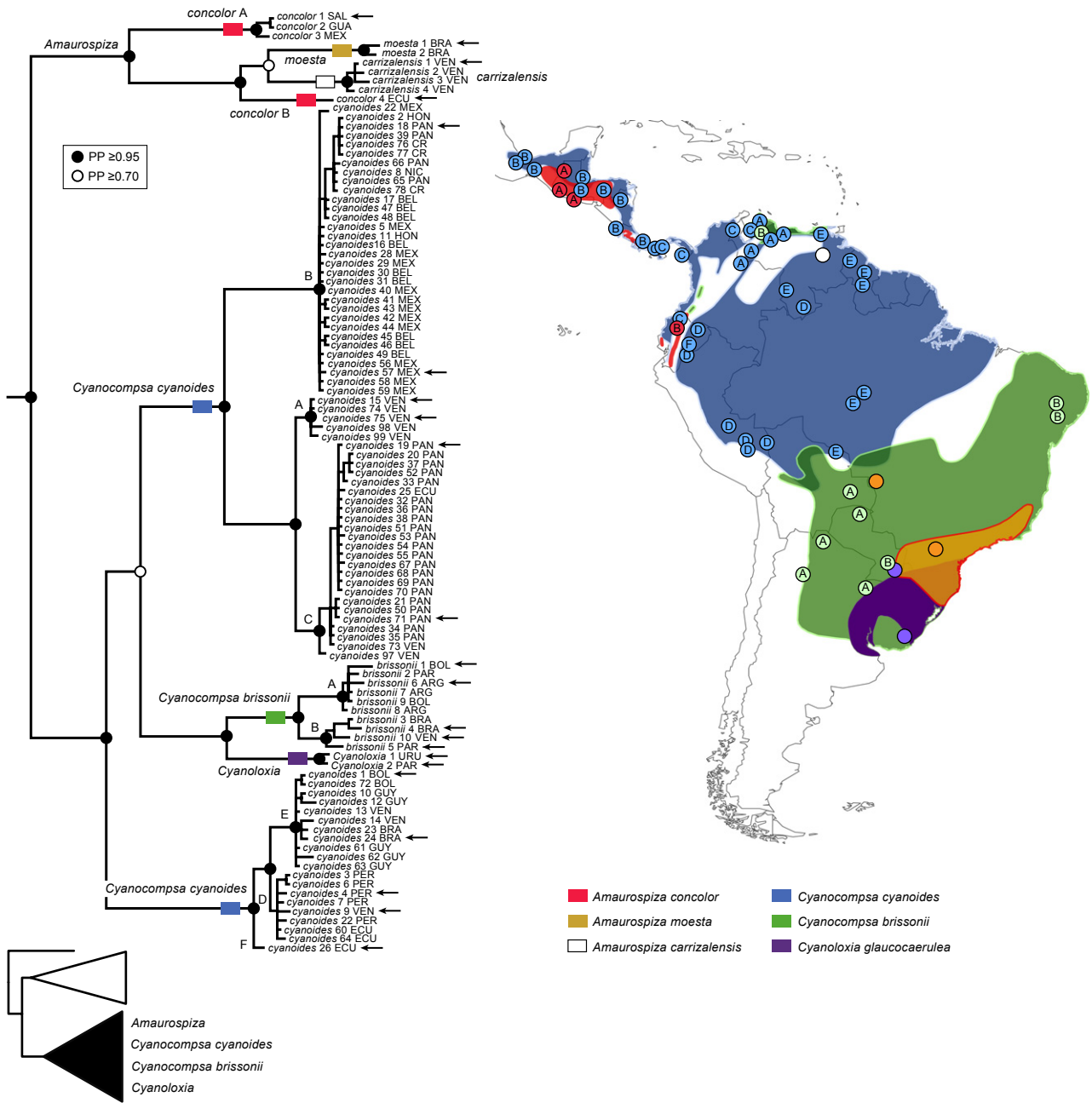


Figure 2 The blue cardinalids of South America and adjacent countries. The map on the right shows the breeding distributions of the six currently recognized species in this region (from Ridgely *et al.*, 2007) and the localities of the specimens sampled. The mitochondrial phylogeny on the left was used to infer phylogeographical structure, and major geographical lineages are noted. Bayesian posterior probability (PP) support for nodes is indicated by coded dots. Multilocus data were generated from specimens marked with an arrow. Additional locality data can be found in Appendix S1.

reconstruct a time-calibrated multilocus species tree using 1–4 individuals ($n = 37$) from each currently recognized species or phylogeographical lineage inferred from our complete mtDNA data and the three outgroups. We used a subset of individuals in order to capture genetic diversity but reduce computational burden and sequencing costs. Best-fit models of evolution were estimated using jMODELTEST (*ND2*: GTR + I + G; *ACO1*, *FGB-15*: GTR + G; *MYC*: HKY + G).

We used a Yule speciation prior and relaxed uncorrelated lognormal clock for each gene tree. To calibrate our species tree, we used the *ND2* substitution rate of 1.25×10^{-2} substitutions/site/Myr (2.5% change between lineages per Myr) from Smith & Klicka (2010), and of 1.35×10^{-3} substitutions/site/Myr for autosomal (*MYC*, *FGB-15*) and 1.45×10^{-3} substitutions/site/Myr for sex-linked (*ACO1*) intron rates (Ellegren, 2007). We specified a lognormal

distribution and a relatively wide logarithmic standard deviation of 0.2 for each gene, thus encompassing alternative substitution rates (e.g. Lerner *et al.*, 2011). Analyses were run for 100 million generations and sampled every 1000 generations. TRACER was used to confirm acceptable mixing and likelihood stationarity, appropriate burn-in, and adequate effective sample sizes (> 200). After discarding the first 10 million generations (10%) as burn-in, the parameter values of the samples from the posterior distribution were summarized on the maximum clade credibility tree using TREEANNOTATOR 1.7.4 (Drummond & Rambaut, 2007).

We reconstructed a second species tree from the full mtDNA dataset to estimate divergences based on all 163 specimens of blue cardinalids and the three outgroups. Although mtDNA provides only a single-gene estimate of a species tree, *BEAST can estimate divergence times from mtDNA data that account for gene divergences that may pre-date species divergences (Heled & Drummond, 2010). This approach allowed us to run biogeographical analyses using time-calibrated species trees estimated from two distinct datasets. Following the same approach as outlined above, each specimen was assigned to a currently recognized species or geographical lineage inferred from our mtDNA gene tree. We calibrated the tree using the same ND2 substitution rate of 1.25×10^{-2} substitutions/site/Myr, and analyses were run for 100 million generations. TRACER was used to confirm acceptable mixing and stationarity, appropriate burn-in, and adequate effective sample sizes. The first 10 million generations were discarded as burn-in, and the posterior parameter values were summarized on the maximum clade credibility tree using TREEANNOTATOR.

Ancestral area reconstruction

The ancestral range at each divergence event was reconstructed using Bayesian binary Markov chain Monte Carlo (MCMC) analysis (BBM) as implemented in RASP 2.0b (Yu *et al.*, 2011). We ran independent analyses on 40,000 post-burn-in trees produced from our multilocus species tree and mtDNA species tree reconstructions to account for phylogenetic uncertainty and topological differences between these two phylogenies. Each sample from our species trees was assigned to one or more of three broad biogeographical regions (following Winker, 2011): (A) temperate North America (including the northern Chihuahuan Desert), (B) Middle America, and (C) South America (including central and eastern Panama). The probabilities for nodes in each phylogeny with > 0.90 posterior probability (PP) were estimated to incorporate information from most nodes (17/25) of the tree but minimize phylogenetic 'noise' from poorly supported relationships. The number of areas was set to 3, a F81 + G model (the most complex model available in RASP) was used, and analyses were conducted for 1 million generations using 10 chains, sampling every 100 generations. *Spiza americana* was set as the outgroup. The first 25% of generations were discarded as burn-in.

Attempts to infer the origin of migratory bird species have been contentious (Zink, 2002; Joseph, 2005). Consistent with the hypothesis that Cardinalidae is derived from a North American ancestor (Barker *et al.*, 2004; Klicka *et al.*, 2007), we chose to assign migratory species to biogeographical areas based on their breeding distributions. However, this assumes that the distributions of blue cardinalids have been static through time, and that current breeding distributions reflect areas of species origins. To test these assumptions, we ran two additional analyses using the multilocus dataset in RASP (Appendix S2). In the first of these, we assigned wintering areas rather than breeding areas as points of origin. In the second, we redefined areas of origin to include both breeding and wintering areas. Finally, because outgroup choice and their distributions can influence ancestral area reconstructions, we ran one more analysis using the multilocus dataset to assess the effect of including four more outgroups on ancestral area reconstructions (Appendix S2).

Diversification rates

We analysed temporal shifts in diversification rates within blue cardinalids using maximum likelihood-based diversification-rate analysis (Rabosky, 2006a) and divergence dates estimated from both the multilocus and the mtDNA datasets. The fits of different birth–death models implementing two constant rates (pure birth and birth–death) and three variable rates (exponential and logistic density-dependent and two-rate pure birth) were computed with LASER 2.3 (Rabosky, 2006b). Model fit was measured using AIC scores, and the significance of the change in AIC scores between the best rate-constant and best rate-variable model was determined through simulations implemented in LASER. Log-likelihood and AIC values were calculated for three models (SPVAR, EXVAR and BOTHVAR; Rabosky & Lovette, 2008) that permit differential extinction and speciation rates. We repeated the analyses and generated lineage-through-time (LTT) plots to visualize the tempo of diversification within the two major geographical clades (North American and predominantly South American) inferred from our multilocus species tree reconstructions.

RESULTS

Phylogeographical estimation

Within blue cardinalids, we identified two major mtDNA clades with 25 evolutionary lineages (Figs 1 & 2). A northern 'North American' clade contained all *Passerina* species and a single member of *Cyanocompsa* (*C. parellina*; Fig. 1), rendering *Cyanocompsa* polyphyletic. Support for this clade was weak (PP = 0.56). All *Passerina* species lacked strong phylogeographical structure, with the exception of *P. versicolor*, which formed three geographical lineages. The Middle American endemic *Cyanocompsa parellina* was also structured into three geographically isolated lineages. Considerably more

structured variation occurred in the strongly supported southern clade, which contained the three *Amaurospiza* species, *Cyanoloxia*, and the remaining two members of *Cyanocompsa* (*C. brissonii* and *C. cyanooides*; Fig. 2). *Amaurospiza concolor* showed distinct Middle American and South American lineages, with the latter more closely related to the other South American representatives of the genus. *Amaurospiza moesta* and *A. carrizalensis* each exhibited shallow genetic structure. *Cyanocompsa cyanooides*, *C. brissonii* and *Cyanoloxia glaucoacaerulea* were part of a three-way polytomy comprising (1) *Cyanocompsa cyanooides* east of the Andes, which formed three shallow lineages; (2) *Cyanocompsa cyanooides* west of the Andes and along the eastern flanks of the northern Andes, which were further divided into three well-diverged lineages

from Middle America and northern South America; and (3) a third group in which the South American endemics *Cyanocompsa brissonii* (two lineages) and *Cyanoloxia glaucoacaerulea* (monotypic, single lineage) were revealed to be sister taxa (Fig. 2). Our dense intraspecific sampling suggested that, in general, species diversity is underestimated.

Species trees and divergence times

Complete genetic data could not be obtained for six specimens used in our multilocus species tree analyses (Appendix S1). In particular, we could not obtain any nuclear data for the divergent sample *A. concolor* B (Fig. 2), so this specimen was represented by only mtDNA data. Our multilocus species

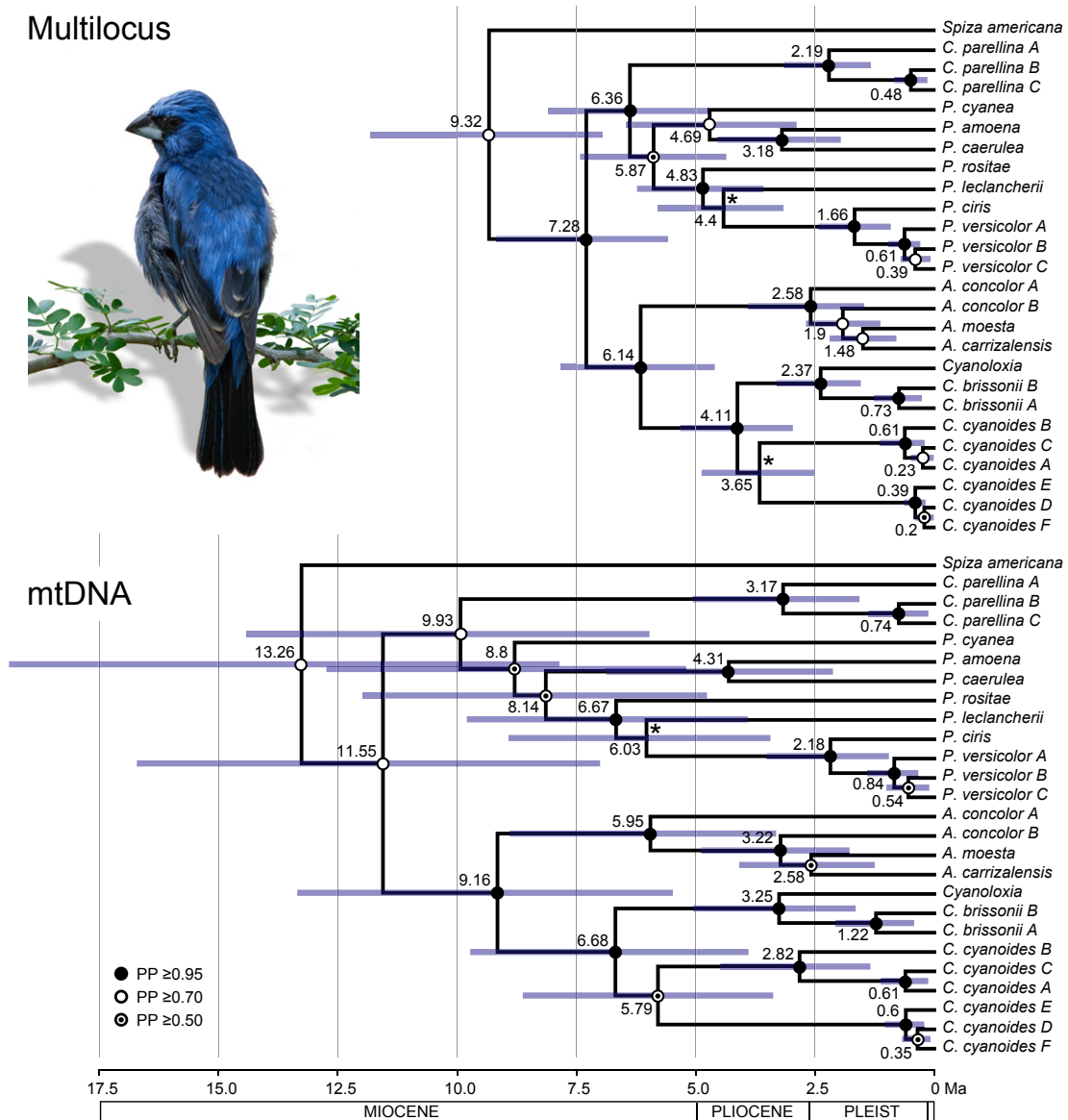


Figure 3 Species tree reconstructions for blue cardinalids estimated from multilocus and mtDNA datasets using *BEAST. Bars indicate the 95% highest posterior densities of divergence dates. The mean estimated dates above nodes and the scale bar are in millions of years ago (Ma). Bayesian posterior probability (PP) support for nodes is indicated by coded dots; nodes that received less than 0.50 support are marked with an asterisk. Pleist = Pleistocene. Photo of *Cyanocompsa brissonii* by Arjan Haverkam.

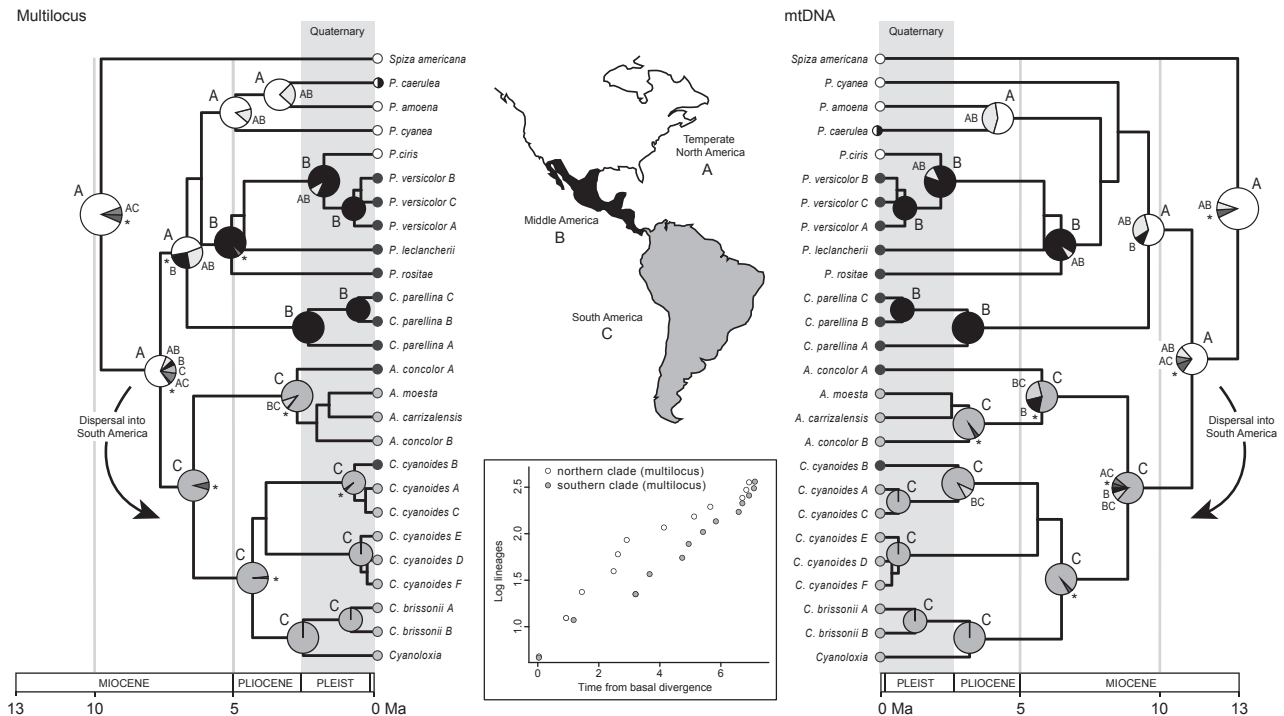


Figure 4 Time-calibrated species trees for blue cardinalids showing ancestral area reconstructions derived from multilocus and mtDNA datasets. Pie charts indicate the relative probability of ancestral areas for nodes in the species tree that received ≥ 0.90 posterior probability support. Asterisks refer to alternative area reconstructions with $\leq 5\%$ RASP probability. Pleist = Pleistocene. The inset shows lineage-through-time plots for the northern (*Passerina* and *Cyanocompsa parellina*) and southern (*Amaurospiza*, *Cyanocompsa cyanoides*, *Cyanocompsa brissonii* and *Cyanoloxia*) clades derived from divergence dates obtained from the multilocus species tree.

tree produced a time-calibrated phylogenetic reconstruction for blue cardinalids with moderate resolution and nodal support (60% of nodes with PP > 0.95; Fig. 3). Importantly, our multilocus and mtDNA species tree topologies were nearly identical and differed only in the placement of *P. cyanea* (Fig. 3). This species was placed as sister to *P. amoena* + *P. caerulea* (multilocus species tree, PP = 0.91) or as basal to all other *Passerina* (mtDNA species tree, PP = 0.55). Support for the North American clade also increased from a PP of 0.56 in the mtDNA gene tree (Fig. 1) to 0.93 and 0.95 in the mtDNA and multilocus species trees, respectively.

Estimates of divergence differed between mtDNA data and the multilocus species tree (Fig. 3). The basal split of blue cardinalids from *Spiza americana*, for example, differed by 4 Myr. Mean estimated divergence dates in the mtDNA species tree were always older, and 95% highest posterior density (HPD) intervals were much wider than the multilocus species tree estimates, as expected (Edwards & Beerli, 2000).

Results from our multilocus species tree suggested a late Miocene divergence of blue cardinalids from the sister species *S. americana* (mean estimated date 9.3 Ma, 95% HPD: 11.8–6.9 Ma). The main northern and southern clades subsequently diverged at *c.* 7.3 Ma (95% HPD: 9.2–5.6 Ma). Diversification within the North American clade began with the early divergence of *Cyanocompsa parellina* at *c.* 6.4 Ma (95% HPD: 8.1–4.7 Ma), and continued throughout the

Pliocene and into the Pleistocene; five divergences occurred within the Pliocene, and four occurred within the Pleistocene. In contrast, divergences within the predominantly South American clade occurred primarily during the Pleistocene. After the early divergence of *Amaurospiza* from the rest of the South American lineages at 6.1 Ma (95% HPD: 7.8–4.6 Ma), two divergences occurred during the Pliocene, followed by nine divergences scattered throughout the Pleistocene.

Ancestral area reconstruction

RASP analyses of the multilocus and mtDNA datasets were similar (Fig. 4), so we used the former. The inferred origin of blue cardinalids based on breeding distributions as areas was in temperate North America (66% RASP probability). One of the two major daughter clades probably originated through subsequent dispersal into South America during the late Miocene. This dispersal event involved a South American ancestor of two clades (*Amaurospiza*, and *Cyanocompsa cyanoides* + *Cyanocompsa brissonii* + *Cyanoloxia glaucoacaerulea*) and was supported by a RASP probability of 92%. The ancestors of two lineages, *A. concolor* B and *Cyanocompsa cyanoides* B, later dispersed back into Middle America. The second major daughter clade probably originated from a temperate North American ancestor of *Passerina* and *Cyanocompsa*

Table 1 Summary of diversification models fitted to the branching times derived from multilocus and mtDNA species tree reconstructions for blue cardinalids. Log-likelihood (lnL) values and Akaike information criterion (AIC) scores for each model are provided. The AIC scores from the best-fitting constant or variable model were determined using simulations and are given in bold text. Rate-variable models were exponential and logistic density-dependent (DDX, DDL), two-rate pure birth (Yule2), time-varying speciation (SPVAR), time-varying extinction (EXVAR), and time-varying speciation and extinction (BOTHVAR).

	Rate-constant models		Rate-variable models					
	Pure birth	Birth–death	DDX	DDL	Yule2	SPVAR	EXVAR	BOTHVAR
Multilocus								
lnL	3.6617	3.6670	3.7547	3.7119	4.7896	3.6832	3.6670	3.6896
AIC	−5.3235	−3.3341	−3.5093	−3.42389	−3.5793	−1.3664	−1.3341	0.6208
mtDNA								
lnL	−8.0865	−8.0865	−7.3309	−7.5628	−6.4231	−7.7036	−8.0907	−7.6570
AIC	18.1730	20.1730	18.6617	19.1256	18.8461	21.4071	22.1814	23.3140

Table 2 Summary of diversification models fitted to the branching times within the two major clades (northern and southern) of blue cardinalids derived from the multilocus species tree reconstructions. Log-likelihood (lnL) values and Akaike information criterion (AIC) scores for each model are provided. The AIC scores from the best-fitting constant or variable model were determined using simulations and are given in bold text. Rate-variable models were exponential and logistic density-dependent (DDX, DDL), two-rate pure birth (Yule2), time-varying speciation (SPVAR), time-varying extinction (EXVAR), and time-varying speciation and extinction (BOTHVAR).

	Rate-constant models		Rate-variable models					
	Pure birth	Birth–death	DDX	DDL	Yule2	SPVAR	EXVAR	BOTHVAR
Northern clade								
lnL	−7.49523	−7.49538	−6.42670	−6.66959	−5.75476	−7.06603	−7.49524	−7.03466
AIC	16.99048	18.99048	16.85340	17.33919	17.50952	20.13207	20.99048	22.06934
Southern clade								
lnL	−3.576302	−3.23708	−3.54427	−3.57631	−2.57868	−3.23735	−3.23784	−3.23735
AIC	9.152603	10.47416	11.08854	11.15263	11.15737	12.47471	12.47568	14.47471

parellina (47% probability), although alternative reconstructions all contained a Middle American ancestor. Middle America was later colonized by *Cyanocompsa parellina* and several species of *Passerina* (*P. versicolor*, *P. lechlancherii*, *P. rositae* and *P. caerulea*).

Our reconstructions using alternative distributions as areas of origin for migratory species (1 – wintering areas, 2 – combined wintering and breeding areas) indicated, as expected, that the above results were highly contingent upon our initial assumptions of static breeding distributions accurately reflecting areas of origin. When the areas of origin for migrants included only wintering ranges, the inferred origin for blue cardinalids switched from temperate North America to a more probable Middle America + South America origin. When the phylogeny was rooted with multiple outgroups, the probability of confidently inferring an ancestral area for blue cardinalids decreased, and all area combinations received low support (additional details in Appendix S2).

Diversification rates

Birth–death likelihood analyses of lineage diversification rates within all blue cardinalids failed to reject the null hypothesis of rate constancy for both datasets ($P = 0.85$, multilocus species tree; $P = 0.49$, mtDNA species tree; Table 1). Under the

best-fit rate-constant model of pure birth, the lineage diversification rate in blue cardinalids remained constant through time, with diversification rates estimated at 0.1731 (multilocus) and 0.2824 (mtDNA) divergence events per lineage per million years. Models considering variable rates of extinction and speciation did not provide a better fit to the data.

Additional analyses and LTT plots suggested differences in lineage accumulation through time between the North American (northern) clade and the mainly southern South American (southern) clade (Table 2). Although the null hypothesis of rate constancy could not be rejected for either dataset ($P = 0.31$, northern; $P = 0.92$, southern), the best-fit pure birth model for each dataset differed from 0.2235 (northern) to 0.3081 (southern) divergence events per lineage per Myr.

DISCUSSION

Given our data, and recognizing that *Spiza* is the closest living relative to the blue cardinalids (Klicka *et al.*, 2007; Barker *et al.*, 2012), our ancestral area reconstructions suggest that the group originated in North America, which generally agrees with earlier work indicating that the entire cardinalid group is derived from a North American ancestor (Barker *et al.*, 2004; Klicka *et al.*, 2007). Nonetheless, *Spiza americana* (the only species in the genus) is an obligate grassland specialist, breeding throughout the plains of North America and

wintering in open habitats in Middle America and northernmost South America. It often spends the non-breeding season in large, nomadic flocks, avoiding drought-stricken regions, and, not infrequently, breeds in unexpected areas (Temple, 2002). This life history strategy makes it impossible to discern where the ancestor of *S. americana* may have been distributed *c.* 9 Ma, when it and the ancestor of blue cardinalids diverged from a common ancestor. Attempting to estimate accurately the ancestral state of such a deep node with no closely related outgroup taxa (see Klicka *et al.*, 2007) is perhaps a red herring, given the likelihood of dynamic range shifts and migration patterns in such mobile birds (e.g. Klicka *et al.*, 2003).

Instead, it is critical to note that blue cardinalids have a long history in both North and South America; our ancestral area reconstruction (Figs 3 & 4) indicates that they occurred on both continents before a late Pliocene closure of the Isthmus of Panama (Coates & Obando, 1996). At the end of the Miocene (*c.* 6 Ma), blue cardinalids comprised a northern and a southern clade that began to diversify essentially independently in North America (including Middle America) and in South America, respectively, at rates that we estimated to be roughly constant and similar, although the South American clade diversified at a slightly higher overall rate. The rate constancy for both clades provides an alternative perspective on avian diversification in the New World, compared with earlier studies that coupled elevated rates of speciation with particular palaeogeographical or palaeoclimatic events (e.g. Weir & Schluter, 2004; Weir, 2006; Chaves *et al.*, 2011; but see Zink *et al.*, 2004). Furthermore, the slightly higher rate of diversification of blue cardinalids in South America relative to North America is in contrast to earlier claims of higher diversification rates in high-latitude relative to low-latitude vertebrates (e.g. Weir & Schluter, 2007), and reiterates the importance of using well-sampled phylogeographical datasets when comparing rates of diversification among tropical and temperate regions (Ribas *et al.*, 2012). Instead, our data are consistent with other recent observations showing steady lineage accumulation up to the present in Neotropical birds (Derryberry *et al.*, 2011; Patel *et al.*, 2011; d'Horta *et al.*, 2012; Smith *et al.*, 2013).

Diversification across North America

Consistent with our reconstructed origin in temperate North America, most of the temporally deep divergences between extant taxa of blue cardinalids are within the North American clade (Fig. 3). Interspecific divergences between *Passerina* species are particularly old. Based on mean estimates, diversification within *Passerina* began during the late Miocene, and five of the seven species diverged prior to the start of the Pleistocene. The sister species *P. ciris* and *P. versicolor* probably split more recently during the Pleistocene. Phylogeographical lineages developed during the Pleistocene within *P. versicolor* and *Cyanocompsa parvilinea*. Although four extant species of blue cardinalids breed in temperate North Amer-

ica, the majority of lineages in the North American clade (67%) are restricted to tropical and subtropical Middle America (Fig. 1). Within blue cardinalids, mean estimated divergence dates between Middle American lineages occurred during the late Miocene (*P. rositae* from *P. leclancherii* + *P. ciris* + *P. versicolor*) and Pliocene (*P. leclancherii* from the clade of *P. ciris* + *P. versicolor*). These results highlight the roles of both pre-Quaternary and Quaternary periods in shaping phylogenetic and phylogeographical structure in birds across the North American continent.

Diversification in South America

Expansion out of North America and into South America during the late Miocene (Fig. 4) provided new opportunities for diversification in blue cardinalids. Colonization *c.* 6 Ma pre-dates most estimates of the Isthmus of Panama closure at 4–2.5 Ma (Coates & Obando, 1996), although recent geological evidence may indicate a Miocene isthmian closure (Farris *et al.*, 2011; Montes *et al.*, 2012). Regardless, our finding of a late Miocene colonization of South America adds to a growing list of terrestrial vertebrate lineages thought to have dispersed between North and South America well before 4 Ma (e.g. Bermingham & Martin, 1998; Pinto-Sánchez *et al.*, 2012). This early dispersal is not surprising, given the prevalence of seasonal migration in the group and its sister, *S. americana*.

Despite reaching South America some 6 to 7 Ma, most diversification within the southern blue cardinalids occurred more recently and at a near-constant rate. Posterior credibility intervals for 10 of the 12 (83%) divergences in our multilocus species tree include the Pleistocene; based on mean estimates, 9 of the 12 (75%) divergences occurred within the Pleistocene. Our ancestral area reconstructions indicate that *A. concolor* and *Cyanocompsa cyanoides* recolonized North America at two different times (Fig. 4). One lineage of *A. concolor* (lineage A) crossed the isthmus back into Middle America near the Pliocene–Pleistocene boundary (mean estimated date 2.6 Ma; 95% HPD: 3.9–1.5 Ma). Around 2 Myr later, one lineage of *Cyanocompsa cyanoides* (lineage B) dispersed out of South America and into Middle America (mean estimated date 0.6 Ma; 95% HPD = 1.1–0.2). Similar complex biogeographical scenarios have been recovered in other passerines from the Darien and Talamancan highlands (e.g. *Chlorospingus* bush-tanagers, Weir *et al.*, 2008; *Myadestes solitaires*, Miller *et al.*, 2007).

A less parsimonious alternative for the colonization of South America is a possibility. *Amaurozpiza* are smaller, uncommon cardinalids with extremely patchy distributions because of their affinity for bamboo (Ridgely & Tudor, 1994). In contrast, *Cyanocompsa cyanoides* (and *Cyanoloxia*) are larger, heavy-billed cardinalids that are more common and occupy more widespread habitats. Given these differences in size, ecology and distribution, these lineages might have diverged while in Middle America, with each lineage independently colonizing South America at a later date. This

scenario would require three independent colonizations of South America: the *Cyanocompsa cyanooides* A/C lineages, the *Cyanocompsa cyanooides* E/D/F lineages, and the *Cyanocompsa brissonii*/*Cyanoloxia* lineages. However, the first two divergences would have occurred around 3–4 Ma, consistent with traditional interpretations of an inter-continental land-bridge connection (see Smith & Klicka, 2010).

Seasonal migration and constant diversification despite major geo-climatic events

Several studies have emphasized the role of particular geological events (e.g. Andean uplift: Brumfield & Capparella, 1996; formation of the Panamanian isthmus: DaCosta & Klicka, 2008; Smith & Klicka, 2010) or palaeoclimatic events (e.g. Pleistocene cooling: Weir & Schluter, 2004) in promoting the diversification of New World bird lineages. However, blue cardinalids represent one among several recent examples of New World avian groups (e.g. furnariid ovenbirds; Derryberry *et al.*, 2011) in which the reconstruction of diversification through time (Fig. 4) shows no discernible sign of pulsations despite such events.

Likewise, the role of seasonal migration in the diversification of blue cardinalids presents a paradox. The sister taxon to the blue cardinalids, *S. americana*, is an obligate seasonal migrant that breeds in North America and winters as far south as northern South America. Migration is asymmetrically exhibited in the ingroup, in which five of eight species in the northern clade are also seasonal migrants, whereas just one of six southern clade species shows any recognized seasonal movement (*Cyanoloxia* is a partial austral migrant; del Hoyo *et al.*, 2011). Although long-distance seasonal migration in birds has generally been considered to decrease rates of avian differentiation (Montgomery, 1896; Mayr, 1963), it is an adaptation that may in some cases stimulate speciation (Winker, 2000). It is thus of interest that in the blue cardinalids the prevalence of this trait in the northern clade did not apparently depress the speciation rate; or, viewed from the other side, the largely sedentary life history trait exhibited in the extant lineages of the southern clade did not significantly enhance it (Fig. 4, inset). We can only speculate on the extent to which the enhanced mobility that accompanies seasonal migration has contributed to the apparently smooth rate of diversification observed in the blue cardinalids, but our study serves to highlight the importance of considering ecological and behavioural characteristics in tandem with palaeogeological events when attempting to understand the diversification history of widespread, mobile taxonomic groups.

Taxonomic implications

Results from our dense range-wide sampling and molecular dataset shed new light on the systematics of blue cardinalids. The 14 biological species currently recognized within this group can be further divided into 11 additional geographically and genetically structured units. At a minimum, the following

taxonomical recommendations are in order. *Amaurospiza concolor* as presently configured is paraphyletic. The Middle American forms have priority regarding the specific epithet *concolor* (*Amaurospiza concolor* [Costa Rica] Cabanis, 1861). We therefore suggest that the geographically and genetically distinctive South American form *A. c. aequatorialis* (*A. concolor* B in Fig. 3) be elevated to species status: *A. aequatorialis* [Ecuador] Sharpe, 1888. Likewise, the current taxonomic treatment of *Cyanocompsa cyanooides* obscures the fact that lineages east and west of the Andes in this group have been evolving independently for more than 3 Myr (Figs 2 & 3). Here again, the lineage occurring west of the Andes has priority (*Coccyborus cyanooides* [Panama] Lafresnaye, 1847), differing from the morphologically distinctive form distributed widely throughout most of the Amazon Basin, *Cyanocompsa c. rothschildii* (*Guiraca rothschildii* [Suriname] Bartlett, 1890), and we recommend recognizing *rothschildii* as a separate species, *Cyanocompsa rothschildii*. Given the abrupt contact zones of *Cyanocompsa cyanocompsa* (*sensu stricto*) phylogroups in both western Panama and northern Venezuela (Fig. 2), it is conceivable that future detailed study will uncover the existence of reproductive isolation, and, hence, additional biological species in this group. At a broader level, our multilocus species tree reconstruction (Fig. 3) strongly suggests that *Cyanocompsa parrellina* is the sister species to *Passerina*, rendering *Cyanocompsa* polyphyletic. This problem can be solved by merging *C. parrellina* (type species, Cabanis, 1861) into *Passerina* (Vieillot, 1816) and the remaining members of *Cyanocompsa* into *Cyanoloxia* (Bonaparte, 1850), eliminating the genus *Cyanocompsa* altogether through priority.

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SUPPORTING INFORMATION

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

Appendix S1 Locality data for specimens.

Appendix S2 Expanded methods and results for ancestral area reconstructions.

DATA ACCESSIBILITY

All genetic data generated for this study are deposited in Dryad: doi:10.5061/dryad.tb50 m.

BIOSKETCH

Robert W. Bryson Jr is interested in the origin and distribution of genetic diversity in taxa distributed in south-western North America and the Neotropics.

Author contributions: J.K. and B.T.S. conceived the idea; B.T.S., M.J.M., K.W., J.L.P. and J.K. obtained most of the specimens; R.W.B., B.T.S. and M.J.M. generated the genetic data; R.W.B. and J.C. analysed the data; R.W.B., B.T.S. and J.K. led the writing, with other authors contributing; and all authors read and approved the manuscript.

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