

1 **Why are tropical mountain passes ‘low’ for some species?**

2 **Genetic and stable-isotope tests for differentiation, migration, and expansion in**  
3 **elevational generalist songbirds**

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24 **Abstract**

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

47 three out of four elevational generalist species underwent genetic divergence despite  
48 gene flow, two of four make seasonal elevational movements, and three of four have  
49 recently expanded. In different ways, each species defies the tendency for tropical birds  
50 to have long-term stable distributions and sedentary habits. We conclude that tropical  
51 elevational generalism is rare due to evolutionary instability.

52

53 **Keywords:** elevational range limits, elevational migration, stable isotopes, divergence  
54 with gene flow, Andean birds.

## 55 **Introduction**

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

72         The tendency for Andean songbird species to have narrow elevational ranges is  
73 strong, as evidenced by their average elevational range breadth of only ~1250 m on a  
74 habitable elevational gradient spanning >5000 m (Parker et al. 1996). These narrow  
75 elevational distributions reflect firmly established elevational limits to species  
76 distributions. However, a small number of species defy this pattern, particularly on the  
77 west slope of the Andes. Among tropical Andean songbirds, ~4% of species inhabit

78 elevational ranges broader than 3000 m (Parker et al. 1996) and represent exceptions  
79 to Janzen's Rule because they encounter a broad range of climatic conditions. We offer  
80 three non-mutually exclusive explanations for the existence of these broad elevational  
81 ranges at tropical latitudes. The first explanation is that genetic fit with the environment  
82 is facilitated by cryptic population genetic structure (Milá et al. 2009, 2010); such  
83 structure can be either genome-wide or limited to functional loci that may be subject to  
84 natural selection that is strong enough to overcome the homogenizing effects of gene  
85 flow. A second possible explanation is that individuals make elevational movements to  
86 track resources; such movement could prevent specialization by inhibiting spatially  
87 variable selection and the isolation of subpopulations along an elevational gradient. A  
88 third possible explanation is that population expansion across elevational gradients  
89 occurs periodically, but the resulting mismatch between genes and environment leads  
90 to subsequent specialization via range contraction or genetic differentiation. Tests of  
91 these mechanisms could help explain the rarity of tropical elevational generalism.

92         Several studies suggest that bird species can diversify along elevational  
93 gradients (McCormack and Smith 2008, Cheviron and Brumfield 2009, Milá et al. 2009,  
94 McCormack and Berg 2010, Galen et al. 2015). Whether this diversification can lead to  
95 speciation without cessation of gene flow is uncertain, but it is theoretically possible  
96 (Hua 2016). While hypoxia, cold temperatures, and high UV exposure associated with  
97 high elevations are known to cause rapid evolutionary emergence of novel phenotypes  
98 (e.g. Beall et al. 2010, Simonson et al. 2010, Galen et al. 2015), homogenizing gene  
99 flow between high and low populations is expected to inhibit functional divergence and  
100 speciation (Rundle and Nosil 2005). Differentiation with gene flow has been shown

101 under some circumstances (e.g. Kirkpatrick and Barton 1997, Milá et al. 2009,  
102 Gutiérrez-Pinto et al. 2014, Benham and Witt 2016), but gene flow generally limits the  
103 extent of local adaptation. For example, Benham and Witt (2016) found that the degree  
104 of hummingbird bill size differentiation across a climatic gradient was constrained where  
105 habitats were contiguous. For sedentary elevational generalists, selection that varies  
106 along elevational gradients should lead to differentiation between high- and low-  
107 elevation populations at functional loci (Storz and Kelly 2008, Natarajan et al. 2015).  
108 The latter process can lead to speciation if functional alleles have pleiotropic effects on  
109 reproductive isolation (Hua 2016). Discontiguous habitat along an elevational gradient  
110 could facilitate functional and neutral divergence via isolation. Alternatively, elevational  
111 movements could directly hinder such divergence.

112         Elevational migration comprises short-distance movements to track elevation-  
113 specific resource pulses that are important for reproduction (Loiselle and Blake 1991,  
114 Johnson and Maclean 1994, Boyle 2017). It has been documented in numerous animal  
115 species (Hunt et al. 1999, McGuire and Boyle 2013, Voigt et al. 2013), particularly birds  
116 (Loiselle and Blake 1991, Chaves-Campos et al. 2003, Hobson et al. 2003, Boyle 2010,  
117 Newsome et al. 2015, Villegas et al. 2016). If elevational generalist species undertake  
118 seasonal movements, they may be able to track resource pulses or temperature niches  
119 (Boyle 2017), but individuals would also experience variable air density,  $PO_2$ , and UV  
120 radiation that vary predictably with elevation during all seasons (West 1996). These  
121 individual movements would reduce the spatial variability of selection and facilitate gene  
122 flow that inhibits elevational divergence (Arguedas and Parker 2000). Despite the  
123 dramatic elevational gradients of the New World tropics, previous studies in the region

124 have found limited evidence of elevational migration, and most elevational movements  
125 that have been documented are small in magnitude (Hobson et al. 2003, Boyle 2010,  
126 Hardesty and Fraser 2010, Boyle et al. 2010, Villegas et al. 2016). In contrast, partial or  
127 full elevational migration may be more common at temperate and subtropical latitudes in  
128 the Andes (e.g., Newsome et al. (2015)). Remarkably, the frequency and extent of  
129 elevational migration for most tropical Andean bird species remains unstudied,  
130 especially in small-bodied passerine species for which satellite-tracking technologies  
131 have yet to be applied.

132         Hydrogen isotope ( $\delta^2\text{H}$ ) values of bird tissues can be used to characterize  
133 latitudinal and elevational movements (Hobson 1999, Bowen et al. 2005). The  $\delta^2\text{H}$  of  
134 precipitation varies predictably with respect to a variety of physicochemical processes  
135 (Dansgaard 1964, Estep and Dabrowski 1980, Estep 1981, Rubenstein and Hobson  
136 2004). As water vapor rises on the windward side of a mountain range, it cools and  
137 condenses, and water containing the heavier isotope of hydrogen (deuterium) is the first  
138 to condense. This produces a systematic relationship between elevation and  $\delta^2\text{H}$  of  
139 local precipitation, resulting in lapse rates of 4 – 8‰ per 100 m (Poage 2001).  $\delta^2\text{H}$   
140 values of primary producers reflect local precipitation, and consumers integrate  $\delta^2\text{H}$   
141 values of food and water such that their tissues have  $\delta^2\text{H}$  that is higher than their food  
142 but lower than their water (Estep and Dabrowski 1980, Hobson et al. 1999, Birchall et  
143 al. 2005, Wolf et al. 2013). A few studies have utilized the elevational lapse rate in the  
144  $\delta^2\text{H}$  of precipitation to assess elevational movements (Hobson et al. 2003, Hardesty and  
145 Fraser 2010, Newsome et al. 2015). Most  $\delta^2\text{H}$ -based studies have analyzed feathers, a  
146 metabolically inert tissue that records ecological information only during molt, which

147 may only last a few weeks (Hobson et al. 2003, Pérez and Hobson 2007, Wunder 2012,  
148 Hobson et al. 2012). More recently, a multi-tissue approach comparing  $\delta^2\text{H}$  values of  
149 metabolically active tissues (i.e. blood, muscle, liver) with metabolically inert feathers  
150 offers the potential to reveal utilization of high versus low elevation resources during  
151 different periods of the annual life cycle. (Mazerolle and Hobson 2005, Hardesty and  
152 Fraser 2010, Newsome et al. 2015, Villegas et al. 2016).

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

165

## 166 **Methods.**

### 167 **Morphometric analyses**

168 To test for genetic differentiation across the elevational range at the loci underlying  
169 functional morphological traits, we compared sizes of four traits. For four focal species,



170 *C. cinereum*, *S. magellanicus*, *T. aedon*, and *Z. capensis*, we compared morphological  
171 measurements between populations at high (>3000 m) and low (<1000 m) elevations.  
172 We measured culmen, wing chord, tail, and tarsus from museum specimens listed in  
173 Appendix 5. We used PCA to visualize the morphometric data, and MANOVA or non-  
174 parametric Kruskal-Wallis tests to compare high and low groups.

175 To help interpret the results of this study, we assessed relative flight capabilities  
176 of our four study species. To do so, we compared relative flight muscle mass and hand-  
177 wing index, measures that are known to correlate with flight ability and dispersal  
178 propensity (Kipp 1959, Dawideit et al. 2009, Burney and Brumfield 2009, Claramunt et  
179 al. 2012, Wright et al. 2014, 2016).

180

### 181 **mtDNA population differentiation**

182 To test for mtDNA differentiation across the elevational range, we analyzed published  
183 (Cheviron and Brumfield 2009, Galen and Witt 2014) and original mtDNA sequence  
184 data from high-elevation (>3000 m) and low-elevation (<1000 m) specimens listed in  
185 Appendix 7. We tested for elevational population genetic structure by estimating  $F_{st}$  and  
186  $\Phi_{st}$  between elevational zones using Arlequin v3.5 (Excoffier and Lischer 2010).

187

### 188 **Stable isotope measurements**

189 To test for elevational movements, we used mass spectrometry to measure  $\delta^2H$  from  
190 liver, contour feathers, and secondary flight feathers from museum specimens of our  
191 four focal taxa that were collected over the last decade on the west slope of the Andes

192 in central Peru (Appendix 4). Technical details are described in Supplementary Material,  
193 Appendix 1.

194

### 195 **$\delta^2\text{H}$ hypothetical framework**

196 We sought to exploit the ubiquitous trend of decreasing precipitation  $\delta^2\text{H}$  with increasing  
197 elevation (Poage 2001, Gonfiantini et al. 2001) to test for short distance elevational  
198 migration (Hobson et al. 2003, Newsome et al. 2015, Villegas et al. 2016). While few  
199 precipitation  $\delta^2\text{H}$  datasets exist for the west slope of the Peruvian Andes (IAEA/WMO  
200 2015), elevational  $\delta^2\text{H}$  lapse rates for other Andean regions range from 4 to 8‰ per  
201 100m (Niewodniczanski et al. 1981, Rozanski and Araguás-Araguás 1995, Araguás-  
202 Araguás et al. 1998, Poage 2001). Our study system on the west slope of the Peruvian  
203 Andes is influenced, at low elevations, by  $^2\text{H}$ -enriched fog (Scholl et al. 2010) coupled  
204 with sporadic precipitation, and at higher elevations by  $^2\text{H}$ -depleted precipitation  
205 resulting from a combination of temperature-dependent fractionation and Rayleigh  
206 distillation (Dansgaard 1964, Poage 2001). These disparate isotopic inputs likely  
207 produce elevational lapse rates in precipitation  $\delta^2\text{H}$  (4–8‰ per 100m) that are  
208 comparable to those reported by Poage and Chamberlain (2001). Yet, seasonal  
209 changes in precipitation  $\delta^2\text{H}$  that are of equal or greater magnitude than elevational  
210 variation in  $\delta^2\text{H}$  may obscure expected elevational trends in  $\delta^2\text{H}$  of metabolically active  
211 bird tissue (Gonfiantini et al. 2001, IAEA/WMO 2015, Villegas et al. 2016).

212 To account for seasonal variation in precipitation  $\delta^2\text{H}$  values across the west  
213 slope of the Peruvian Andes, we used precipitation  $\delta^2\text{H}$  data collected from 2006–2008  
214 in Marcapomacocha, Peru (~4400m) from the Global Network of Isotopes in

215 Precipitation (GNIP) (IAEA/WMO, 2015). Marcapomacocha, at the top of the transect  
216 where most of our specimens were collected (Fig. S1), is the only site on the west slope  
217 of the Peruvian Andes for which multiple years of monthly measurements of  
218 precipitation  $\delta^2\text{H}$  values exist. We used monthly mean precipitation  $\delta^2\text{H}$  values ( $\delta^2\text{H}_{\text{month}}$ )  
219 obtained from the Marcapomacocha to account for effects of seasonal fluctuations in  
220 precipitation  $\delta^2\text{H}$  in our model of elevational effects on  $\delta^2\text{H}$  of metabolically active bird  
221 liver tissue. The dearth of available data on precipitation  $\delta^2\text{H}$  at other elevations requires  
222 us to assume that seasonal fluctuations in precipitation  $\delta^2\text{H}$  occur similarly across  
223 elevations.

224 Differences in migratory behavior among elevational-generalist songbird species  
225 will be reflected in how their metabolically inert versus active tissues differentially  
226 integrate elevational versus seasonal trends in local precipitation  $\delta^2\text{H}$ . Feather tissues  
227 are grown over the course of a few weeks, typically during the dry season (June to  
228 August; Fig. S2), after which they become metabolically inert. Thus,  $\delta^2\text{H}_{\text{feather}}$  should not  
229 be influenced by the date of sampling, which is captured in our models by  $\delta^2\text{H}_{\text{month}}$ .  
230  $\delta^2\text{H}_{\text{feather}}$  values are expected to vary with elevation of capture in sedentary birds, but not  
231 in migratory ones that often will have shifted in elevation between the date of molt and  
232 the date of sampling. Conversely, liver tissue is metabolically active so  $\delta^2\text{H}_{\text{liver}}$  should  
233 reflect both seasonal ( $\delta^2\text{H}_{\text{month}}$ ) and elevational variation in precipitation  $\delta^2\text{H}$ , regardless  
234 of whether the bird is sedentary or migratory. Thus, regardless of the date on which a  
235 bird was collected, liver  $\delta^2\text{H}$  values should be predicted in part by seasonal fluctuations  
236 in precipitation  $\delta^2\text{H}$ , which are reflected in our models as  $\delta^2\text{H}_{\text{month}}$ .

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

243

#### 244 **$\delta^2\text{H}$ statistical analyses**

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

256

#### 257 **mtDNA test of recent population expansion**

258 To test for recent population expansion, we used DnaSP v5 (Librado and Rozas 2009)  
259 to estimate Tajima's D (Tajima 1996), Fu's F (Fu 1997), and we used mismatch

260 distributions to evaluate the distribution of pairwise divergence between individuals in a  
261 population. Using the mismatch distributions, we calculated the raggedness index ( $r$ ),  
262 with raggedness expected to be elevated under a stable population relative to an  
263 expanding one (Harpending 1994). We inferred population expansion when both  
264 Tajima's  $D$  and Fu's  $F$  were significantly negative and there was no significant  
265 raggedness.

266 Detailed methods are reported in the Supplementary Material, Appendix 1.

267

## 268 **Results**

### 269 **Precipitation $\delta^2\text{H}$**

270 GNIP data (IAEA/WMO 2015) revealed striking seasonal variation for the  
271 Marcapomacocha GNIP site (Fig. S1). Mean  $\delta^2\text{H}_{\text{month}}$  was  $-117 \pm 33\text{‰}$  during the wet  
272 season (Oct–May) and  $-40 \pm 19\text{‰}$  during the dry season (June –Sep). The large  
273 ( $\sim 100\text{‰}$ ) difference between precipitation  $\delta^2\text{H}$  in the wet season and dry season (Fig.  
274 S1) represents a confounding factor that requires careful consideration when attempting  
275 to interpret  $\delta^2\text{H}$  values of metabolically active tissues collected along an elevational  
276 gradient.

277

### 278 **Tissue $\delta^2\text{H}$**

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

283 significantly negatively correlated with elevation of collection ( $t$  value: -2.67,  $P = 0.01$ ;  
284 Fig. 1). Similarly, top models for *Z. capensis* feather- $\delta^2\text{H}$  included only elevation of  
285 capture as a predictor variable, and both  $\delta^2\text{H}_{\text{contour}}$  ( $t$  value: -2.96,  $P < 0.01$ ) and  
286  $\delta^2\text{H}_{\text{secondary}}$  ( $t$  value: -3.99,  $P < 0.001$ ) were significantly negatively correlated with  
287 elevation of capture (Fig. 1; Table 1).

288  $\delta^2\text{H}_{\text{liver}}$  varied significantly among species ( $F$  value: 18.59,  $\text{DF} = 3$ ,  $P < 0.001$ ),  
289 with values for the two insectivorous species (*C. cinereum* and *T. aedon*) significantly  
290 higher than those of the two granivorous species (*S. magellanicus* and *Z. capensis*). For  
291 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  
292 a predictor variable (Table 1). *C. cinereum*  $\delta^2\text{H}_{\text{liver}}$  values were positively correlated with  
293  $\delta^2\text{H}_{\text{month}}$  ( $F$ : 4.6,  $\text{DF} = 37$ ,  $P = 0.04$ ) (Fig. 2). *S. magellanicus*  $\delta^2\text{H}_{\text{liver}}$  values were  
294 positively correlated with  $\delta^2\text{H}_{\text{month}}$  ( $F$ : 11.6,  $\text{DF} = 16$ ,  $P < 0.001$ ), latitude ( $F$ : 11.6,  $\text{DF} =$   
295 16,  $P = 0.01$ ), and negatively correlated with elevation ( $F$ : 11.6,  $\text{DF} = 16$ ,  $P = 0.01$ ) (Fig.  
296 1). *T. aedon*  $\delta^2\text{H}_{\text{liver}}$  values were positively correlated with  $\delta^2\text{H}_{\text{month}}$  ( $F$ : 6.7,  $\text{DF} = 26$ ,  $P <$   
297 0.001). *Z. capensis*  $\delta^2\text{H}_{\text{liver}}$  values were positively correlated with  $\delta^2\text{H}_{\text{month}}$  ( $F$ : 16.1,  $\text{DF} =$   
298 27,  $P < 0.01$ ) (Fig. 2), negatively correlated with elevation of capture ( $F$ : 16.1,  $\text{DF} = 27$ ,  
299  $P < 0.001$ ) (Fig. 1), and negatively correlated with latitude of capture ( $F$ : 16.1,  $\text{DF} = 27$ ,  
300  $P < 0.01$ ).

301 Comparisons of  $\delta^2\text{H}$  values among tissue types are reported in Supplementary  
302 Table S2. Interspecific comparisons of  $\delta^2\text{H}_{\text{feather}}$  values are reported in supplementary  
303 Table S3.

304

305 **Morphometric comparisons**

306 Mean morphometric measurements (culmen, tail, tarsus, and wing chord) are reported  
307 for low (< 1000 m) and high (> 3000 m) elevational bins for each species in Table S4.  
308 Wing length differed between elevations for *S. magellanicus* ( $\chi^2$ : 22.2, DF = 1,  $P$  =  
309 <0.001); tail length differed between elevations for *T. aedon* ( $F$ : 6.7, DF = 1,  $P$  = 0.02)  
310 (Table S4). We found no measurement differences between elevational groups for *C.*  
311 *cinereum* or *Z. capensis*. The principal component analysis illustrates the overall  
312 findings of morphological differentiation in *S. magellanicus* and *T. aedon*, but not in the  
313 other two species (Fig. 3).

314 Hand-wing-index differed in all pairwise comparisons between species except  
315 those of *Z. capensis* with *C. cinereum* and *T. aedon*, respectively (Fig. S4). Those data  
316 indicate highest vagility in *S. magellanicus*, followed by *C. cinereum*, *Z. capensis*, and *T.*  
317 *aedon*. Species variation in flight muscle mass (corrected for body size) was consistent  
318 with the latter finding (Fig. S4). Flight muscle mass differed between species in all  
319 comparisons except the one between *Z. capensis* and *C. cinereum*.

320

### 321 **mtDNA structure and demography**

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

329

## 330 **Discussion**

331 Elevational movement, elevational genetic differentiation, and evidence of recent  
332 population expansion occur to varying degrees in our four study taxa, suggesting that  
333 each may play a role in causing exceptions to Janzen's Rule. Tissue  $\delta^2\text{H}$  patterns  
334 associated with elevational movement were observed in two species (*C. cinereum* and  
335 *S. magellanicus*) that had no mtDNA population structure, only one of which (*S.*  
336 *magellanicus*) showed morphological differentiation. Two other species showed isotopic  
337 patterns associated with sedentary habits; one of those species exhibited mtDNA  
338 population structure (*Z. capensis*) while the other exhibited morphological differentiation  
339 (*T. aedon*). Three out of the four focal species showed signs of recent population  
340 expansion by all three indices tested. In the following sections, we examine tissue  $\delta^2\text{H}$   
341 patterns associated with elevational movement and we consider how differing  
342 environmental processes might obscure or maintain these patterns. We delineate  
343 subcategories of elevational generalists (sedentary and migratory) to illustrate potential  
344 evolutionary consequences of short distance migration along environmental gradients.

345

### 346 **Tissue $\delta^2\text{H}$ patterns**

347 Variation in *T. aedon* and *Z. capensis* feather  $\delta^2\text{H}$  with elevation of capture (Fig.  
348 1) conforms with expected elevational patterns in precipitation  $\delta^2\text{H}$ , which suggests  
349 these species are generally sedentary. Both species are terrestrial foragers with  
350 morphological characteristics indicating low to moderate vagility (Table S1). For both  
351 species, the best linear models to explain variation in  $\delta^2\text{H}_{\text{contour}}$  and  $\delta^2\text{H}_{\text{secondary}}$  included



352 only elevation as a predictor variable (Table 1). This indicates that individuals of these  
353 species had molted at or near the elevation of capture. It further suggests that the  
354 season of molt was consistent among individuals such that the elevational signal was  
355 not overwhelmed by seasonal fluctuations in precipitation  $\delta^2\text{H}$ . For the other two  
356 species, *S. magellanicus* and *C. cinereum*, elevation did not explain variation in feather  
357  $\delta^2\text{H}$ , suggesting that individuals of those species underwent elevational movements  
358 between the time of molt and the time of sampling. A plausible alternative explanation  
359 would be that the season of molt is more variable among individuals of the latter two  
360 species, but there is no evidence for that in our molt data (Fig. S2), so we favor the  
361 conclusion that *S. magellanicus* and *C. cinereum* are elevational migrants.

362 Unlike feather  $\delta^2\text{H}$  values,  $\delta^2\text{H}_{\text{liver}}$  integrate seasonal variation in precipitation  $\delta^2\text{H}$   
363 because this tissue is metabolically active and has a rapid isotopic incorporation rate,  
364 integrating ecological information over 1–2 weeks prior to capture for an endotherm the  
365 size of a songbird (Martínez del Rio et al. 2009, Wolf et al. 2009).  $\delta^2\text{H}_{\text{liver}}$  showed  
366 significant positive associations with  $\delta^2\text{H}_{\text{month}}$  for all species (Fig. 2; Table 1). Elevation  
367 of capture was included in the best models for  $\delta^2\text{H}_{\text{liver}}$  for all species except *C. cinereum*  
368 (Table 1), though elevation was significant only for *Z. capensis* (Fig. 1). It is possible  
369 that the lack of elevational trend in  $\delta^2\text{H}_{\text{liver}}$  for *C. cinereum* might have occurred due to  
370 elevational movements within the weeks before sampling, but the generally modest  
371 relationship between elevation and  $\delta^2\text{H}_{\text{liver}}$  may have other causes (see below).

372 Any seasonal variation in precipitation  $\delta^2\text{H}$  that was not captured by our temporal  
373 index ( $\delta^2\text{H}_{\text{month}}$ ) may have dampened the expected elevational trends in  $\delta^2\text{H}_{\text{liver}}$ .  $\delta^2\text{H}_{\text{month}}$   
374 provided an index of seasonal flux in precipitation  $\delta^2\text{H}$  that was derived from three years

375 (2006-2008) of data at a single high elevation site, Marcapomacocha (~4400 meters). It  
376 is possible that the Marcapomacocha data poorly represented seasonal fluctuations in  
377 precipitation  $\delta^2\text{H}$  at other elevations or in other years. Thus, future studies would greatly  
378 benefit from the collection of additional precipitation  $\delta^2\text{H}$  data along Andean elevation  
379 gradients. Additional problems with interpretation of our models could have occurred if  
380 sampling during the wet or dry season were concentrated at high or low elevation;  
381 however, we consider these potential sources of bias unlikely to have driven our results  
382 because our  $\delta^2\text{H}$  data came from specimens that were collected across the entire  
383 elevational gradient during both wet and dry periods for all species (Fig. 1, Fig. 2).

384

### 385 **Morphological and genetic differentiation**

386 The division between sedentary and migratory modes of elevational generalism  
387 reflected in tissue  $\delta^2\text{H}$  patterns is likely mirrored in flight capabilities. Depending on  
388 foraging strategies and local ecologies, sedentary birds are predicted to be less vagile  
389 than their migratory counterparts, traits that should be reflected in the flight apparatus  
390 (flight muscle size and hand wing index; Fig. S4) and foraging stratum (Table S1).  
391 Larger flight muscle mass, higher hand wing index, and less terrestrial foraging ecology  
392 in *C. cinereum* and *S. magellanicus* relative to *T. aedon* and *Z. capensis* generally  
393 support this dichotomy, although there was some overlap in hand-wing index in *Z.*  
394 *capensis*–*T. aedon*/*C. cinereum* comparisons (Fig. S4) and flight muscle size between  
395 *C. cinereum* and *Z. capensis* (Table S1; Fig. S4).

396 Differences in sedentary and elevational-migratory habits should be further  
397 reflected in their respective levels of within-species population-genetic structure. Given

398 enough time, we expect sedentary elevational generalists to have developed genetic  
399 structure between high- and low-elevation populations. Conversely, migratory behavior  
400 in elevational generalists should maintain or enhance gene flow, effectively washing out  
401 any incipient population structure. We expect that tests for population level  
402 differentiation within this two-mode framework will provide insight into the ecologies and  
403 evolutionary trajectories of bird species that are elevational generalists.

404 Our morphological tests showed significant differentiation between high and low  
405 elevation *T. aedon* in tail length (Fig. 3 and S5). A trend of larger appendages at higher  
406 elevations has been previously reported in another Andean bird, the Torrent Duck  
407 (Gutiérrez-Pinto et al. 2014). The morphometric disparity, in combination with our  $\delta^2\text{H}$   
408 data, is in agreement with our hypothesis regarding the link between sedentary habit  
409 and elevational genetic differentiation. In contrast, *C. cinereum* exhibited no  
410 differentiation between high- and low-elevation specimens in the four characters we  
411 measured. This lack of differentiation is consistent with our predictions for an elevational  
412 generalist that is also an elevational migrant. *S. magellanicus* showed significant  
413 morphological differentiation in wing chord length between high- and low-elevation  
414 specimens. This morphological population structure conflicts with an isotopic pattern  
415 indicating elevational movement. The larger wing-chord sizes at high elevation could be  
416 the result of selection on wing-size that was strong enough to overcome gene flow  
417 (Smith et al. 2004, Gutiérrez-Pinto et al. 2014, Benham and Witt 2016). An alternative  
418 possibility is that the traits we measured exhibit high levels of phenotypic plasticity in  
419 response to elevation-specific pressures, but we consider this possibility to be unlikely.  
420 Phenotypically plastic traits could produce similar elevational patterns in the absence of

421 genetic population structure by changes in gene expression alone (Przybylo et al. 2000,  
422 Cheviron et al. 2008), but there is evidence that morphometric traits remain highly  
423 heritable despite this possibility (Boag 1983, Keller et al. 2001).

424 Population structure can persist locally along contiguous elevational distributions,  
425 effectively selecting against unfit immigrants (Cheviron and Brumfield 2009, Cheviron et  
426 al. 2014). Yet, if average dispersal distances are large, these clines are unlikely to form.  
427 To test for genetic differentiation, we analyzed *ND2* or *ND3* mtDNA sequence data from  
428 all four species.  $F_{st}$  and  $\Phi_{st}$  values confirmed previously reported population structure  
429 between high and low elevation *Z. capensis*, corroborating a sedentary habit for this  
430 species (Fig. 4) (Cheviron and Brumfield 2009).  $F_{st}$  and  $\Phi_{st}$  values were not significant  
431 for *T. aedon*, which was somewhat surprising considering the sedentary lifestyle  
432 suggested by our isotopic and morphometric data. Analysis of  $\beta$ -hemoglobin gene  
433 variation in *T. aedon* across the same elevational transect studied here found  
434 substantial elevational population structure (Galen et al. 2015). mtDNA analyzed here  
435 was unstructured with respect to elevation, as were the vast majority of nuclear protein-  
436 coding genes analyzed by Galen et al. (2015). As in *T. aedon*, mtDNA sequence data  
437 from *C. cinereum* and the *S. magellanicus* showed no population structure between  
438 high- and low-elevation groups.

439 Signals of recent demographic expansions, as indicated by mismatch  
440 distributions, Tajima's  $D$ , and Fu's  $F$  test statistics (Table 2, Fig. 4) were present in three  
441 of the four focal species (*S. magellanicus*, *T. aedon*, and *Z. capensis*). These  
442 demographic expansions, if accompanied by expansions of the elevational range,  
443 potentially explain exceptions to Janzen's Rule (1967). Considering the physical

444 landscape of the western Andes, this expansion likely originated in high elevation  
445 environments that are diverse and productive relative to dry coastal zones that are  
446 depauperate and may have fewer competitors. Published phylogenies for tanagers  
447 (including *C. cinereum*) (Burns et al. 2014), siskins (including *S. magellanicus*)  
448 (Beckman and Witt 2015), South American *T. aedon* populations (Galen and Witt 2014,  
449 Galen et al. 2015) and *Z. capensis* populations (Lougheed et al. 2013) are all consistent  
450 with montane origins and subsequent, downslope range expansions in western Peru.  
451 As a caveat, it should be noted that false inference of population expansion from  
452 mtDNA haplotype frequency spectra can be caused by other demographic events, such  
453 as selective sweeps (Fay and Wu 2000, Wakeley and Aliacar 2001, Przeworski 2002).  
454 Moreover, the high prevalence of apparent range expansions among these elevational  
455 generalists contrasts with previous findings for Andean cloud-forest specialist species;  
456 haplotype frequency spectra consistent with population expansion were found in only a  
457 small fraction of subpopulations for *Thamnophilus caerulescens* (Brumfield 2005),  
458 *Metallura tyrianthina* (Benham et al. 2015, Benham and Witt 2016), and *Premnoplex*  
459 *brunnescens* (Valderrama et al. 2014). Two species of brush-finches (*Buarremon*) that  
460 are restricted to mid-elevations appear to have undergone recent expansions, but the  
461 evidence was considered to be equivocal (Cadena 2007).

462 Our mtDNA data provides insights into the timing of the inferred range  
463 expansions. Fossil-calibrated divergence rates such as the oft-used 2% per million  
464 years (Lovette 2004, Weir and Schluter 2008) are known to overestimate the ages of  
465 recent events (Arbogast et al. 2002, Ho et al. 2015). Therefore, we used a pedigree-  
466 based substitution rate ( $3.13 \times 10^{-7}$  mutations/site/year) derived from chicken mtDNA

467 genomes to estimate dates of population expansion (Alexander et al. 2015). Applying  
468 this rate to our mtDNA data, we estimated expansion to have occurred ~3.5 Kya (*T.*  
469 *aedon*) to ~34 Kya (*S. magellanicus*).

470

#### 471 **Possible anthropological influence on expansion**

472         Considering that our estimates of the timing of population expansion are as  
473 recent as ~3.5 Kya, it is possible that these expansions may have coincided with human  
474 activity in this region. The lower west slope of the central Peruvian Andes is one of the  
475 driest places on Earth, with very limited natural bird habitats away from the immediate  
476 vicinity of rivers and streams sourced from the high Andes. Isolated patches of 'lomas'  
477 vegetation that depend on water from persistent coastal fog comprise one exception  
478 (Rundel and Dillon 1998). With the exception of lomas patches, the expansion of bird  
479 habitats away from rivers would have occurred only recently, following the  
480 implementation of sophisticated irrigation systems by the Paracas people, which also  
481 occurred ~3.5 Kya (Hesse and Baade 2009). Regardless of whether it directly caused  
482 signals of expansion in our genetic data, the expansion of bird populations spurred by  
483 water diversion and irrigation on formerly arid land should be considered likely.

484 Currently, *T. aedon* and *Z. capensis*, though widespread in undeveloped areas, are  
485 facultative human commensalists (Ruiz et al. 2002, Newhouse et al. 2008). Whether or  
486 not agricultural development facilitated expansion to lower and dryer portions of the  
487 western Andean slopes, our findings of recent expansion, individual movements, and  
488 ongoing diversification indicate that evolutionary instability is inherent to broad  
489 elevational ranges, at least for tropical songbird species.

490

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496

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744 Appendix 1–8.
- 745  
746

747 **Table 1.** Comparison of models to explain  $\delta^2\text{H}_{\text{tissue}}$  values as a function of elevation  
 748 (elev), seasonal variation in precipitation  $\delta^2\text{H}$  ( $\delta^2\text{H}_{\text{month}}$ ), and latitude (lat) for each of the  
 749 four-focal species.  $^2\text{H}_{\text{month}}$  was excluded from comparisons for  $\delta^2\text{H}_{\text{feather}}$  values (dark  
 750 gray boxes). All combinations of predictor variables were tested against  $\delta^2\text{H}_{\text{liver}}$ . Models  
 751 that scored lower than nested versions of themselves were removed, following Arnold  
 752 (2010). For each species and tissue type, models with lowest  $\text{AIC}_c$ ,  $\Delta\text{AIC}_c$  of 0, and  
 753 highest weight are bolded.

species	model	$\delta^2\text{H}_{\text{contour}}$			$\delta^2\text{H}_{\text{secondary}}$			$\delta^2\text{H}_{\text{liver}}$		
		$\text{AIC}_c$	$\Delta\text{AIC}_c$	weight	$\text{AIC}_c$	$\Delta\text{AIC}_c$	weight	$\text{AIC}_c$	$\Delta\text{AIC}_c$	weight
<i>C. cinereum</i>	~ 1	<b>299.83</b>	<b>0.00</b>	<b>1.00</b>	<b>322.68</b>	<b>0.00</b>	<b>1.00</b>	328.89	2.24	0.25
	~ elev									
	~ elev + lat									
	~ $\delta^2\text{H}_{\text{month}}$							<b>326.66</b>	<b>0.00</b>	<b>0.75</b>
	~ elev + $\delta^2\text{H}_{\text{month}}$									
	~ $\delta^2\text{H}_{\text{month}}$ + lat									
	~ elev + $\delta^2\text{H}_{\text{month}}$ + lat									
<i>S. magellanicus</i>	~ 1	<b>188.59</b>	<b>0.00</b>	<b>1.00</b>	<b>176.93</b>	<b>0.00</b>	<b>1.00</b>	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							<b>167.74</b>	<b>0.00</b>	<b>0.81</b>
<i>T. aedon</i>	~ 1	249.30	4.76	0.08	257.72	0.90	0.39	275.11	9.15	0.00
	~ elev	<b>244.55</b>	<b>0.00</b>	<b>0.92</b>	<b>256.83</b>	<b>0.00</b>	<b>0.61</b>			
	~ 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							<b>265.95</b>	<b>0.00</b>	<b>0.35</b>
<i>Z. capensis</i>	~ 1	272.86	5.72	0.05	264.56	11.11	0.00	264.71	23.80	0.00
	~ elev	<b>267.14</b>	<b>0.00</b>	<b>0.95</b>	<b>253.45</b>	<b>0.00</b>	<b>1.00</b>	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							<b>240.91</b>	<b>0.00</b>	<b>0.96</b>

754

755 **Table 2.** Summary of mtDNA analyses by species for low (<1000 m) and high (>3000 m) elevation specimens.  
 756 Haplotypes (N H-Type), proportion of polymorphic sites (P-site), nucleotide diversity ( $\pi$ ), Tajima's D (D), Fu's F (F),  
 757 raggedness (r), Fst and  $\phi$ st are reported. Significant values accompanied by asterisks.  
 758

Species	N	N H-type	P-site (n/total)	$\pi$	D	F	r	Fst	$\phi$ st
<i>Conirostrum cinereum</i>	High=15 Low=15	12	0.012 (11/892)	0.0024	-0.322 ns	-3.19 ns	0.04 ns p=0.10	0.01 ns	-0.003 ns
<i>Spinus magellanicus</i>	High=20 Low=11	8	0.027 (13/475)	0.0020	-2.37 **	-4.37 **	0.17 ns p=0.40	-0.02 ns	-0.007 ns
<i>Troglodytes aedon</i>	High=22 Low=18	23	0.032 (29/918)	0.0023	-2.30 **	-21.91 ***	0.04 ns p=0.10	-0.0004 ns	0.015 ns
<i>Zonotrichia capensis</i>	High=31 Low=29	15	0.044 (17/384)	0.0025	-2.21 **	-12.87 **	0.09 ns p=0.15	0.17 ***	0.18 ***

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762 **Figure 1.**  $\delta^2\text{H}$  values contour feather (green), secondary flight feather (blue), and liver  
763 (red) plotted against elevation of capture for *C. cinereum*, *S. magellanicus*, *T. aedon*,  
764 and *Z. capensis*; sample sizes for each species are reported in Table 2; statistics for  
765 linear relationships are provided in Table 3. Best-fit lines are shown for linear  
766 regressions that are significant at  $p < 0.05$  and gray bands represent 95% confidence  
767 intervals.

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769 **Figure 2.**  $\delta^2\text{H}_{\text{liver}}$  plotted against 2006–2008 monthly mean precipitation  $\delta^2\text{H}$  ( $\delta^2\text{H}_{\text{month}}$ )  
770 values from Marcapomacocha, Peru (IAEA/WMO, 2015); sample sizes for each species  
771 are reported in Table 2. Best-fit lines are shown for linear regressions that are  
772 significant at  $p < 0.05$ . Gray bands represent 95% confidence intervals.

773

774 **Figure 3.** Principal component analyses of four morphological measurements: bill  
775 length, tarsus, wing chord, and tail length in millimeters for *C. cinereum* ( $n = 21$ ), *S.*  
776 *magellanicus* ( $n = 34$ ), *T. aedon* ( $n = 22$ ), and *Z. capensis* ( $n = 11$ ), grouped into (red)  
777 high elevation ( $>3000\text{m}$ ) and (blue) low elevation ( $<1000\text{m}$ ).

778

779 **Figure 4.** Mismatch distributions obtained from mtDNA loci (*ND2* or *ND3*) of high  
780 (>3000 m) and low (<1000 m) elevation specimens of *C. cinereum*, *S. magellanicus*, *T.*  
781 *aedon*, and *Z. capensis*. Haplotype networks colored by elevation group: high elevation  
782 (red), low elevation (blue) accompany each mismatch distribution. Double-asterisks  
783 indicate that both Tajima's D and Fu's F tests for population expansion, based on  
784 haplotype frequency spectra, were significant for that species.

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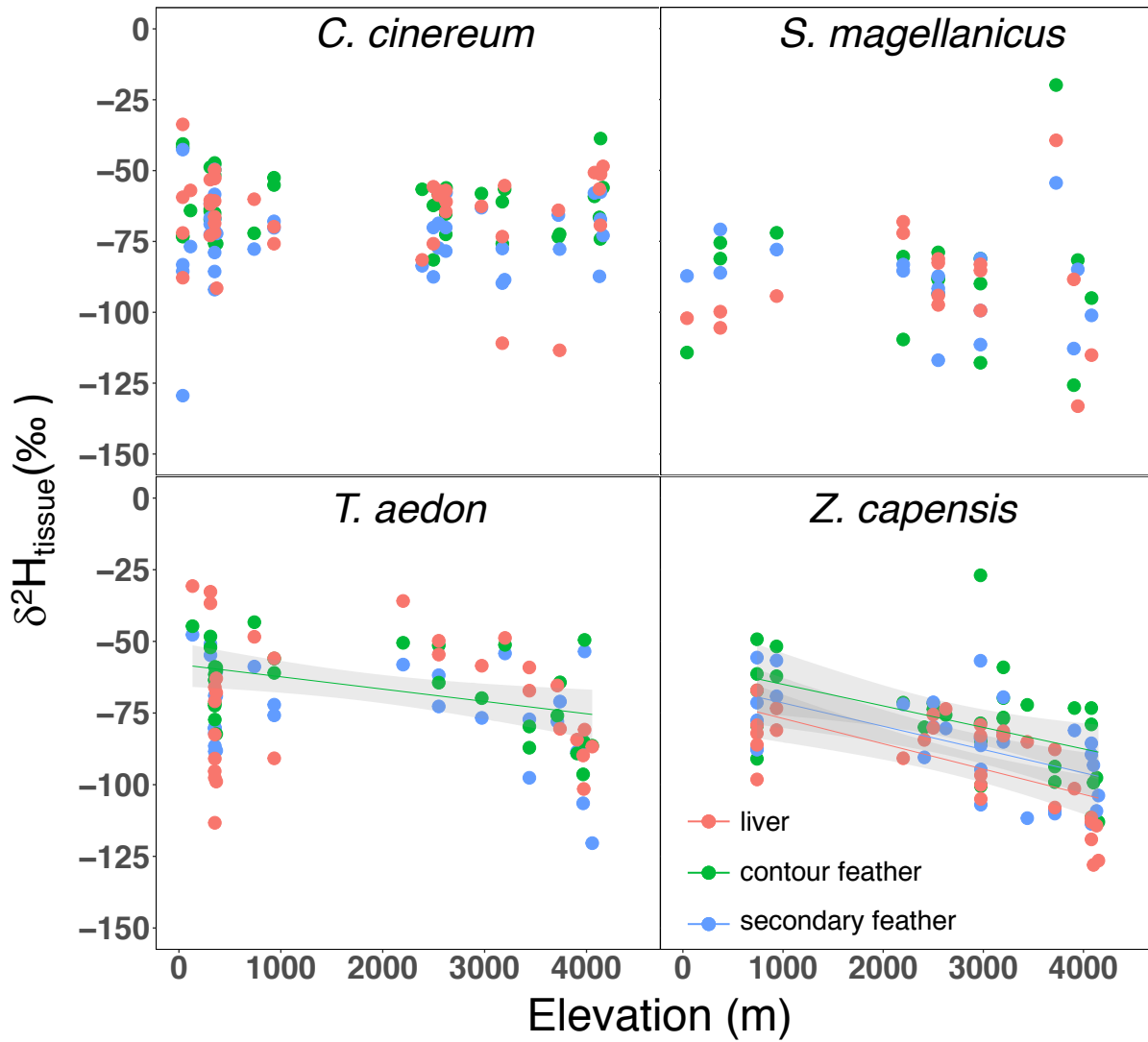
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802 **Figure 1.**



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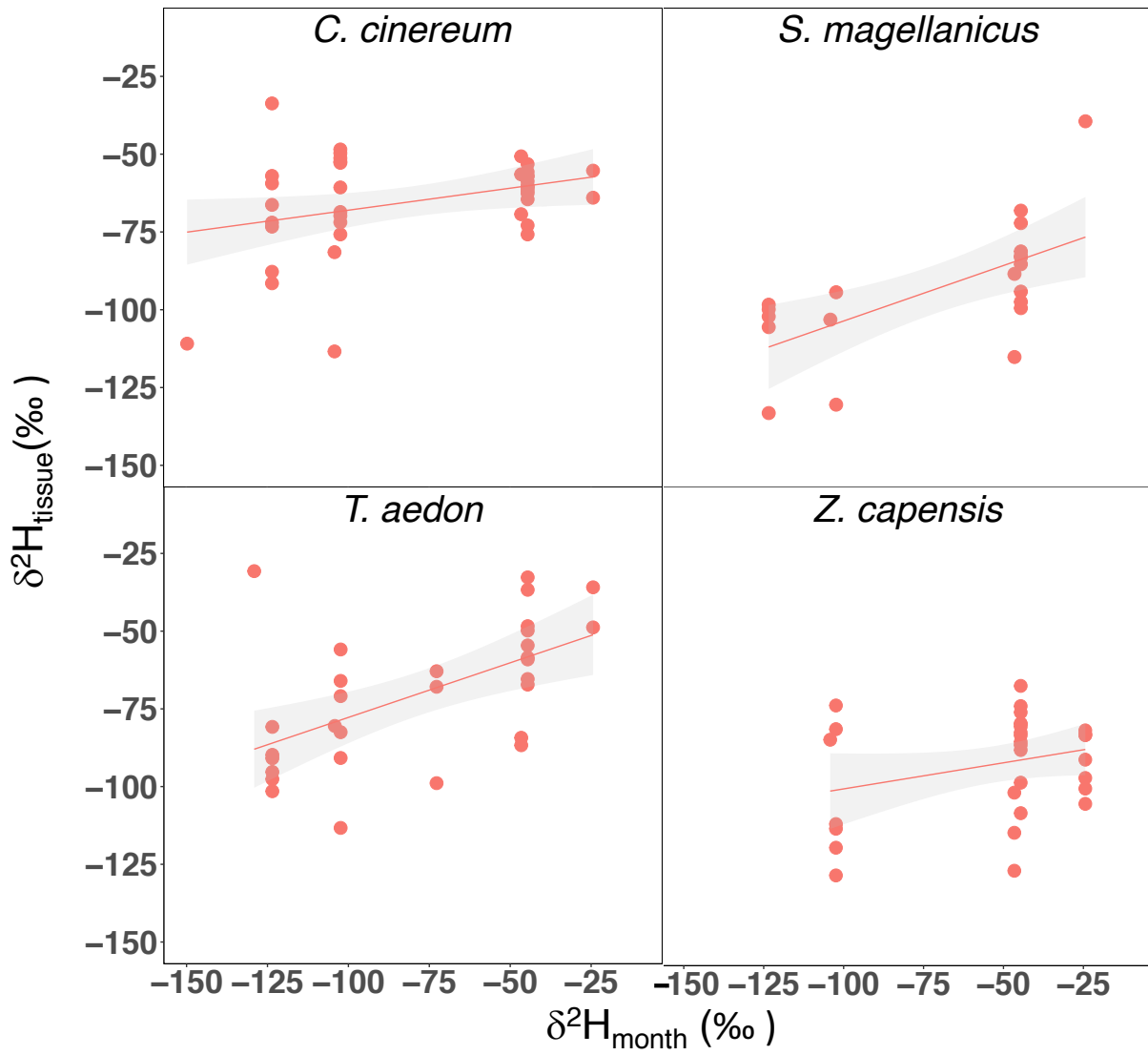
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811 **Figure 2.**



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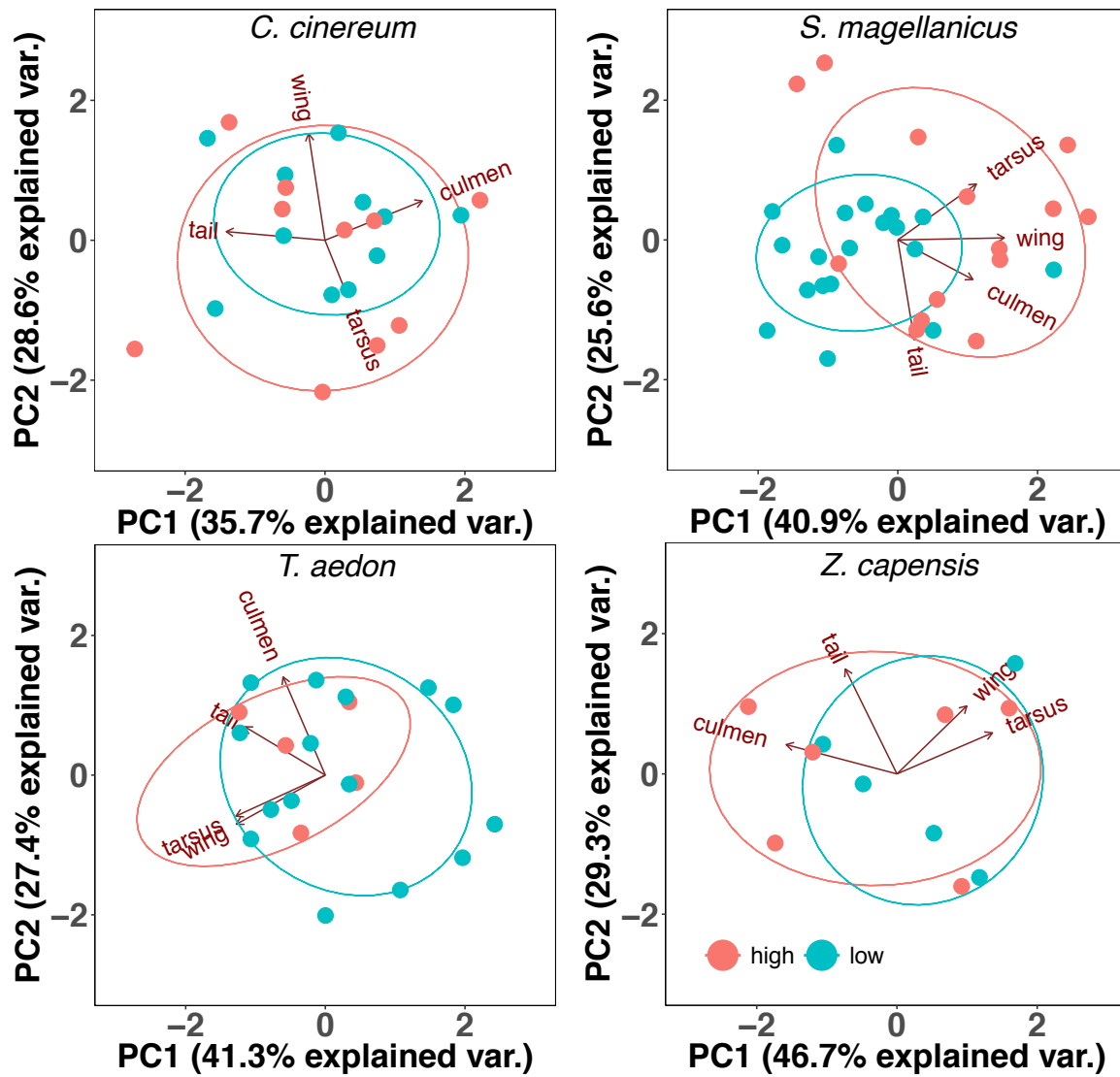
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819 **Figure 3.**



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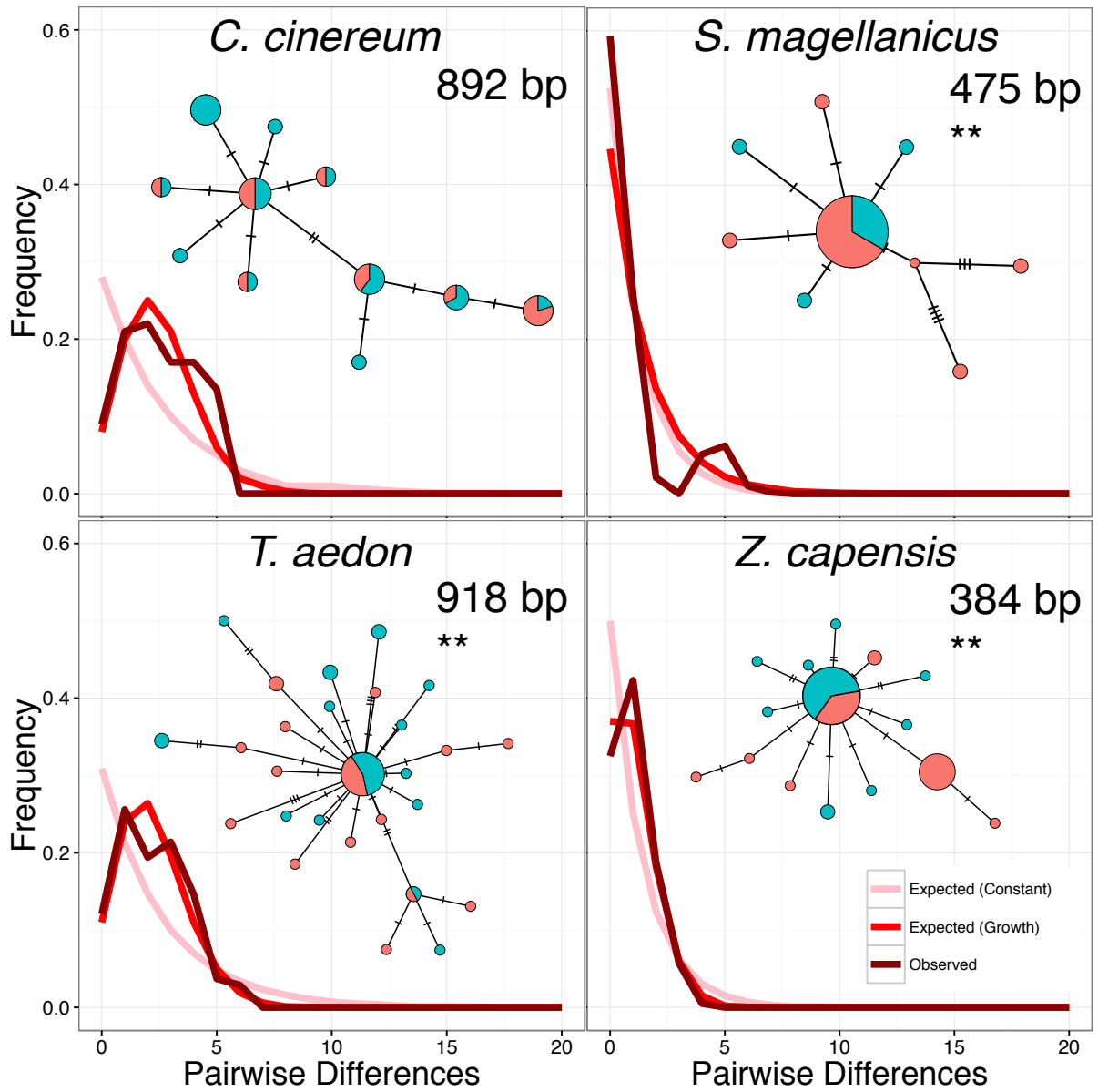
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827 **Figure 4.**



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