





LETTER

Demographic consequences of foraging ecology explain genetic diversification in Neotropical bird species

Matthew J. Miller,^{1,2*} 
 Eldredge Bermingham,^{2,3} Benjamin
 L. Turner,²  Justin C.
 Touchon,^{2,4}  Andrew B.
 Johnson^{1,5} and Kevin Winker¹ 

Abstract

Despite evidence that species' traits affect rates of bird diversification, biogeographic studies tend to prioritise earth history in Neotropical bird speciation. Here we compare mitochondrial genetic differentiation among 56 co-distributed Neotropical bird species with varying ecologies. The trait 'diet' best predicted divergence, with plant-dependent species (mostly frugivores and nectivores) showing lower levels of genetic divergence than insectivores or mixed-diet species. We propose that the greater vagility and demographic instability of birds whose diets rely on fruit, seeds, or nectar – known to vary in abundance seasonally and between years – relative to birds that eat primarily insects, drives episodic re-unification of otherwise isolated populations, resetting the divergence 'clock'. Testing this prediction using coalescent simulations, we find that plant-dependent species show stronger signals of recent demographic expansion compared to insectivores or mixed-diet species, consistent with this hypothesis. Our study provides evidence that localised ecological phenomena scale up to generate larger macroevolutionary patterns.

Keywords

diet, genetic differentiation, population demographics, stable isotopes, trophic level, tropical biodiversity.

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INTRODUCTION

Globally, avian diversity is greatest in the Neotropical lowlands: nearly one in four of the world's species breeds in this ecoregion (Orme *et al.* 2005). The observation of congruent geographic patterns of Neotropical species endemism and turnover has been recognised for nearly two centuries (Wallace 1852; Chapman 1917; Cracraft 1985). Thus, hypotheses to explain species formation in Neotropical lowland birds have usually focused on geographic barriers to explain diversification. These include wide rivers (Sick 1967; Thom & Aleixo 2015; Naka & Brumfield 2018), the uplift of the Andes mountain range (Chapman 1917; Brumfield & Capparella 1996; Weir & Price 2011) and Pleistocene climate oscillations that reduced continuous lowland forest to islands within a matrix of open, non-forested habitat (Haffer 1969; Smith *et al.* 2012; Silva *et al.* 2019).

Molecular data support the notion that limited dispersal over these barriers can result in founder populations that ultimately lead to new lineages (Miller *et al.* 2008; Smith *et al.* 2014). Indeed, limited dispersal ability is linked with subspecies richness in birds (Belluire *et al.* 2000) and may promote speciation in the Neotropical lowlands (Claramunt *et al.* 2012; Salisbury *et al.* 2012; Sheard *et al.* 2020). While dispersal across barriers has been shown to be associated with diversification in some Neotropical birds (Smith *et al.* 2014), more

generally, high rates of dispersal homogenise populations and dampen differentiation (Bohonak 1999). Thus, it is appropriate to consider that a lack of dispersal drives diversification in Neotropical lowland birds (Crouch *et al.* 2019). This is perhaps most visible in the observation that many Neotropical bird species are differentiated across geographic space where physical barriers to dispersal are not obvious (Lovette 2004; Cheviron *et al.* 2005; Vázquez-Miranda *et al.* 2009; Miller *et al.* 2011; Milá *et al.* 2012), suggesting that additional forces beyond geography, that is, avian traits themselves, might limit or promote dispersal, with consequences for differentiation.

Several species traits are commonly suggested as important correlates to dispersal among Neotropical lowland birds. Habitat type is known to affect dispersal, with closed-forest birds often showing substantial dispersal limitation (Willis 1974; Develey & Stouffer 2001; Moore *et al.* 2008). In contrast, Neotropical lowland birds that occur in non-forest habitats show higher dispersal ability (Hayes & Sewlal 2004; Lees & Peres 2009; Cadena *et al.* 2011). Resident canopy birds also show substantial seasonal fluctuations in abundance, likely due to seasonal tracking of food resources (Greenberg 1981), whereas understory birds often maintain year-round territories and have remarkably constant temporal densities (Greenberg & Gradwohl 1986). Heavier birds tend to disperse over greater distances compared to lighter birds (Brown *et al.* 1978; Gotelli & Graves 1990). Finally, Neotropical birds with diets

¹Department of Biology & Wildlife, University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775, USA

²Smithsonian Tropical Research Institute, Apartado, Balboa, Ancón 0843-03092, Republic of Panama

³Patricia and Phillip Frost Museum of Science, Miami, FL 33129, USA

⁴Biology Department, Vassar College, 124 Raymond Ave., Poughkeepsie, NY 12604, USA

⁵Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM 87131, USA

*Correspondence: E-mail: biodiversity@onehealth-genomics.org

dominated by seasonally variable plant reproductive parts (fruits, seeds, flowers and nectar) have repeatedly been shown to have greater dispersal tendencies than birds with primarily arthropod-based diets (Willis 1974; Martin & Karr 1986; Loisele & Blake 1991; Şekercio lu *et al.* 2002; Laurance *et al.* 2004; Gorrell *et al.* 2005; Boyle 2011; Pizo & dos Santos 2011; de Lima & Manhães 2017). Obviously, many of these species' traits are correlated (Levey & Stiles 1992). For example, large-bodied frugivorous birds are most often found in the canopy (Barlow *et al.* 2007) where fruits are most abundant and diverse (Schaefer *et al.* 2002), whereas small insectivores are most abundant in the forest understorey (Terborgh *et al.* 1990; Marra & Remsen 1997; Şekercio lu *et al.* 2002).

A few studies have attempted to understand the consequences of species' traits associated with dispersal on patterns of genetic differentiation across space. Burney & Brumfield (2009) found that birds encountered in forest understorey showed greater genetic differentiation across well-known South American geographic barriers than bird species in forest canopies. Harvey *et al.* (2017) found that bird species that inhabit floodplains showed shallower genetic divergence across Amazonian rivers than bird species that live in upland forests. While these studies measured differentiation across geographic barriers, it is plausible that the effects of these factors would also exist across unbroken landscapes. What has been lacking thus far is adequately discriminating among correlated species traits, and, more importantly, proposing and testing a mechanism underlying trait-based differences in genetic differentiation.

In addition to differences in dispersal ability, Neotropical birds with differing foraging ecologies also have different demographic patterns. Generally, Neotropical birds whose diets are dominated by arthropods are notable for demographic stability (Faaborg *et al.* 1984; Greenberg & Gradwohl 1986; Şekercio lu *et al.* 2002; Woltmann & Sherry 2011; Sherry *et al.* 2020), likely due to the relative year-round and year-over-year availability of invertebrate food resources, despite changes in seasonal abundance of particular arthropod prey taxa (Jahn *et al.* 2010). On the other hand, species dependent on calorie-rich, but seasonal, plant reproductive parts such as fruit, seeds, and nectar often experience large population size fluctuations due to variations in resource availability (Stiles 1980; Faaborg *et al.* 1984; Stiles 1992; Brawn *et al.* 1995; Ryder & Sillett 2016; Macario *et al.* 2017). The tendency for plant-dependent bird species to have greater seasonal movements across the landscape, and their propensity for boom-bust population dynamics, could result in episodic reticulation of otherwise isolated populations, in effect 'resetting the clock' for differentiation time. This hypothesised mechanism might produce fundamentally different patterns of genetic differentiation between populations in birds with arthropod versus plant-based diets (Figure 1). Here we use DNA sequence data from populations of 56 co-distributed resident, nonmigratory bird species, sampled from Belize at the northern end of the Caribbean lowlands of Central America to Panama in the south, a landscape with no obvious major geographic barriers to avian movement. We identify avian species' traits associated with genetic differentiation across this Neotropical landscape and test our hypothesised mechanism for potential divergence differences due to diet.

MATERIALS AND METHODS

We obtained tissue samples from 56 species (representing 54 genera and 19 families) of non-migratory lowland Neotropical birds from two locations: Toledo District, Belize and the central provinces (Coclé, Colón, Panamá) of Panama, the end-points of the largely forested Caribbean slope of Central America, a region lacking obvious geographic barriers to lowland birds (Figure S1). The vast majority of these samples came from community surveys based on mist netting in forest and forest edges and thus include a diversity of Neotropical lowland birds. Long-term research at the Belize site has revealed no seasonal migrations among these tropical resident species, movements that we know well from other sites in or near Neotropical montane systems (Winker *et al.* 1997, 1999). Our sample of species represents 41% of the resident terrestrial bird community shared between Belize and Panama. More than 99% of our samples are associated with catalogued museum specimens. Sample sizes for each population varied from 1 to 20 individuals (total $N = 619$); variation in number reflects the relative commonness of the species and ease of capture. Specimen numbers and GenBank accession numbers are in Table S1.

We selected the complete mitochondrial NADH dehydrogenase subunit 2 gene (ND2, 1041 bp) as our standard genetic

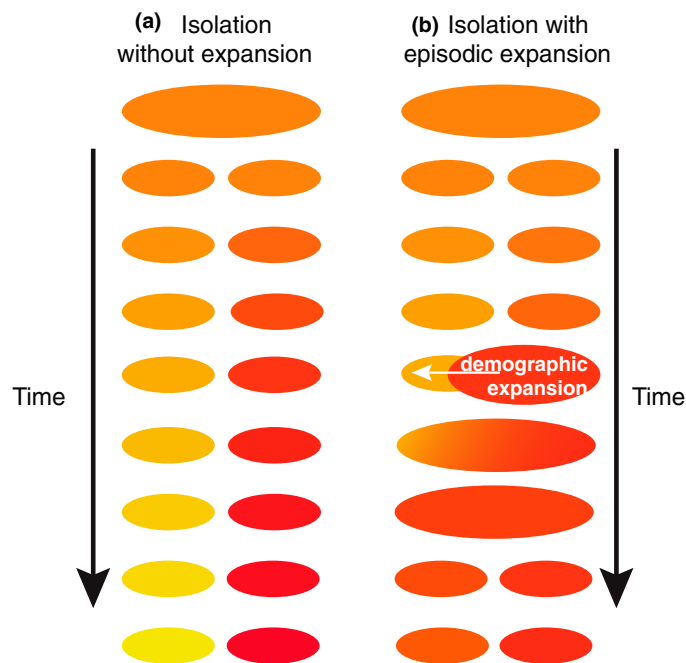


Figure 1 Graphical model of the effect of episodic demographic expansion on estimated divergence time between two genetically isolated populations. Species (a) has stable demographics and limited dispersal, typical of Neotropical avian insectivores. Species (b) has local populations that tend to be demographically variable (e.g. boom-bust), and has greater tendencies for vagility and dispersal, as is typical of many Neotropical avian frugivores and nectivores. Although both species in the model experience similar rates of genetic divergence with time, the episodic demographic expansion of species (b) results in the re-unification of isolated populations, in effect resetting the clock for genetic divergence between two populations.

measure to evaluate population-level genetic variation between sampling points in Belize and Panama for all 56 species. For studies such as ours with over 600 samples, mitochondrial markers remain a versatile genetic marker for taxonomically and geographically broad comparative studies because of ease of amplification across taxa, homology, level of resolution and low cost (Bowen *et al.* 2014). Furthermore, ND2 has been shown to be among the most efficient genes for recovering the mitochondrial evolutionary history (Meiklejohn *et al.* 2014). We first used the sequence data to calculate net nucleotide divergence (D_A ; Nei 1987), which measures the average pairwise divergence between the two populations after controlling for intra-population variation.

Based on published literature, we classified species' ecological traits by forest use (interior or forest edge/open), vertical stratum (canopy or understory) and diet (principally plant-based, including fruits, seeds or nectar; arthropods; or those consuming a mixture of plant and animal sources). Following earlier studies (Burney & Brumfield 2009; Salisbury *et al.* 2012), we used Stotz *et al.* (1996) to classify species by habitat type and vertical stratum. We classified diets based on descriptions in Stiles & Skutch (1989). Two of us independently and blindly used this source to categorise each species' diet. We had a 95% repeatability. Differences were resolved using del Hoyo *et al.* (1992–2011) and examination of stomach contents. Body mass (ln-transformed) was also included among our parameters, because this trait has been suggested to influence life history (Calder 1984; Brown *et al.* 1978), and among a variety of animals, rates of molecular evolution decrease with increasing body mass (Martin & Palumbi 1993; Gillooly *et al.* 2005; Nabholz *et al.* 2016; but see Gillman *et al.* 2012). We tested for differences among population net genetic distance values (D_A) based on four predictors: (1) forest use (interior forest species vs. non-forest and edge species); (2) foraging stratum (canopy vs. understory); (3) and diet, classified using three categories: frugivores, granivores and nectivores (i.e. species whose diet is based on calorie-rich, but seasonal, plant resources; hence 'frugivores/nectivores'); insectivores (i.e. species whose diet is primarily arthropod-based); or mixed-diet species (i.e. those that regularly consume both arthropods and fruit, seeds and/or nectar); and (4) body mass. The classification data are in Table S2.

All statistical analyses were conducted in R v3.4.1 (R Core Development Team 2017). Genetic divergence values (D_A) were non-normally distributed (Figure S2) and included values of 0. We analysed D_A using generalised linear mixed models (GLMM) assuming an underlying gamma error distribution, which fit our data better than normal, lognormal, Weibull, or exponential distributions. Taxonomic family was included as a random effect in all models to remove variation due to phylogenetic relatedness. Because a gamma error distribution can only accommodate non-zero values, we added a small constant (0.00005) to all values. To ensure that the constant did not influence our results, we also ran analyses excluding species with values of D_A that were zero ($N = 12$). Excluding species with $D_A = 0$ had no effect on our results.

We tested all additive combinations of our four explanatory predictors and compared models using Akaike's Information

Criterion (AIC). In addition, we estimated the relative importance of our four predictor variables (Burnham & Anderson 2004) by generating a confidence set of models whose Akaike weights (w) summed to 0.95. The relative importance of a given predictor was calculated as the sum of w for all models containing that predictor in the candidate model set divided by the cumulative w in the set. To better understand the relation between our three diet classes and genetic differentiation, we conducted pairwise *post hoc* comparisons among diet classes using the `glht` function in the `multcomp` package (Hothorn *et al.* 2014). Lastly, because a previous study suggested that foraging stratum rather than diet best explained the degree of genetic variation across geographic barriers in Amazonia (Burney & Brumfield 2009), we repeated our GLMM analysis explicitly testing the effects of diet, foraging stratum and their interaction on D_A .

To independently assess the role of diet, we used stable nitrogen isotopes ($^{15}\text{N}/^{14}\text{N}$) obtained from feathers of specimens in the Smithsonian Tropical Research Institute (STRI) Bird Collection collected in central Panama to estimate trophic level for 35 of the 56 species in our dataset. (Specimens from central Panama were lacking in the STRI Bird Collection for the remaining 21 species.) Feathers from 109 specimens were analysed at the STRI stable isotope facility, using an isotope ratio mass spectrometer (Delta V Advantage) coupled with a ConFloIII continuous flow interface to a Flash HT elemental analyzer (Thermo, Bremen, Germany).

Nitrogen isotope ratios ($\delta^{15}\text{N}$) were expressed in standard notation in parts-per-thousand as:

$$\delta^{15}\text{N} = \left[\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{sample}} - \left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{standard}} \right] \times 1000.$$

These ratios were converted to trophic level estimates following Herrera *et al.* (2003):

$$TL_{\text{consumer}} = 1 + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{producer}}) / FRAC_{\text{feather}},$$

where TL_{consumer} is the trophic level. An estimate of $\delta^{15}\text{N}_{\text{producer}}$ ($\delta = 1.88$) was obtained by averaging the $\delta^{15}\text{N}$ value of 27 samples of fruits and flowers obtained from Panama. For $FRAC_{\text{feather}}$ (the fractionation rate of nitrogen between bird diet and feathers) we used a value of 4.7, which was the average fractionation value observed by Bearhop *et al.* (2002). We fit a GLMM with a gamma error distribution to D_A using TL_{consumer} as the predictor. Details on the stable isotope procedures, including a list of samples used, are provided in Methods S1.

Because 80% of species' populations did not share haplotypes (see Results), we could not calculate gene flow for most species in the dataset. Instead, we approached population demographics by testing for signals of recent population expansion using the sequence data in two ways. First, we calculated the R_2 summary statistic (Ramos-Onsins & Rozas 2002), which measures the ratio of singletons to the overall number of segregating sites in a population, for 45 of 46 populations (16 species in Belize, 29 species in Panama) in which the sample size was six or greater (*Tiaris olivaceus* from Panama were excluded because the population lacked nucleotide variation, and thus R_2 could not be calculated). Small values of R_2 are expected for populations that have recently

expanded, and significance of the observed R_2 value was compared to the distribution of values observed from 10,000 coalescent simulations assuming no population expansion. As this is a within-population metric, we evaluated R_2 independently for the Belizean and Panamanian populations of a given species. Calculation of observed R_2 and coalescent simulations were performed in DnaSP v5.10 (Librado & Rozas 2009).

Second, we estimated g , the exponent of the growth rate formula in LAMARC v.2.1.8 (Kuhner 2006). Zero values of g indicate constant population size, whereas positive and negative values indicate exponential population growth and decline respectively. Under the assumption of equivalent substitution rates, larger g values indicate a larger rate of population growth. We ran LAMARC on the same 45 populations for which we calculated R_2 . LAMARC was used in Bayesian mode under the following conditions: three replicates of an initial run of 40 chains with 500 samples recorded with an interval of 50 rearrangements between samples and a burn-in of 2000 followed by a final run of eight chains with 20,000 samples with an interval of 50 rearrangements between samples and a burn-in of 4000. Lower and upper logarithmic priors for theta were set at 1×10^{-5} and 5×10^{-2} , whereas lower and upper linear priors for g were set at -1000 and $15\,000$ respectively. We used Tracer v1.7 (Rambaut *et al.* 2018) to examine outfiles for stationarity and curvfiles for evidence of convergence.

Of the 45 populations analysed, 37 converged and were used in subsequent analyses. Among these, values of g ranged from -117 to 9136 and were right skewed. We added 118 to all values to make them positive, and then we evaluated normal, lognormal, negative binomial, geometric, exponential, and Weibull error distributions, finding that a negative binomial distribution was the best fit. The relationship among population (Belize vs. Panama), diet (frugivore/nectivore vs. mixed/insectivore) and g was examined using a GLM with an underlying negative binomial error distribution.

RESULTS

We generated 619 ND2 sequences from 56 Neotropical resident bird species. Among these 56 species, net nucleotide divergence (D_A) between Belize and Panama varied from 0.0000 to 0.0437 (Table S2), with values substantially right skewed (Figure S2). Eleven of 56 species shared common haplotypes between our sampling points in Belize and Panama (Figure S3). However, a significantly greater proportion of frugivores and nectivores (10 of 22, 45%) had shared haplotypes between populations than either insectivores or mixed-diet species (1 of 34, 3%; Fisher's exact test: $P = 0.00015$; Table S2).

Median D_A for frugivores and nectivores was 0.0009 and 0.0010 respectively, while median D_A for species with mixed diets and insectivores was 0.0131 and 0.0111. Among models of ecological characters to explain interspecific variation in D_A , the best model was 'stratum + diet', with the lowest AICc value and an Akaike weight of 0.27. Four other models had Δ AICc values within two of the best model (Table 1), and 'diet' was included among the factors in all these models; further, model parameter significance found 'diet' to be

approximately two times as important as either 'stratum' or 'habitat' and over five times as important as 'body mass' (Table 2). Models with single parameters, for example, 'stratum', 'habitat' or 'body mass', were not significant, however the model with 'diet' alone was (Figure 2). Finally, a GLMM with all four predictors found that among all predictors only 'diet' was significantly related to variation in D_A ($P < 0.0004$; Figure 2). When we explicitly compared the effects of foraging stratum and diet class on D_A , we found that only diet was a significant predictor of nucleotide divergence (GLMM, $P = 0.0016$). Neither foraging stratum nor the interaction between stratum and diet were significant (GLMM, foraging stratum, $P = 0.41$, diet \times stratum, $P = 0.29$). Pairwise post hoc comparisons of the three diet classes (frugivore/nectivore; insectivore; mixed-diet) found a significant difference between frugivores/nectivores and insectivores ($P < 0.002$) and between frugivores/nectivores and mixed-diet species ($P = 0.005$) but no significant difference between insectivores and species with mixed-diets ($P = 0.92$). Thus, in subsequent analyses we considered species of frugivores and nectivores as a single category: frugivores/nectivores, while considering species with mixed diets and insectivores as a second category: mixed-diet/insectivores.

To provide a complementary and independent perspective on the relationship between diet and D_A , we estimated trophic levels (TL) using stable isotopes obtained from bird feathers. Among the 35 species analysed, estimated TL_{consumer} varied from 1.73 to 2.77. Our earlier diet classification agreed with $\delta^{15}\text{N}$ -estimated trophic levels (GLMM, $P = 0.007$): TL_{consumer} for frugivores and nectivores (2.17 ± 0.05 , mean \pm SE) was significantly lower than that of insectivores and mixed-diet species (2.37 ± 0.06). Corroborating our previous finding using diet categories, D_A increased significantly with estimated TL_{consumer} (Figure 3).

To test our hypothesis that diet results in different demographic histories between species with primarily plant-based

Table 1 Model selection for a set of generalised linear mixed models incorporating four ecological predictors of net nucleotide divergence (D_A) between Belizean and Panamanian populations among 56 species of Neotropical birds. (d.f. = degrees of freedom; AICc = corrected Akaike Information Criterion, w = Akaike weight). * indicates models included in the $w = 0.95$ candidate model set used to establish predictor relative importance (see Table 2)

Model	d.f.	AICc	w
<i>stratum + diet</i>	6	-465.12	0.27*
<i>diet</i>	5	-464.51	0.20*
<i>habitat + diet</i>	6	-464.31	0.18*
<i>habitat + stratum + diet</i>	7	-463.78	0.14*
<i>stratum + diet + mass</i>	7	-462.78	0.08*
<i>habitat + diet + mass</i>	7	-461.88	0.05*
<i>habitat + stratum + diet + mass</i>	8	-461.13	0.04*
<i>stratum</i>	5	-459.30	0.01
<i>habitat</i>	5	-458.72	0.01
<i>mass</i>	5	-458.09	<0.01
<i>habitat + stratum</i>	6	-457.18	<0.01
<i>stratum + mass</i>	6	-457.02	<0.01
<i>habitat + mass</i>	6	-456.52	<0.01
<i>habitat + stratum + mass</i>	7	-454.96	<0.01

Table 2 Relative importance of four predictors of D_A (Table 1). Importance was calculated by selecting a candidate subset of the models with the lowest AICc values with summed Aikake weights (w) equal to 0.95. The relative importance of a predictor is determined by summing w for all models containing that predictor in the candidate model set and dividing that value by the overall w of the set. Diet is the most important predictor of D_A , being found in all models in the candidate model set

Predictor	Relative predictor importance
<i>diet</i>	1.00
<i>stratum</i>	0.55
<i>habitat</i>	0.42
<i>body mass</i>	0.18

diets and those with diets based largely on arthropods, we measured population size changes using coalescence-based estimates of population growth. Among the 45 populations (from 31 species) for which we calculated R_2 , 18 had significant R_2 values, indicating recent population expansion (Table S3). Populations of frugivores and nectivores were more likely to have a significant R_2 value: 12 of 20 (60%) populations of frugivores/nectivores had significant R_2 values, while only 6 of 25 (23%) insectivore/mixed-diet populations had significant R_2 values. The difference between the two groups was significant (Fisher's exact test: $P = 0.016$).

Similarly, g (a coalescent measure of population growth or decline) varied among 37 populations from -117 to 9136 (Table S3) and was best predicted by population (Belize or Panama) and diet (AIC = 655.20). This model was better than either a model with population (AIC = 657.93) or diet (AIC = 660.04) alone and better than a model with population \times diet (AIC = 657.10). Similarly, significance testing found that both diet (GLM, $\chi^2 = 6.40$, $P = 0.011$) and population (GLM, $\chi^2 = 9.27$, $P = 0.002$) were significant predictors of g , with frugivores/nectivores and populations from Belize having more population expansion than insectivores/mixed diet species or populations from Panama respectively (Figure 4). The interaction between diet and population was not significant (GLM, $\chi^2 = 0.13$, $P = 0.71$).

DISCUSSION

We found that in the absence of obvious geographic barriers to dispersal over evolutionary time scales, diet is the principal ecological determinant of population genetic divergence among 56 species of Neotropical resident birds sampled at the endpoints of Central America. Bird species consuming plant products such as fruit, seeds and nectar have significantly less mitochondrial divergence between Belize and Panama than species consuming solely arthropods and species with mixed arthropod- and plant-based diets. This result was robust when

stable isotope-based estimates of trophic level were used as a proxy for qualitative diet classes. Genetic divergence was not related to habitat use, canopy stratum or body mass. While

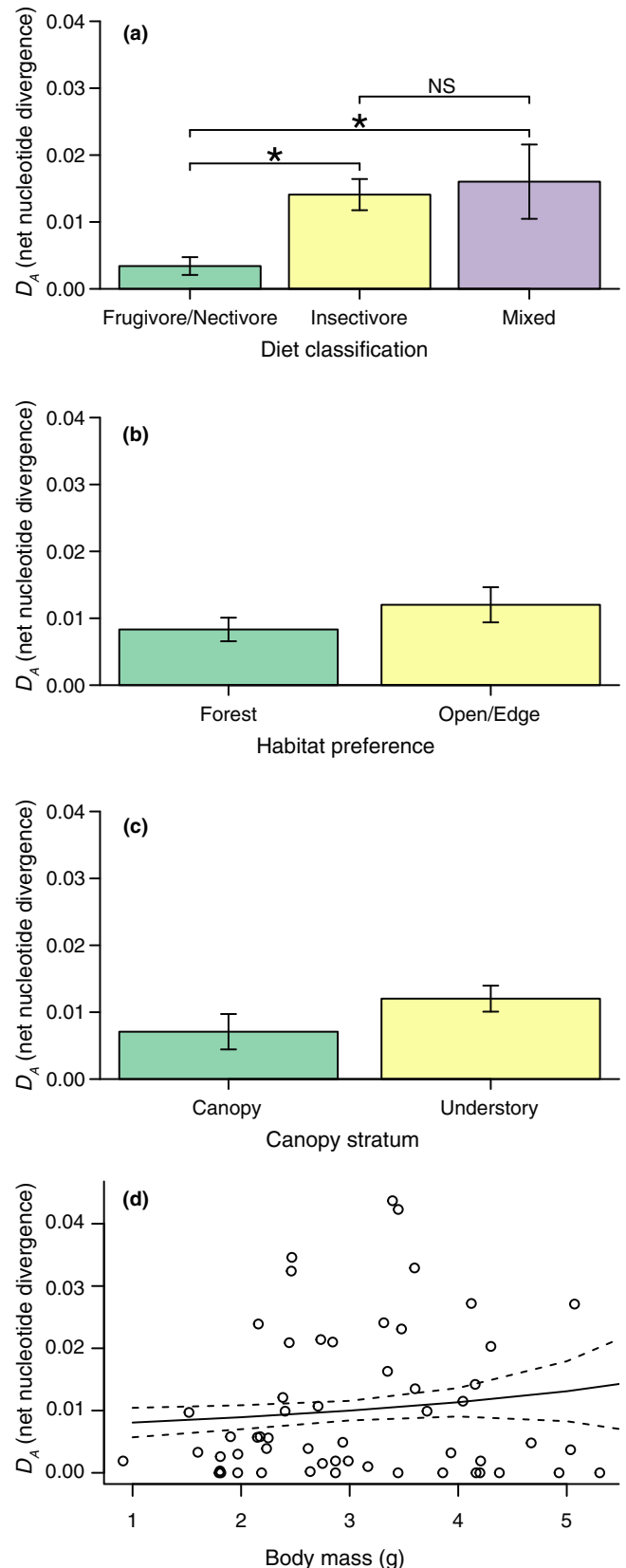


Figure 2 D_A (net nucleotide divergence) as a function of four predictors. (a) diet: frugivores have significantly lower D_A than insectivores and mixed diet species; (b) habitat: no significant difference exists between forest and open/edge species; (c) foraging stratum: no significant difference exists between canopy and understory species; (d) body mass: no relationship exists between D_A and mass.

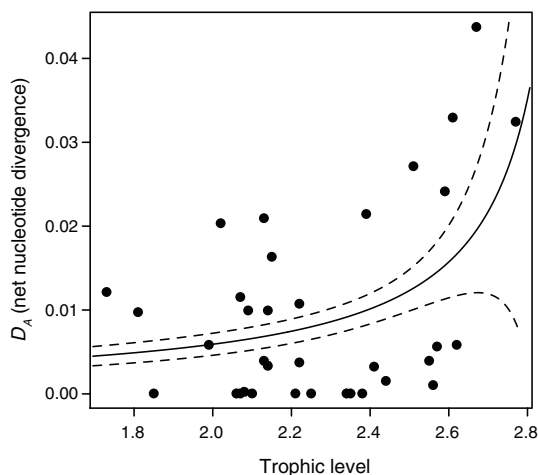


Figure 3 Net nucleotide divergence (D_A) between Belize and Panama increases with trophic level (estimated from stable nitrogen isotopes in feathers) for 35 species of Neotropical birds. The solid line represents the predicted fit from a GLMM with underlying gamma error distribution, and the dashed lines represent the 95% confidence interval. This result corroborates our finding that diet is the primary predictor of genetic differentiation among Central America resident landbirds.

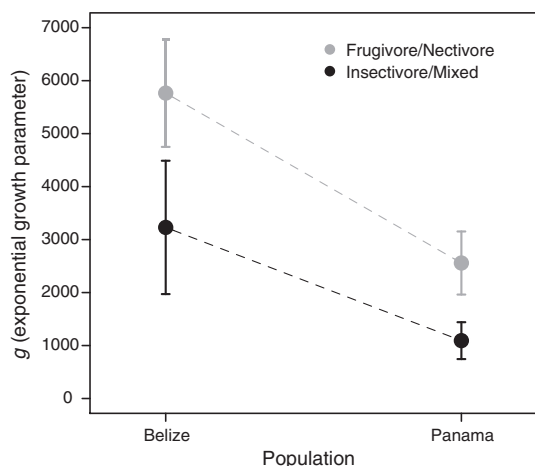


Figure 4 Relationship between population and diet and the exponential growth parameter (g). Populations of frugivores and nectivores had significantly higher g values than populations of species with arthropod-based or mixed diets, irrespective of a significant effect of population on g .

our study used a single molecular marker, mitochondrial DNA (mtDNA), this marker has often been used for comparative studies with many taxa requiring a homologous genetic basis, even in the genomic era (Bowen *et al.* 2014). Among many Neotropical birds, the ability to move relatively large distances is related to reliance on seasonally variable resources, whereas many other Neotropical species do not need to track resources seasonally across the landscape, and thus apparently have lower levels of dispersal. Our results thus confirm dispersal limitation as a key driver of avian differentiation in the Neotropics (Burney & Brumfield 2009; Claramunt *et al.* 2012; Salisbury *et al.* 2012). This is also

consistent with the latitudinal gradient in avian dispersal ability (Sheard *et al.* 2020).

Boom-bust dynamics scale up to evolutionary time scales

A focus on dispersal alone, however, neglects an important role that demographic patterns play in evolutionary diversification (Harvey *et al.* 2019). In addition to lower dispersal ability, ornithologists have repeatedly reported that Neotropical insectivore bird populations have greater local demographic stability (e.g. Willis 1974; Greenberg & Gradwohl 1986; Şekercio lu *et al.* 2002), while frugivore populations appear to undergo boom–bust demographic patterns (Faaborg *et al.* 1984; Martin & Karr 1986; Stiles 1992; Brawn *et al.* 1995) that may be associated with inter-annual resource availability. Based on these demographic differences between frugivores/nectivores and insectivores, we predicted that bouts of demographic expansion should occur more frequently in frugivores and nectivores, which if extreme enough, should result in episodic gene flow between otherwise isolated populations, in effect ‘resetting the clock’ on population divergence (Figure 1). Indeed, results from our population expansion tests found that frugivores and nectivores were significantly more likely to show genetic evidence of recent demographic expansion than mixed-diet and insectivorous species (Figure 4).

Few species in our study shared haplotypes between Belize and Panama, a necessary but not sufficient indicator of ongoing gene flow (Figure S3). However, shared haplotypes do not always indicate current gene flow – populations with prior gene flow will share haplotypes because of shared history. Only over hundreds or thousands of generations can we expect the processes of drift and mutation to result in no shared haplotypes between populations no longer experiencing gene flow (Slatkin 1985; Bohonak 1999). The lack of shared haplotypes for the majority of species in our study indicates that most species of Neotropical birds are unable to regularly maintain long-distance gene flow even across landscapes with no obvious physical barriers to dispersal. However, we did find that a significantly greater proportion of frugivore and nectivore species shared haplotypes between Belize and Panama than did insectivore or mixed-diet species, consistent with our boom–bust model (Figure 1). Previously, we found greater mtDNA haplotype diversity in the range centre than at range edges among nine species of Neotropical birds sampled across their distributions (Miller *et al.* 2010), providing additional evidence for distance-based limitation of gene flow in Neotropical birds. This would imply that ecological attributes observable over just a few years have consequences on evolutionary time scales. Indeed, we found an effect of geography (e.g. Belize vs. Panama) along with diet on estimated population growth (Figure 4). This agrees with Late Pleistocene palaeoclimatic models for Middle America that show that most of the Panamanian lowlands remained warm and moist (e.g. forested) during the last glacial cycle and subsequent deglaciation (Shadik *et al.* 2017), whereas the lowlands of northern Middle America experienced wet and arid cycles during this period (Escobar *et al.* 2012), meaning that most

Belizean forest bird populations derive from recolonisation events during the Holocene.

Our study finds that few Middle American lowland birds are able to maintain gene flow over long distances, and that among the many species we studied, foraging ecology predicts the degree of genetic differentiation between populations. This finding, along with previous studies, clearly demonstrates an important role for avian ecological traits in historical patterns of Neotropical bird diversification in addition to the role of extrinsic, physical barriers (e.g. rivers, rocks and refugia). Furthermore, our hypothesis that demographic fluctuations and dispersal differences associated with foraging guild would have predictable consequences for population divergence (Figure 1) was supported by genetic evidence. Our study demonstrates how integration of isotopes, genomic markers and model-selection hypothesis testing can provide better insight into the role of diet and its demographic correlates and consequences as an important driver of patterns of differentiation in Neotropical birds.

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AUTHORSHIP

MJM, EB and KW designed the study; MJM, BLT, ABJ and KW collected the data; MJM, BLT and JCT analysed the data, MJM wrote the first draft and all authors contributed substantially to subsequent revisions.

DATA ACCESSIBILITY STATEMENT

All data and R code are archived on the Open Science Framework (<https://doi.org/10.17605/osf.io/dghjy>) and are also available on the public github repository: <http://github/mjmillerlab/bp>. Readers should get in contact with the journal if they discover issues with data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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