


## RESEARCH ARTICLE

# Why are tropical mountain passes “low” for some species? Genetic and stable-isotope tests for differentiation, migration and expansion in elevational generalist songbirds

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## Abstract

1. Most tropical bird species have narrow elevational ranges, likely reflecting climatic specialization. This is consistent with Janzen's Rule, the tendency for mountain passes to be effectively “higher” in the tropics. Hence, those few tropical species that occur across broad elevational gradients (elevational generalists) represent a contradiction to Janzen's Rule.
2. Here, we aim to address the following questions. Are elevational generalists being sundered by diversifying selection along the gradient? Does elevational movement cause these species to resist diversification or specialization? Have they recently expanded, suggesting that elevational generalism is short-lived in geological time?
3. To answer these questions, we tested for differentiation, movement and expansion in four elevational generalist songbird species on the Andean west slope. We used morphology and mtDNA to test for genetic differentiation between high- and low-elevation populations. To test for elevational movements, we measured hydrogen isotope ( $\delta^2\text{H}$ ) values of metabolically inert feathers and metabolically active liver.
4. Morphology differed for House Wren (*Troglodytes aedon*) and Hooded Siskin (*Spinus magellanicus*), but not for Cinereous Conebill (*Conirostrum cinereum*) and Rufous-collared Sparrow (*Zonotrichia capensis*) respectively. mtDNA was structured by elevation only in *Z. capensis*.  $\delta^2\text{H}$  data indicated elevational movements by two tree- and shrub-foraging species with moderate-to-high vagility (*C. cinereum* and *S. magellanicus*), and sedentary behaviour by two terrestrial-foraging species with low-to-moderate vagility (*T. aedon* and *Z. capensis*). In *S. magellanicus*, elevational movements and lack of mtDNA structure contrast with striking morphological divergence, suggesting strong diversifying selection on body proportions across the c. 50 km gradient. All species except *C. cinereum* exhibited mtDNA haplotype variation consistent with recent population expansion across the elevational gradient,

potentially concurrent with Holocene anthropogenic habitat conversion for agriculture.

5. In different ways, each species defies the tendency for tropical birds to have long-term stable distributions and sedentary habits. We conclude that tropical elevational generalism is rare due to evolutionary instability.

#### KEYWORDS

Andean birds, divergence with gene flow, elevational migration, elevational range limits, stable isotopes

## 1 | INTRODUCTION

Elevational gradients cause profound eco-climatic variation across short distances. As a result, mountains are important hotspots of biodiversity (e.g. Grytnes & Vetaas, 2002; McCain, 2003; Sanders, 2002) and drivers of diversification (e.g. Benham & Witt, 2016; Bertrand et al., 2016; Freeman, 2015; Galen et al., 2015; Ribas, Moyle, Miyaki, & Cracraft, 2007). As elevation increases, organisms must cope with reduced temperature, humidity, air density, and partial pressure of oxygen ( $PO_2$ ) as well as increased exposure to UV radiation. The seasonally stable climatic gradients imposed by tropical mountains have been implicated in global latitudinal biodiversity gradients via Janzen's Rule (Janzen, 1967). Janzen's Rule holds that mountain passes are effectively "higher" in the tropics because seasonal thermal stability on tropical slopes has led to elevational specialization and discouraged dispersal across elevations. With increased specialization and reduced dispersal, tropical mountains promote allopatric diversification (Cadena et al., 2011). As a result, tropical species should have narrower elevational ranges than temperate ones. Although empirical support for that prediction is mixed (McCain, 2009), narrow elevational distributions are the predominant pattern for tropical montane landbirds, including songbirds (Jankowski, Londoño, Robinson, & Chappell, 2013; Parker, Stotz, & Fitzpatrick, 1996; Terborgh, 1971).

The tendency for Andean songbird species to have narrow elevational ranges is strong, as evidenced by their average elevational range breadth of only c. 1,250 m on a habitable elevational gradient spanning >5,000 m (Parker et al., 1996). These narrow elevational distributions reflect firmly established elevational limits to species distributions. Among tropical Andean songbirds, only c. 4% of species inhabit elevational ranges broader than 3,000 m (Parker et al., 1996); these species represent exceptions to Janzen's Rule because they encounter a broad range of climatic conditions. Several of the species with broad elevational ranges are found on the west slope of the Peruvian Andes, an area that experiences more temperature seasonality than the same latitudes on the east slope, but it is still dramatically less seasonal than temperate latitudes, in the southern Andes or elsewhere (Fick & Hijmans, 2017). The vast majority of terrestrial bird species on the west slope of the Peruvian Andes do have restricted elevational ranges as predicted by Janzen's Rule (Parker et al., 1996).

We offer three non-mutually exclusive explanations for the existence of these broad elevational ranges at tropical latitudes. The first explanation is that genetic fit with the environment is facilitated by cryptic population genetic structure (Milá, Warren, Heeb, & Thébaud, 2010; Milá, Wayne, Fitz, & Smith, 2009); such structure can be either genome wide or limited to functional loci that may be subject to natural selection that is strong enough to overcome the homogenizing effects of gene flow. A second possible explanation is that individuals make elevational movements to track resources; such a movement could prevent specialization by inhibiting spatially variable selection and the isolation of subpopulations along an elevational gradient. A third possible explanation is that population expansion across elevational gradients occurs periodically, but the resulting mismatch between genes and environment leads to subsequent specialization via range contraction or genetic differentiation. Tests of these mechanisms could help explain the rarity of tropical elevational generalism.

Several studies suggest that bird species can diversify along elevational gradients (Cheviron & Brumfield, 2009; Galen et al., 2015; McCormack & Berg, 2010; McCormack & Smith, 2008; Milá et al., 2009). Whether this diversification can lead to speciation without cessation of gene flow is uncertain, but it is theoretically possible (Hua, 2016). While hypoxia, cold temperatures and high UV exposure associated with high elevations are known to cause rapid evolutionary emergence of novel phenotypes (e.g. Beall et al., 2010; Galen et al., 2015; Simonson et al., 2010), homogenizing gene flow between high and low populations is expected to inhibit functional divergence and speciation (Rundle & Nosil, 2005). Differentiation with gene flow has been shown under some circumstances (e.g. Benham & Witt, 2016; Gutiérrez-Pinto et al., 2014; Kirkpatrick & Barton, 1997; Milá et al., 2009), but gene flow generally limits the extent of local adaptation. For example, Benham and Witt (2016) found that the degree of hummingbird bill size differentiation across a climatic gradient was constrained where habitats were contiguous. For sedentary elevational generalists, selection that varies along elevational gradients should lead to differentiation between high- and low-elevation populations at functional loci (Natarajan et al., 2015; Storz & Kelly, 2008). The latter process can lead to speciation if functional alleles have pleiotropic effects on reproductive isolation (Hua, 2016). Discontiguous habitat along an elevational gradient could facilitate functional and neutral

divergence via isolation. Alternatively, elevational movements could directly hinder such divergence.

Elevational migration comprises short-distance movements to track elevation-specific resource pulses that are important for reproduction (Boyle, 2017; Johnson & Maclean, 1994; Loiselle & Blake, 1991). It has been documented in numerous animal species (Hunt, Brodie, Carithers, Goldstein, & Janzen, 1999; McGuire & Boyle, 2013; Voigt, Helbig-Bonitz, Kramer-Schadt, & Kalko, 2013), particularly birds (Boyle, 2010; Chaves-Campos, Arévalo, & Araya, 2003; Hobson et al., 2003; Loiselle & Blake, 1991; Newsome, Sabat, Wolf, Rader, & del Rio, 2015; Villegas, Newsome, & Blake, 2016). If elevational generalist species undertake seasonal movements, they may be able to track resource pulses or temperature niches (Boyle, 2017), but individuals would also experience variable air density,  $PO_2$  and UV radiation that vary predictably with elevation during all seasons (West, 1996). These individual movements would reduce the spatial variability in selection and facilitate gene flow that inhibits elevational divergence (Arguedas & Parker, 2000). Despite the dramatic elevational gradients of the New World tropics, previous studies in the region have found limited evidence of elevational migration, and most elevational movements that have been documented are small in magnitude (Boyle, 2010; Boyle, Norris, & Guglielmo, 2010; Hardesty & Fraser, 2010; Hobson et al., 2003; Villegas et al., 2016). In contrast, partial or full elevational migration may be more common at temperate and subtropical latitudes in the Andes (e.g. Newsome et al., 2015). Remarkably, the frequency and extent of elevational migration for most tropical Andean bird species remains unstudied, especially in small-bodied passerine species for which satellite-tracking technologies have yet to be applied.

Hydrogen isotope ( $\delta^2H$ ) values of bird tissues can be used to characterize latitudinal and elevational movements (Bowen, Wassenaar, & Hobson, 2005; Hobson, 1999). The  $\delta^2H$  of precipitation varies predictably with respect to a variety of physicochemical processes (Dansgaard, 1964; Estep, 1981; Estep & Dabrowski, 1980; Rubenstein & Hobson, 2004). As water vapour rises on the windward side of a mountain range, it cools and condenses, and water containing the heavier isotope of hydrogen (deuterium) is the first to condense. This produces a systematic relationship between elevation and  $\delta^2H$  of local precipitation, resulting in lapse rates of 4‰–8‰ per 100 m (Poage & Chamberlain, 2001).  $\delta^2H$  values of primary producers reflect local precipitation, and consumers integrate  $\delta^2H$  values of food and water such that their tissues have  $\delta^2H$  that is higher than their food but lower than their water (Birchall, O'Connell, Heaton, & Hedges, 2005; Estep & Dabrowski, 1980; Hobson, Atwell, & Wassenaar, 1999; Wolf, Newsome, Fogel, & Martínez del Rio, 2013). A few studies have utilized the elevational lapse rate in the  $\delta^2H$  of precipitation to assess elevational movements (Hardesty & Fraser, 2010; Hobson et al., 2003; Newsome et al., 2015). Most  $\delta^2H$ -based studies have analysed feathers, a metabolically inert tissue that records ecological information only during moult, which may only last a few weeks (Hobson, Van Wilgenburg, Wassenaar, & Larson, 2012; Hobson et al., 2003; Pérez & Hobson, 2007; Wunder, 2012). More recently, a multi-tissue approach comparing  $\delta^2H$  values of metabolically active tissues (i.e. blood, muscle, liver) with metabolically inert feathers offers the potential to

reveal utilization of high- vs. low-elevation resources during different periods of the annual life cycle (Hardesty & Fraser, 2010; Mazerolle & Hobson, 2005; Newsome et al., 2015; Villegas et al., 2016).

Here, we test our explanations for broad elevational distributions in four tropical songbird species: Cinereous Conebill (*Conirostrum cinereum*), Hooded Siskin (*Spinus magellanicus*), House Wren (*Troglodytes aedon*) and Rufous-collared Sparrow *Zonotrichia capensis*. We used morphological and genetic data to test for genetic differentiation and signals of expansion along the gradient. We examined  $\delta^2H$  in metabolically inert (feathers) and active (liver) tissues to test for elevational movements in species with varying foraging strata and dispersal abilities. Our analyses suggest elevational movement in *C. cinereum* and *S. magellanicus*, genetic differentiation in *S. magellanicus*, *T. aedon* and *Z. capensis*, and recent demographic expansion in all species except *C. cinereum*. These patterns of elevational movement and diversification are consistent, in part, with all three proposed explanations for the relative rarity of tropical elevational generalists.

## 2 | MATERIALS AND METHODS

### 2.1 | Morphometric analyses

To test for genetic differentiation across the elevational range at the loci underlying functional morphological traits, we compared sizes of four traits. For four focal species, *C. cinereum*, *S. magellanicus*, *T. aedon* and *Z. capensis*, we compared morphological measurements between populations at high (>3,000 m) and low (<1,000 m) elevations. We measured culmen, wing chord, tail and tarsus from museum specimens. We used PCA to visualize the morphometric data, and compared high and low groups.

To help interpret the results of this study, we assessed relative flight capabilities of our four study species. To do so, we compared relative flight muscle mass and hand-wing index, measures that are known to correlate with flight ability and dispersal propensity (Burney & Brumfield, 2009; Claramunt, Derryberry, Remsen, & Brumfield, 2012; Dawideit, Phillimore, Laube, Leisler, & Böhning-Gaese, 2009; Kipp, 1959; Wright, Gregory, & Witt, 2014; Wright, Steadman, & Witt, 2016).

### 2.2 | mtDNA population differentiation

To test for mtDNA differentiation across the elevational range, we analysed published (Cheviron & Brumfield, 2009; Galen & Witt, 2014) and original mtDNA sequence data from high-elevation (>3,000 m) and low-elevation (<1,000 m) specimens listed in Appendix S4. We tested for elevational population genetic structure by estimating  $F_{st}$  and  $\phi_{st}$  between elevational zones using Arlequin v3.5 (Excoffier & Lischer, 2010).

### 2.3 | Stable isotope measurements

To test for elevational movements, we used mass spectrometry to measure  $\delta^2H$  from liver, contour feathers and secondary flight feathers from museum specimens of our four focal taxa that were collected

over the last decade on the west slope of the Andes in central Peru. Technical details are described in Appendix S1.

## 2.4 | $\delta^2\text{H}$ hypothetical framework

We sought to exploit the ubiquitous trend of decreasing precipitation  $\delta^2\text{H}$  with increasing elevation (Gonfiantini, Roche, Olivry, Fontes, & Zuppi, 2001; Poage & Chamberlain, 2001) to test for short-distance elevational migration (Hobson et al., 2003; Newsome et al., 2015; Villegas et al., 2016). It should be noted, that our analyses cannot distinguish between nomadism (stochastic movements that include changes in elevation) and elevational migration (predictable seasonal movement across elevation).

While few precipitation  $\delta^2\text{H}$  datasets exist for the west slope of the Peruvian Andes (IAEA/WMO, 2015), elevational  $\delta^2\text{H}$  lapse rates for other Andean regions range from 4‰ to 8‰ per 100 m (Araguás-Araguás, Froehlich, & Rozanski, 1998; Niewodniczanski, Grabczak, Baranski, & Rzepka, 1981; Poage & Chamberlain, 2001; Rozanski & Araguás-Araguás, 1995). Our study system on the west slope of the Peruvian Andes is influenced, at low elevations, by  $\delta^2\text{H}$ -enriched fog (Scholl, Eugster, & Burkard, 2010) coupled with sporadic precipitation, and at higher elevations by  $\delta^2\text{H}$ -depleted precipitation resulting from a combination of temperature-dependent fractionation and Rayleigh distillation (Dansgaard, 1964; Poage & Chamberlain, 2001). These disparate isotopic inputs likely produce elevational lapse rates in precipitation  $\delta^2\text{H}$  (4‰–8‰ per 100 m) that are comparable to those reported by Poage and Chamberlain (2001). Yet, seasonal changes in precipitation  $\delta^2\text{H}$  values that are of equal or greater magnitude than elevational variation in  $\delta^2\text{H}$  may obscure expected elevational trends in  $\delta^2\text{H}$  of metabolically active bird tissue (Gonfiantini et al., 2001; IAEA/WMO, 2015; Villegas et al., 2016).

To account for seasonal variation in precipitation  $\delta^2\text{H}$  values across the west slope of the Peruvian Andes, we used precipitation  $\delta^2\text{H}$  data collected from 2006 to 2008 in Marcapomacocha, Peru (c. 4,400 m) from the Global Network of Isotopes in Precipitation (GNIP) (IAEA/WMO, 2015). Marcapomacocha, at the top of the transect where most of our specimens were collected (Figure S1), is the only site on the west slope of the Peruvian Andes for which multiple years of monthly measurements of precipitation  $\delta^2\text{H}$  values exist. We assembled monthly mean precipitation  $\delta^2\text{H}$  values ( $\delta^2\text{H}_{\text{month}}$ ) that were obtained from Marcapomacocha during weak El Niño (2006–2007) and moderate La Niña years (2007–2008). We used the monthly mean values to account for effects of seasonal fluctuations in precipitation  $\delta^2\text{H}$  in our model of elevational effects on  $\delta^2\text{H}$  of metabolically active bird liver tissue. The dearth of available data on precipitation  $\delta^2\text{H}$  at other elevations and years required us to assume that seasonal fluctuations in precipitation  $\delta^2\text{H}$  occur similarly across elevations through time.

Differences in migratory behaviour among elevational generalist songbird species will be reflected in how their metabolically inert vs. active tissues differentially integrate elevational vs. seasonal trends in local precipitation  $\delta^2\text{H}$ . Feather tissues are grown over the course of a few weeks, typically during the dry season (June to August; Figure S2), after which they become metabolically inert. Thus,  $\delta^2\text{H}_{\text{feather}}$  should not be influenced by the date of sampling, which is captured in our

models by  $\delta^2\text{H}_{\text{month}}$ .  $\delta^2\text{H}_{\text{feather}}$  values are expected to vary with elevation of capture in sedentary birds, but not in migratory ones that will have often shifted in elevation between the date of moult and the date of sampling. Conversely, liver tissue is metabolically active so  $\delta^2\text{H}_{\text{liver}}$  should reflect both seasonal ( $\delta^2\text{H}_{\text{month}}$ ) and elevational variation in precipitation  $\delta^2\text{H}$ , regardless of whether the bird is sedentary or migratory. Thus, regardless of the date on which a bird was collected, liver  $\delta^2\text{H}$  values should be predicted in part by seasonal fluctuations in precipitation  $\delta^2\text{H}$ , which are reflected in our models as  $\delta^2\text{H}_{\text{month}}$ .

Elevational movements of individual birds are predicted to influence the relationship between tissue  $\delta^2\text{H}$  and elevation. If elevational migration occurs, we expect that any correlation between  $\delta^2\text{H}$  and elevation of capture would be diminished in metabolically inert tissues, and potentially also in metabolically active ones. Because elevational migrant species are less likely to have grown feathers at the elevation of capture, we expect  $\delta^2\text{H}$  values for inert feathers to lack a trend with elevation of capture.

## 2.5 | $\delta^2\text{H}$ statistical analyses

We compared intra- and interspecific  $\delta^2\text{H}_{\text{tissue}}$  values using nonparametric tests when applicable. To test for elevational and seasonal effects on  $\delta^2\text{H}$ , we evaluated sets of linear models for each species and tissue type using  $\text{AIC}_c$ . For  $\delta^2\text{H}_{\text{liver}}$ , we compared models containing all possible combinations of the intercept and three continuous predictor variables: elevation, precipitation  $\delta^2\text{H}_{\text{month}}$  for the sampling date and latitude. For  $\delta^2\text{H}_{\text{feather}}$ , we excluded models that included precipitation  $\delta^2\text{H}_{\text{month}}$  because metabolically inert tissues should be independent of precipitation  $\delta^2\text{H}$  at the date of capture. We included latitude as a potentially confounding variable, but we did not consider models with latitude as the sole predictor variable. Furthermore, we excluded models that performed worse by  $\text{AIC}_c$  than a nested version of the same model (Arnold, 2010).

## 2.6 | mtDNA test of recent population expansion

To test for recent population expansion, we used DnaSP v5 (Librado & Rozas, 2009) to estimate Tajima's  $D$  (Tajima, 1996), Fu's  $F$  (Fu, 1997) and we used mismatch distributions to evaluate the distribution of pairwise divergence between individuals in a population. Using the mismatch distributions, we calculated the raggedness index ( $r$ ), with raggedness expected to be elevated under a stable population relative to an expanding one (Harpending, 1994). We inferred population expansion when both Tajima's  $D$  and Fu's  $F$  were significantly negative and there was no significant raggedness.

Detailed methods are reported in the Appendix S1.

# 3 | RESULTS

## 3.1 | Precipitation $\delta^2\text{H}$

The GNIP data (IAEA/WMO, 2015) revealed striking seasonal variation for the Marcapomacocha GNIP site (Figure S1). Mean  $\delta^2\text{H}_{\text{month}}$

was  $-117 \pm 33\text{‰}$  during the wet season (Oct–May) and  $-40 \pm 19\text{‰}$  during the dry season (June–Sep). The large (c. 100%) difference between precipitation  $\delta^2\text{H}$  in the wet season and dry season (Figure S1) represents a confounding factor that requires careful consideration when attempting to interpret  $\delta^2\text{H}$  values of metabolically active tissues collected along an elevational gradient.

### 3.2 | Tissue $\delta^2\text{H}$

Comparisons of linear models to explain tissue  $\delta^2\text{H}$  values are reported in Table 1. Neither latitude nor elevation explained variation in  $\delta^2\text{H}_{\text{feather}}$  for *C. cinereum* or *S. magellanicus*. In contrast, top models for both feather types of *T. aedon* included elevation of capture as the sole predictor variable, although only  $\delta^2\text{H}_{\text{contour}}$  values were

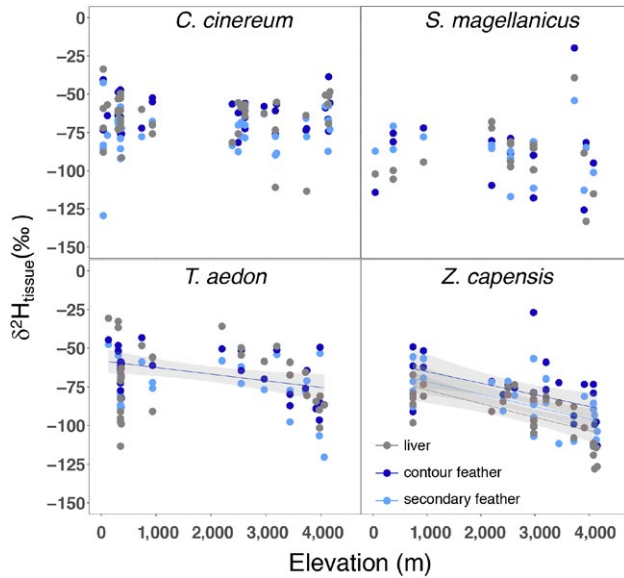
significantly negatively correlated with elevation of collection ( $t$  value:  $-2.67$ ,  $p = .01$ ; Figure 1). Similarly, top models for *Z. capensis*  $\delta^2\text{H}_{\text{feather}}$  included only elevation of capture as a predictor variable, and both  $\delta^2\text{H}_{\text{contour}}$  ( $t$  value:  $-2.96$ ,  $p < .01$ ) and  $\delta^2\text{H}_{\text{secondary}}$  ( $t$  value:  $-3.99$ ,  $p < .001$ ) were significantly negatively correlated with elevation of capture (Figure 1; Table 1).

The  $\delta^2\text{H}_{\text{liver}}$  varied significantly among species ( $F$  value: 18.59,  $df = 3$ ,  $p < .001$ ), with values for the two insectivorous species (*C. cinereum* and *T. aedon*) significantly higher than those of the two granivorous species (*S. magellanicus* and *Z. capensis*). For all four species, the best models with  $\delta^2\text{H}_{\text{liver}}$  as a response variable included  $\delta^2\text{H}_{\text{month}}$  as a predictor variable (Table 1). *Conirostrum cinereum*  $\delta^2\text{H}_{\text{liver}}$  values were positively correlated with  $\delta^2\text{H}_{\text{month}}$  ( $F$ : 4.6,  $df = 37$ ,  $p = .04$ ) (Figure 2). *Spinus magellanicus*  $\delta^2\text{H}_{\text{liver}}$  values were positively

**TABLE 1** Comparison of models to explain  $\delta^2\text{H}_{\text{tissues}}$  values as a function of elevation (elev), seasonal variation in precipitation  $\delta^2\text{H}$  ( $\delta^2\text{H}_{\text{month}}$ ) and latitude (lat) for each of the four focal species.  $\delta^2\text{H}_{\text{month}}$  was excluded from comparisons for  $\delta^2\text{H}_{\text{feather}}$  values (dark grey boxes). All combinations of predictor variables were tested against  $\delta^2\text{H}_{\text{liver}}$ . Models that scored lower than nested versions of themselves were removed, following Arnold (2010). For each species and tissue type, models with lowest AIC<sub>c</sub>,  $\Delta\text{AIC}_c$  of 0 and highest weight are bolded

Species	Model	$\delta^2\text{H}_{\text{contour}}$			$\delta^2\text{H}_{\text{secondary}}$			$\delta^2\text{H}_{\text{liver}}$		
		AIC <sub>c</sub>	$\Delta\text{AIC}_c$	Weight	AIC <sub>c</sub>	$\Delta\text{AIC}_c$	Weight	AIC <sub>c</sub>	$\Delta\text{AIC}_c$	Weight
<i>Conirostrum cinereum</i>	~ 1	299.83	0.00	1.00	322.68	0.00	1.00	328.89	2.24	0.25
	~ elev									
	~ elev + lat									
	~ $\delta^2\text{H}_{\text{month}}$							326.66	0.00	0.75
	~ elev + $\delta^2\text{H}_{\text{month}}$									
	~ $\delta^2\text{H}_{\text{month}}$ + lat									
	~ elev + $\delta^2\text{H}_{\text{month}}$ + lat									
<i>Spinus magellanicus</i>	~ 1	188.59	0.00	1.00	176.93	0.00	1.00	182.08	14.34	0.00
	~ elev									
	~ elev + lat									
	~ $\delta^2\text{H}_{\text{month}}$							174.54	6.80	0.03
	~ elev + $\delta^2\text{H}_{\text{month}}$							172.62	4.87	0.07
	~ $\delta^2\text{H}_{\text{month}}$ + lat							172.05	4.30	0.09
	~ elev + $\delta^2\text{H}_{\text{month}}$ + lat							167.74	0.00	0.81
<i>Troglodytes aedon</i>	~ 1	249.30	4.76	0.08	257.72	0.90	0.39	275.11	9.15	0.00
	~ elev	244.55	0.00	0.92	256.83	0.00	0.61			
	~ elev + lat									
	~ $\delta^2\text{H}_{\text{month}}$							266.23	0.27	0.31
	~ elev + $\delta^2\text{H}_{\text{month}}$							266.03	0.07	0.34
	~ $\delta^2\text{H}_{\text{month}}$ + lat									
	~ elev + $\delta^2\text{H}_{\text{month}}$ + lat							265.95	0.00	0.35
<i>Zonotrichia capensis</i>	~ 1	272.86	5.72	0.05	264.56	11.11	0.00	264.71	23.80	0.00
	~ elev	267.14	0.00	0.95	253.45	0.00	1.00	250.44	9.53	0.01
	~ elev + lat							248.86	7.95	0.02
	~ $\delta^2\text{H}_{\text{month}}$							264.35	23.44	0.00
	~ elev + $\delta^2\text{H}_{\text{month}}$							249.16	8.25	0.02
	~ $\delta^2\text{H}_{\text{month}}$ + lat									
	~ elev + $\delta^2\text{H}_{\text{month}}$ + lat							240.91	0.00	0.96





**FIGURE 1**  $\delta^2\text{H}$  values contour feather (dark blue), secondary flight feather (light blue) and liver (grey) plotted against elevation of capture for *Conirostrum cinereum*, *Spinus magellanicus*, *Troglodytes aedon* and *Zonotrichia capensis*; sample sizes for each species are reported in Appendix S1; statistics for linear relationships are provided in Table 1. Best-fit lines are shown for linear regressions that are significant at  $p < .05$  and grey bands represent 95% confidence intervals

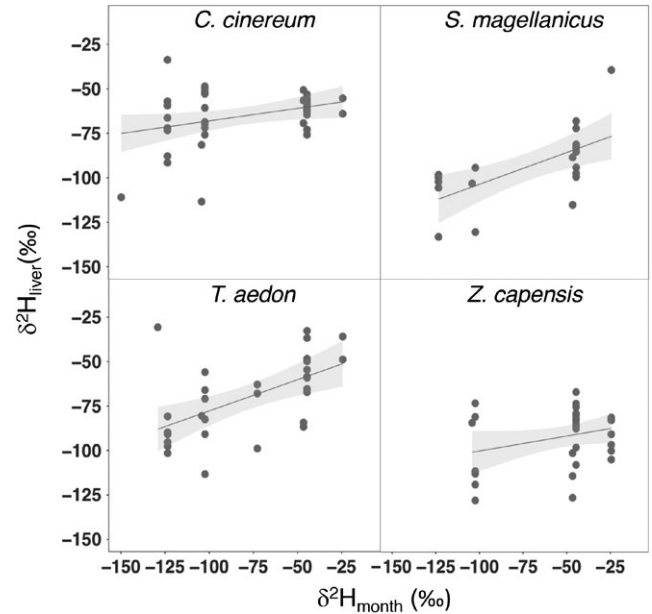
correlated with  $\delta^2\text{H}_{\text{month}}$  ( $F: 11.6, df = 16, p < .001$ ), latitude ( $F: 11.6, df = 16, p = .01$ ) and negatively correlated with elevation ( $F: 11.6, df = 16, p = .01$ ) (Figure 1). *Troglodytes aedon*  $\delta^2\text{H}_{\text{liver}}$  values were positively correlated with  $\delta^2\text{H}_{\text{month}}$  ( $F: 6.7, df = 26, p < .001$ ). *Zonotrichia capensis*  $\delta^2\text{H}_{\text{liver}}$  values were positively correlated with  $\delta^2\text{H}_{\text{month}}$  ( $F: 16.1, df = 27, p < .01$ ) (Figure 2), negatively correlated with elevation of capture ( $F: 16.1, df = 27, p < .001$ ) (Figure 1) and negatively correlated with latitude of capture ( $F: 16.1, df = 27, p < .01$ ).

Comparisons of  $\delta^2\text{H}$  values among tissue types are reported in Table S2. Interspecific comparisons of  $\delta^2\text{H}_{\text{feather}}$  values are reported in Table S3 and Figure S3.

### 3.3 | Morphometric comparisons

Mean morphometric measurements (culmen, tail, tarsus and wing chord) are reported for low ( $<1,000$  m) and high ( $>3,000$  m) elevational bins for each species in Table S4. Wing length differed between elevations for *S. magellanicus* ( $\chi^2: 22.2, df = 1, p < .001$ ); tail length differed between elevations for *T. aedon* ( $F: 6.7, df = 1, p = .02$ ) (Table S4). We found no measurement differences between elevational groups for *C. cinereum* or *Z. capensis*. The principal component analysis illustrates the overall findings of morphological differentiation in *S. magellanicus* and *T. aedon*, but not in the other two species (Figure 3).

Hand-wing index differed in all pairwise comparisons between species except those of *Z. capensis* with *C. cinereum* and *T. aedon* respectively (Figure S4). Those data indicate highest vagility in *S. magellanicus*,



**FIGURE 2**  $\delta^2\text{H}_{\text{liver}}$  plotted against 2006–2008 monthly mean precipitation  $\delta^2\text{H}$  ( $\delta^2\text{H}_{\text{month}}$ ) values from Marcapomacocha, Peru (IAEA/WMO, 2015); sample sizes for each species are reported in Table 2. Best-fit lines are shown for linear regressions that are significant at  $p < .05$ . Grey bands represent 95% confidence intervals

followed by *C. cinereum*, *Z. capensis* and *T. aedon*. Species variation in flight muscle mass (corrected for body size) was consistent with the latter finding (Figure S4). Flight muscle mass differed between species in all comparisons except the one between *Z. capensis* and *C. cinereum*.

### 3.4 | mtDNA structure and demography

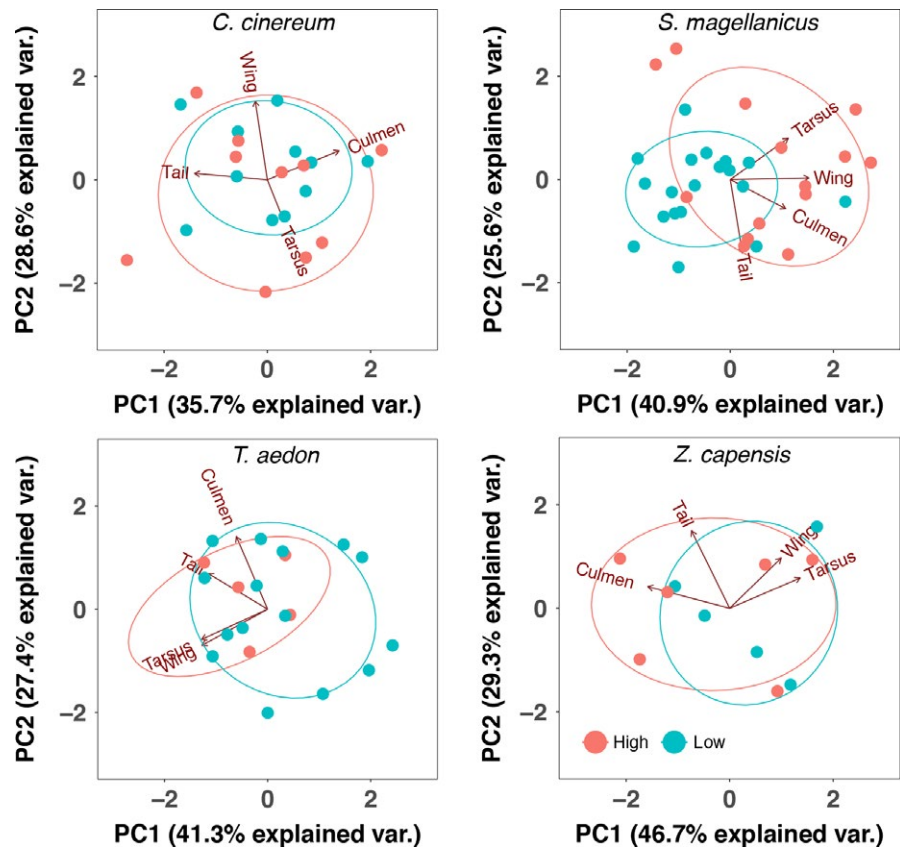
$F_{\text{st}}$  and  $\phi_{\text{st}}$  statistics were only significantly non-zero for the comparison between high- ( $n = 31$ ) and low-elevation ( $n = 29$ ) groups of *Z. capensis* (Table 2). Raggedness of mismatch distributions was not significant for any of the four species (Table 2, Figure 4), which is consistent with the null hypothesis of recent demographic expansion. Tajima's  $D$  and Fu's  $F$  statistics were significantly negative, suggesting recent demographic expansion for *S. magellanicus*, *T. aedon* and *Z. capensis*, but not for *C. cinereum* (Table 2, Figure 4).

## 4 | DISCUSSION

Janzen's Rule predicts narrower elevational ranges as an evolutionary response to decreased seasonality. The climate of the west slope of the Peruvian Andes is far less seasonal than temperate latitude climates, although it exhibits markedly more temperature seasonality than the more humid east slope (Fick & Hijmans, 2017). Consistent with the predictions of Janzen's Rule, most of the few terrestrial Peruvian bird species that have elevational ranges greater than 3,000 m occur on the relatively seasonal west slope. Elevational movement, elevational genetic differentiation and evidence of recent population expansion occur to varying degrees in

**TABLE 2** Summary of mtDNA analyses by species for low (<1,000 m) and high (>3,000 m) elevation specimens. Haplotypes (N H-Type), proportion of polymorphic sites (P-site), nucleotide diversity ( $\pi$ ), Tajima's  $D$  ( $D$ ), Fu's  $F$  ( $F$ ), raggedness ( $r$ ),  $F_{st}$  and  $\phi_{st}$  are reported. Significant values accompanied by asterisks

Species	N	N H-type	P-site (n/total)	$\pi$	$D$	$F$	$r$	$F_{st}$	$\phi_{st}$
<i>Conirostrum cinereum</i>	High = 15 Low = 15	12	0.012 (11/892)	0.0024	-0.322ns	-3.19ns	0.04ns $p = .10$	0.01ns	-0.003ns
<i>Spinus magellanicus</i>	High = 20 Low = 11	8	0.027 (13/475)	0.0020	-2.37**	-4.37**	0.17ns $p = .40$	-0.02ns	-0.007ns
<i>Troglodytes aedon</i>	High = 22 Low = 18	23	0.032 (29/918)	0.0023	-2.30**	-21.91***	0.04ns $p = .10$	-0.0004ns	0.015ns
<i>Zonotrichia capensis</i>	High = 31 Low = 29	15	0.044 (17/384)	0.0025	-2.21**	-12.87**	0.09ns $p = .15$	0.17***	0.18***



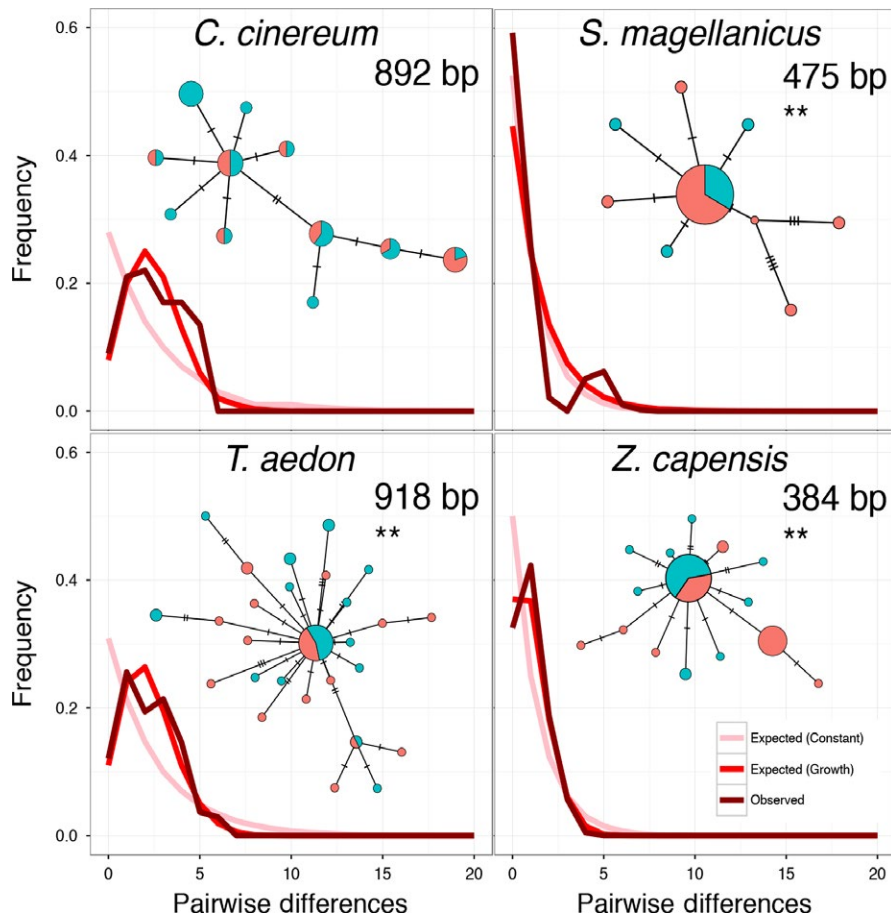
**FIGURE 3** Principal component analyses of four morphological measurements: bill length, tarsus, wing chord and tail length in millimetres for *Conirostrum cinereum* ( $n = 21$ ), *Spinus magellanicus* ( $n = 34$ ), *Troglodytes aedon* ( $n = 22$ ) and *Zonotrichia capensis* ( $n = 11$ ), grouped into (red) high elevation (>3,000 m) and (blue) low elevation (<1,000 m)

our four study taxa, suggesting that each may play a role in causing exceptions to Janzen's Rule. Tissue  $\delta^2H$  patterns associated with elevational movement were observed in two species (*C. cinereum* and *S. magellanicus*) that had no mtDNA population structure, only one of which (*S. magellanicus*) showed morphological differentiation. Two other species showed isotopic patterns associated with sedentary habits; one of those species exhibited mtDNA population structure (*Z. capensis*) while the other exhibited morphological differentiation (*T. aedon*). Three of the four focal species showed signs of recent population expansion by all three indices tested. In the following sections, we examine tissue  $\delta^2H$  patterns associated with elevational movement and we consider how differing environmental processes might obscure or maintain these patterns. We delineate

subcategories of elevational generalists (sedentary and migratory) to illustrate potential evolutionary consequences of short distance migration along environmental gradients.

#### 4.1 | Tissue $\delta^2H$ patterns

Variation in *T. aedon* and *Z. capensis* feather  $\delta^2H$  with elevation of capture (Figure 1) conforms with expected elevational patterns in precipitation  $\delta^2H$ , which suggests these species are generally sedentary. Both species are terrestrial foragers with morphological characteristics indicating low-to-moderate vagility (Table S1). For both species, the best linear models to explain variation in  $\delta^2H_{\text{contour}}$  and  $\delta^2H_{\text{secondary}}$  included only elevation as a predictor variable (Table 1). This indicates



**FIGURE 4** Mismatch distributions obtained from mtDNA loci (ND2 or ND3) of high (>3,000 m) and low (<1,000 m) elevation specimens of *Conirostrum cinereum*, *Spinus magellanicus*, *Troglodytes aedon* and *Zonotrichia capensis*. Haplotype networks coloured by elevation group: high elevation (red), low elevation (blue) accompany each mismatch distribution. Double asterisks indicate that both Tajima's *D* and Fu's *F* tests for population expansion, based on haplotype frequency spectra, were significant for that species

that individuals of these species had moulted at or near the elevation of capture. It further suggests that the season of moult was consistent among individuals such that the elevational signal was not overwhelmed by seasonal fluctuations in precipitation  $\delta^2\text{H}$ . For the other two species, *S. magellanicus* and *C. cinereum*, elevation did not explain variation in feather  $\delta^2\text{H}$ , suggesting that individuals of those species underwent elevational movements between the time of moult and the time of sampling. A plausible alternative explanation would be that the season of moult is more variable among individuals of the latter two species, but there is no evidence for that in our moult data (Figure S2), so we favour the conclusion that *S. magellanicus* and *C. cinereum* are undertaking elevational movements. Such movements could comprise nomadism or true elevational migration.

Unlike feather  $\delta^2\text{H}$  values,  $\delta^2\text{H}_{\text{liver}}$  values integrate seasonal variation in precipitation  $\delta^2\text{H}$  because this tissue is metabolically active and has a rapid isotopic incorporation rate, integrating ecological information over 1–2 weeks prior to capture for an endotherm the size of a songbird (Martínez del Río, Wolf, Carleton, & Gannes, 2009; Wolf, Carleton, & Martínez del Río, 2009).  $\delta^2\text{H}_{\text{liver}}$  showed significant positive associations with  $\delta^2\text{H}_{\text{month}}$  for all species (Figure 2; Table 1). Elevation of capture was included in the best models for  $\delta^2\text{H}_{\text{liver}}$  for all species except *C. cinereum* (Table 1), although elevation was significant only for *Z. capensis* (Figure 1). It is possible that the lack of elevational trend in  $\delta^2\text{H}_{\text{liver}}$  for *C. cinereum* might have occurred due to elevational movements within the weeks before sampling, but the

generally modest relationship between elevation and  $\delta^2\text{H}_{\text{liver}}$  may have other causes (see below).

Any seasonal variation in precipitation  $\delta^2\text{H}$  that was not captured by our temporal index ( $\delta^2\text{H}_{\text{month}}$ ) may have dampened the expected elevational trends in  $\delta^2\text{H}_{\text{liver}}$ .  $\delta^2\text{H}_{\text{month}}$  provided an index of seasonal flux in precipitation  $\delta^2\text{H}$  that was derived from 3 years (2006–2008) of data at a single high-elevation site, Marcapomacocha (c. 4,400 m). It is possible that the Marcapomacocha data poorly represent seasonal fluctuations in precipitation  $\delta^2\text{H}$  at other elevations or in other years. The data spanned both El Niño and La Niña years. Precipitation  $\delta^2\text{H}$  data recorded during these events potentially differs from non-ENSO years, but the Marcapomacocha 2006–2008 data are the best, available from the west slope at present. Future studies would greatly benefit from the collection of additional precipitation  $\delta^2\text{H}$  data along the Andean elevation gradients. Additional problems with interpretation of our models could have occurred if sampling during the wet or dry season were concentrated at high or low elevation; however, we consider these potential sources of bias unlikely to have driven our results because our  $\delta^2\text{H}$  data came from specimens that were collected across the entire elevational gradient during both wet and dry periods for all species (Figures 1 and 2).

## 4.2 | Morphological and genetic differentiation

The division between sedentary and migratory modes of elevational generalism reflected in tissue  $\delta^2\text{H}$  patterns is likely mirrored in flight



capabilities. Depending on foraging strategies and local ecologies, sedentary birds are predicted to be less vagile than their migratory counterparts, traits that should be reflected in the flight apparatus (flight muscle size and hand-wing index; Figure S4) and foraging stratum (Table S1). Larger flight muscle mass, higher hand-wing index and less terrestrial-foraging ecology in *C. cinereum* and *S. magellanicus* relative to *T. aedon* and *Z. capensis* generally support this dichotomy, although there was some overlap in hand-wing index in *Z. capensis*–*T. aedon*/*C. cinereum* comparisons (Figure S4) and flight muscle size between *C. cinereum* and *Z. capensis* (Table S1; Figure S4).

Differences in sedentary and elevational migratory habits should be further reflected in their respective levels of within-species population genetic structure. Given enough time, we expect sedentary elevational generalists to have developed genetic structure between high- and low-elevation populations. Genetic structure could arise as a result of local adaptation and consequent selection for elevational philopatry; isolation by distance alone is unlikely to cause differentiation across these elevational gradients that span 50–100 km because of the proportionately high dispersal distances that are typical of songbirds (Cheviron & Brumfield, 2009; Galen et al., 2015). Migratory or nomadic behaviour in elevational generalists should maintain or enhance gene flow, effectively washing out any incipient population structure. We expect that tests for population-level differentiation within this framework will provide insight into the ecologies and evolutionary trajectories of bird species that are elevational generalists.

Our morphological tests showed significant differentiation between high- and low-elevation *T. aedon* in tail length (Figure 3 and Figure S5). A trend of larger appendages at higher elevations has been previously reported in another Andean bird, the Torrent Duck (Gutiérrez-Pinto et al., 2014). The morphometric disparity, in combination with our  $\delta^2\text{H}$  data, is in agreement with our hypothesis regarding the link between sedentary habit and elevational genetic differentiation. In contrast, *C. cinereum* exhibited no differentiation between high- and low-elevation specimens in the four characters we measured. Although vagile and migratory species can be philopatric and locally differentiated (Fudickar, Greives, Atwell, Stricker, & Ketterson, 2016), this lack of differentiation is consistent with our predictions for an elevational generalist, that is, it also undergoes elevational movements. *Spinus magellanicus* showed significant morphological differentiation in wing chord length between high- and low-elevation specimens, despite its isotopic pattern indicating elevational movements. The larger wing chord sizes at high elevation could be the result of selection on wing size that was strong enough to overcome gene flow (Benham & Witt, 2016; Gutiérrez-Pinto et al., 2014; Smith et al., 2004). An alternative possibility is that the traits we measured exhibit high levels of phenotypic plasticity in response to elevation-specific pressures, but we consider this possibility to be less likely. Phenotypically plastic traits could appear to be differentiated as a result of environmental effects on gene expression alone (Cheviron, Whitehead, & Brumfield, 2008; James, 1983; Przybylo, Sheldon, & Merila, 2000), but there is evidence that morphometric traits are generally highly heritable (Boag, 1983; Keller, Grant, Grant, & Petren, 2001). For the two species that undergo elevational movements, we cannot rule out that spatial segregation

might only occur only during breeding, as occurs among subspecies of *Junco hyemalis* that are sympatric during the non-breeding season (Fudickar et al., 2016); if that is the case, our sampling from throughout the year may have failed to uncover the full extent of elevational differentiation.

Population structure can persist locally along contiguous elevational distributions, effectively selecting against unfit immigrants (Cheviron & Brumfield, 2009; Cheviron et al., 2014). Yet, if average dispersal distances are large, these clines are unlikely to form. To test for genetic differentiation, we analysed ND2 or ND3 mtDNA sequence data from all four species.  $F_{st}$  and  $\phi_{st}$  values confirmed previously reported population structure between high- and low-elevation *Z. capensis*, corroborating a sedentary habit for this species (Figure 4) (Cheviron & Brumfield, 2009). Although this structure could result through isolation by distance, Cheviron and Brumfield (2009) found higher reductions in mitochondrial gene flow between high and low elevation relative to latitudinal control transects, suggesting selection was maintaining the elevational cline.

$F_{st}$  and  $\phi_{st}$  values were not significant for *T. aedon*, which was somewhat surprising considering the sedentary lifestyle suggested by our isotopic and morphometric data. Analysis of  $\beta$ -haemoglobin gene variation in *T. aedon* across the same elevational transect studied here found substantial elevational population structure (Galen et al., 2015). mtDNA analysed here was unstructured with respect to elevation, as were the vast majority of nuclear protein-coding genes analysed by Galen et al. (2015). As in *T. aedon*, mtDNA sequence data from *C. cinereum* and the *S. magellanicus* showed no population structure between high- and low-elevation groups.

Signals of recent demographic expansions, as indicated by mismatch distributions, Tajima's  $D$  and Fu's  $F$  test statistics (Table 2, Figure 4) were present in three of the four focal species (*S. magellanicus*, *T. aedon* and *Z. capensis*). These demographic expansions, if accompanied by expansions of the elevational range, potentially explain exceptions to Janzen's (1967) Rule. Considering the physical landscape of the western Andes, this expansion likely originated in high-elevation environments that are diverse and productive relative to dry coastal zones that are depauperate and may have fewer competitors. Published phylogenies for tanagers (including *C. cinereum*) (Burns et al., 2014), siskins (including *S. magellanicus*) (Beckman & Witt, 2015), South American *T. aedon* populations (Galen & Witt, 2014; Galen et al., 2015) and *Z. capensis* populations (Lougheed et al., 2013) are all consistent with montane origins and subsequent, downslope range expansions in western Peru. As a caveat, it should be noted that false inference of population expansion from mtDNA haplotype frequency spectra can be caused by other demographic events, such as selective sweeps (Fay & Wu, 2000; Przeworski, 2002; Wakeley & Aliacar, 2001). Moreover, the high prevalence of apparent range expansions among these elevational generalists contrasts with previous findings for Andean cloud-forest specialist species; haplotype frequency spectra consistent with population expansion were found in only a small fraction of subpopulations for *Thamnophilus caerulescens* (Brumfield, 2005), *Metallura tyrianthina* (Benham, Cuervo, McGuire, & Witt, 2015; Benham & Witt, 2016) and *Premnoplex brunnescens* (Valderrama, Pérez

Emán, Brumfield, Cuervo, & Cadena, 2014). Two species of brush-finches (*Buarremon*) that are restricted to mid-elevations appear to have undergone recent expansions, but the evidence was considered to be equivocal (Cadena, 2007).

Our mtDNA data provides insights into the timing of the inferred range expansions. Fossil-calibrated divergence rates such as the oft-used 2% per million years (Lovette, 2004; Weir & Schluter, 2008) are known to overestimate the ages of recent events (Arbogast, Edwards, Wakeley, Beerli, & Slowinski, 2002; Ho, Duchêne, Molak, & Shapiro, 2015). Therefore, we used a pedigree-based substitution rate ( $3.13 \times 10^{-7}$  mutations/site/year) derived from chicken mtDNA genomes to estimate dates of population expansion (Alexander et al., 2015). Applying this rate to our mtDNA data, we estimated expansion to have occurred c. 3.5 Kya (*T. aedon*) to c. 34 Kya (*S. magellanicus*).

### 4.3 | Anthropogenic or climatic influence on expansion

The estimated range of dates for population expansion is compatible with causation due to the warming and cooling cycles of the last glacial period, including the dramatic warming that followed Younger Dryas. These climatic events might have caused demographic fluctuations, including upward expansion into habitat that became accessible after final recession of the glaciers, c. 12 Kya (Smith, Seltzer, Farber, Rodbell, & Finkel, 2005).

Considering that the timing of population expansion may have been as recent as c. 3.5 Kya, it is possible that they were caused by downward expansion that coincided with the onset of anthropogenic habitat conversion. The lower west slope of the central Peruvian Andes is one of the driest places on Earth, with very limited natural bird habitats away from the immediate vicinity of rivers and streams sourced from the high Andes. Isolated patches of “lomas” vegetation that depend on water from persistent coastal fog comprise one exception (Rundel & Dillon, 1998). With the exception of lomas patches, the expansion of bird habitats away from rivers would have occurred only recently, following the implementation of sophisticated irrigation systems by the Paracas people, which also occurred c. 3.5 Kya (Hesse & Baade, 2009). Land alteration by humans on the west slope likely established habitat corridors between formerly isolated patches, particularly for generalist, non-forest species. Regardless of whether it directly caused signals of expansion in our genetic data, the expansion of bird populations spurred by water diversion, irrigation and agriculture on formerly arid land should be considered likely. Although all four of our study species are widespread in undeveloped areas, they also occur in human dominated landscapes, and *T. aedon* and *Z. capensis* can be considered facultative human commensalists (Newhouse, Marra, & Johnson, 2008; Ruiz, Rosenmann, Novoa, & Sabat, 2002). Whether or not agricultural development facilitated expansion to lower and dryer portions of the western Andean slopes, our findings of recent expansion, individual movements and ongoing diversification indicate that evolutionary instability is inherent to broad elevational ranges, at least for tropical songbird species.

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### AUTHORS' CONTRIBUTIONS

C.G., C.W. and S.N. devised the ideas and created the methodology and led the writing of the manuscript; C.G., E.J.B., A.C. and S.G. collected the data; C.G., A.C. and S.G. analysed the data. All authors offered crucial contributions to drafts and gave final approval of the manuscript.

### DATA ACCESSIBILITY

GenBank accession numbers reported in Appendix S4. Sample details are documented in the Arctos database (arctosdb.org), and are directly linked from Appendix S4. Data are deposited on Dryad Digital Repository <https://doi.org/10.5061/dryad.0sq68> (Gadek et al., 2017).

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## SUPPORTING INFORMATION

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