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

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 raise questions. Are they being sundered by diversifying selection along the gradient? Does elevational movement cause them to resist diversification or specialization? Have they recently expanded, suggesting that elevational generalism is short-lived in geological time? Here 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. 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*. To test for elevational movements, we measured hydrogen isotope (δ^2H) values of metabolically inert feathers and metabolically active liver. δ^2H data indicated elevational movements by two tree- and shrub-foraging species with moderate-to-high vagility (*C. cinereum* and *S. magellanicus*), and sedentary behavior 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 ~50 km gradient. All species except *C. cinereum* exhibited mtDNA-haplotype variation consistent with recent population expansion across the elevational gradient. In sum,

three out of four elevational generalist species underwent genetic divergence despite gene flow, two of four make seasonal elevational movements, and three of four have recently expanded. 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: elevational range limits, elevational migration, stable isotopes, divergence with gene flow, Andean birds.

Introduction

Elevational gradients cause profound eco-climatic variation across short distances. As a result, mountains are important hotspots of biodiversity (e.g. Sanders 2002, Grytnes and Vetaas 2002, McCain 2003) and drivers of diversification (e.g. Ribas et al. 2007, Freeman 2015, Galen et al. 2015, Benham and Witt 2016, Bertrand et al. 2016). As elevation increases, organisms must cope with reduced temperature, humidity, air density, and partial pressure of oxygen (PO_2), and 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. 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 (Terborgh 1971, Parker et al. 1996, Jankowski et al. 2013).

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

elevational ranges broader than 3000 m (Parker et al. 1996) and represent exceptions to Janzen's Rule because they encounter a broad range of climatic conditions. 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 et al. 2009, 2010); 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 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 (McCormack and Smith 2008, Cheviron and Brumfield 2009, Milá et al. 2009, McCormack and Berg 2010, Galen et al. 2015). 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, Simonson et al. 2010, Galen et al. 2015), homogenizing gene flow between high and low populations is expected to inhibit functional divergence and 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

have found limited evidence of elevational migration, and most elevational movements that have been documented are small in magnitude (Hobson et al. 2003, Boyle 2010, Hardesty and Fraser 2010, Boyle et al. 2010, 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 (δ^2H) values of bird tissues can be used to characterize latitudinal and elevational movements (Hobson 1999, Bowen et al. 2005). The δ^2H of precipitation varies predictably with respect to a variety of physicochemical processes (Dansgaard 1964, Estep and Dabrowski 1980, Estep 1981, Rubenstein and Hobson 2004). As water vapor 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 δ^2H of local precipitation, resulting in lapse rates of 4 – 8‰ per 100 m (Poage 2001). δ^2H values of primary producers reflect local precipitation, and consumers integrate δ^2H values of food and water such that their tissues have δ^2H that is higher than their food but lower than their water (Estep and Dabrowski 1980, Hobson et al. 1999, Birchall et al. 2005, Wolf et al. 2013). A few studies have utilized the elevational lapse rate in the δ^2H of precipitation to assess elevational movements (Hobson et al. 2003, Hardesty and Fraser 2010, Newsome et al. 2015). Most δ^2H-based studies have analyzed feathers, a metabolically inert tissue that records ecological information only during molt, which

may only last a few weeks (Hobson et al. 2003, Pérez and Hobson 2007, Wunder 2012, Hobson et al. 2012). More recently, a multi-tissue approach comparing δ^2H values of metabolically active tissues (i.e. blood, muscle, liver) with metabolically inert feathers offers the potential to reveal utilization of high versus low elevation resources during different periods of the annual life cycle. (Mazerolle and Hobson 2005, Hardesty and Fraser 2010, 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 δ^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.

Methods.

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,

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-Wallace 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 Fst and
186 Φ 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 δ^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

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

δ^2H hypothetical framework

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

To account for seasonal variation in precipitation δ^2H values across the west slope of the Peruvian Andes, we used precipitation δ^2H data collected from 2006–2008 in Marcapomacocha, Peru (~4400m) 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 (Fig. S1), is the only site on the west slope of the Peruvian Andes for which multiple years of monthly measurements of precipitation δ^2H values exist. We used monthly mean precipitation δ^2H values (δ^2H_{month}) obtained from the Marcapomacocha to account for effects of seasonal fluctuations in precipitation δ^2H in our model of elevational effects on δ^2H of metabolically active bird liver tissue. The dearth of available data on precipitation δ^2H at other elevations requires us to assume that seasonal fluctuations in precipitation δ^2H occur similarly across elevations.

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

237 Elevational movements of individual birds are predicted to influence the
238 relationship between tissue δ^2H and elevation. If elevational migration occurs, we expect
239 that any correlation between δ^2H 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 δ^2H values for inert feathers to lack a trend with elevation of capture.
243

244 **δ^2H statistical analyses**

245 We compared intra- and interspecific δ^2H_{tissue} values using ANOVA and Kruskal-Wallis
246 or Wilcoxon Rank-Sum tests where applicable. To test for elevational and seasonal
247 effects on δ^2H we evaluated sets of linear models for each species and tissue type
248 using AIC_c. For δ^2H_{liver}, we compared models containing all possible combinations of the
249 intercept and three continuous predictor variables: elevation, precipitation δ^2H_{month} for
250 the sampling date, and latitude. For $\delta^2H_{feather}$ we excluded models that included
251 precipitation δ^2H_{month} because metabolically inert tissues should be independent of
252 precipitation-δ^2H 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 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 δ^2H**

270 GNIP data (IAEA/WMO 2015) revealed striking seasonal variation for the
271 Marcapomacocha GNIP site (Fig. S1). Mean $\delta 2H_{month}$ was -117±33‰ during the wet
272 season (Oct–May) and -40±19‰ during the dry season (June –Sep). The large
273 (~100‰) difference between precipitation δ^2H in the wet season and dry season (Fig.
274 S1) represents a confounding factor that requires careful consideration when attempting
275 to interpret δ^2H values of metabolically active tissues collected along an elevational
276 gradient.
277

278 **Tissue δ^2H**

279 Comparisons of linear models to explain tissue δ^2H values are reported in Table 1.
280 Neither latitude nor elevation explained variation in feather-δ^2H 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 $\delta^2H_{contour}$ 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-δ^2H included only elevation of
285 capture as a predictor variable, and both $\delta^2H_{contour}$ (*t value*: -2.96, $P < 0.01$) and
286 $\delta^2H_{secondary}$ (*t value*: -3.99, $P < 0.001$) were significantly negatively correlated with
287 elevation of capture (Fig. 1; Table 1).

288 δ^2H_{liver} varied significantly among species (*F value*: 18.59, 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 δ^2H_{liver} as a response variable included δ^2H_{month} as
292 a predictor variable (Table 1). *C. cinereum* δ^2H_{liver} values were positively correlated with
293 δ^2H_{month} (*F*: 4.6, DF = 37, $P = 0.04$) (Fig. 2). *S. magellanicus* δ^2H_{liver} values were
294 positively correlated with δ^2H_{month} (*F*: 11.6, DF = 16, $P < 0.001$), latitude (*F:* 11.6, DF =
295 16, $P = 0.01$), and negatively correlated with elevation (*F:* 11.6, DF = 16, $P = 0.01$) (Fig.
296 1). *T. aedon* δ^2H_{liver} values were positively correlated with δ^2H_{month} (*F:* 6.7, DF = 26, $P <$
297 0.001). *Z. capensis* δ^2H_{liver} values were positively correlated with δ^2H_{month} (*F:* 16.1, DF =
298 27, $P < 0.01$) (Fig. 2), negatively correlated with elevation of capture (*F:* 16.1, DF = 27,
299 $P < 0.001$) (Fig. 1), and negatively correlated with latitude of capture (*F:* 16.1, DF = 27,
300 $P < 0.01$).

301 Comparisons of δ^2H values among tissue types are reported in Supplementary
302 Table S2. Interspecific comparisons of $\delta^2H_{feather}$ 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* (χ^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 Fst and Φst 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).

Discussion

Elevational movement, elevational genetic differentiation, and evidence of recent population expansion occur to varying degrees in our four study taxa, suggesting that each may play a role in causing exceptions to Janzen's Rule. Tissue δ^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 out of the four focal species showed signs of recent population expansion by all three indices tested. In the following sections, we examine tissue δ^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.

Tissue δ^2H patterns

Variation in *T. aedon* and *Z. capensis* feather δ^2H with elevation of capture (Fig. 1) conforms with expected elevational patterns in precipitation δ^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 $\delta^2H_{contour}$ and $\delta^2H_{secondary}$ 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 δ^2H. For the other two
356 species, *S. magellanicus* and *C. cinereum*, elevation did not explain variation in feather
357 δ^2H, 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 δ^2H values, δ^2H_{liver} integrate seasonal variation in precipitation δ^2H
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). δ^2H_{liver} showed
366 significant positive associations with δ^2H_{month} for all species (Fig. 2; Table 1). Elevation
367 of capture was included in the best models for δ^2H_{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 δ^2H_{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 δ^2H_{liver} may have other causes (see below).

372 Any seasonal variation in precipitation δ^2H that was not captured by our temporal
373 index (δ^2H_{month}) may have dampened the expected elevational trends in δ^2H_{liver}. δ^2H_{month}
374 provided an index of seasonal flux in precipitation δ^2H 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 δ^2H at other elevations or in other years. Thus, future studies would greatly
378 benefit from the collection of additional precipitation δ^2H 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 δ^2H 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 δ^2H 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 δ^2H
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. Fst and Φst 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). Fst and Φst 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 β-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 (3.13×10^{-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.

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Supplementary material (Appendix EXXXXX at <www.oikosoffice.lu.se/appendix>). Appendix 1–8.

Table 1. Comparison of models to explain δ^2H_{tissus} values as a function of elevation (elev), seasonal variation in precipitation δ^2H (δ^2H_{month}), and latitude (lat) for each of the four-focal species. $^2H_{month}$ was excluded from comparisons for $\delta^2H_{feather}$ values (dark gray boxes). All combinations of predictor variables were tested against δ^2H_{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_c, ΔAIC_c of 0, and highest weight are bolded.

species	model	$\delta^2H_{contour}$			$\delta^2H_{secondary}$			δ^2H_{liver}		
		AIC_c	ΔAIC_c	weight	AIC_c	ΔAIC_c	weight	AIC_c	ΔAIC_c	weight
C. cinereum	~ 1	**299.83**	**0.00**	**1.00**	**322.68**	**0.00**	**1.00**	328.89	2.24	0.25
	~ elev									
	~ elev + lat									
	~ δ^2H_{month}							**326.66**	**0.00**	**0.75**
	~ elev + δ^2H_{month}									
	~ δ^2H_{month} + lat									
	~ elev + δ^2H_{month} + lat									
S. magellanicus	~ 1	**188.59**	**0.00**	**1.00**	**176.93**	**0.00**	**1.00**	182.08	14.34	0.00
	~ elev									
	~ elev + lat									
	~ δ^2H_{month}							174.54	6.80	0.03
	~ elev + δ^2H_{month}							172.62	4.87	0.07
	~ δ^2H_{month} + lat							172.05	4.30	0.09
	~ elev + δ^2H_{month} + lat							**167.74**	**0.00**	**0.81**
T. aedon	~ 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									
	~ δ^2H_{month}							266.23	0.27	0.31
	~ elev + δ^2H_{month}							266.03	0.07	0.34
	~ δ^2H_{month} + lat									
	~ elev + δ^2H_{month} + lat							**265.95**	**0.00**	**0.35**
Z. capensis	~ 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
	~ δ^2H_{month}							264.35	23.44	0.00
	~ elev + δ^2H_{month}							249.16	8.25	0.02
	~ δ^2H_{month} + lat									
	~ elev + δ^2H_{month} + lat							**240.91**	**0.00**	**0.96**

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 (π), Tajima's D (D), Fu's F (F),
757 raggedness (r), Fst and ϕst are reported. Significant values accompanied by asterisks.
758

Species	N	N H-type	P-site (n/total)	π	D	F	r	Fst	ϕst
Conirostrum cinereum	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
Spinus magellanicus	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
Troglodytes aedon	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
Zonotrichia capensis	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 ***

759
760

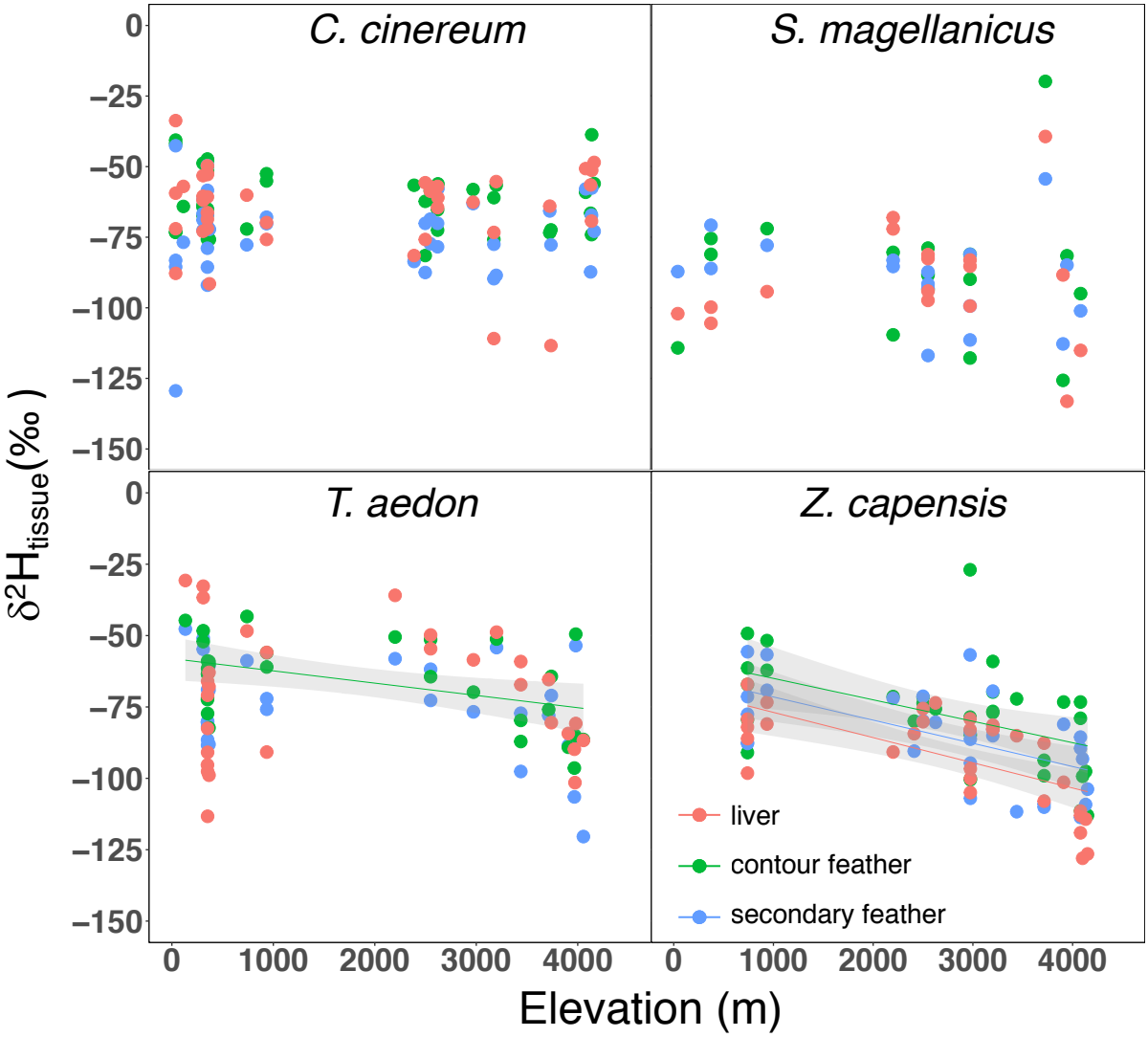
Figure 1. δ^2H values contour feather (green), secondary flight feather (blue), and liver (red) plotted against elevation of capture for *C. cinereum*, *S. magellanicus*, *T. aedon*, and *Z. capensis*; sample sizes for each species are reported in Table 2; statistics for linear relationships are provided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

Figure 2. δ^2H_{liver} plotted against 2006–2008 monthly mean precipitation δ^2H (δ^2H_{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 $p < 0.05$. Gray bands represent 95% confidence intervals.

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

Figure 4. Mismatch distributions obtained from mtDNA loci (*ND2* or ND3) of high (>3000 m) and low (<1000 m) elevation specimens of *C. cinereum, S. magellanicus, T. aedon*, and *Z. capensis*. Haplotype networks colored 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.

Figure 1.


C. cinereum
S. magellanicus
T. aedon
Z. capensis
0
−25
−50
−75
−100
−125
−150
δ2Htissue(‰)
0
1000
2000
3000
4000
Elevation (m)
liver
contour feather
secondary feather

811 **Figure 2.**

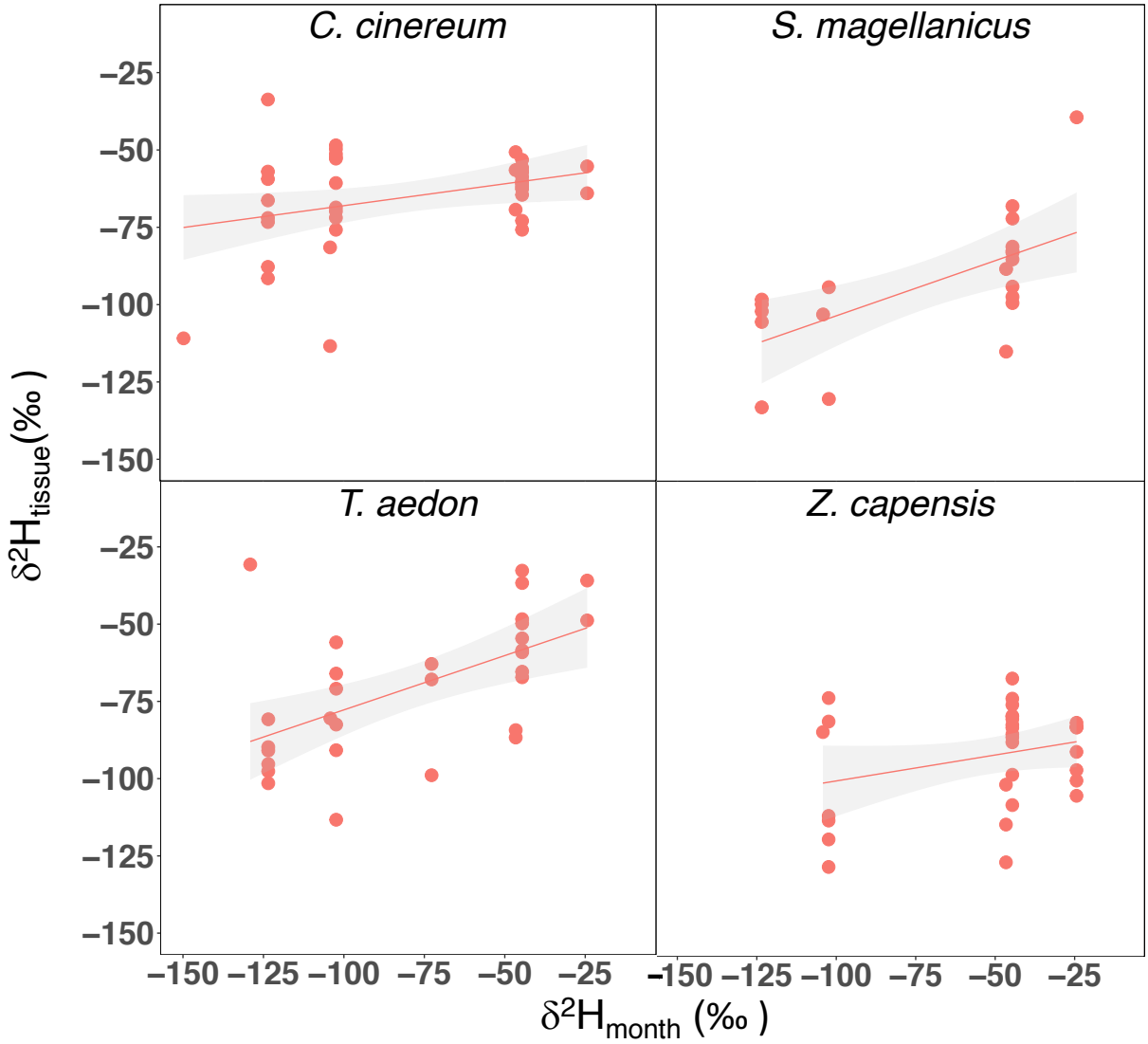


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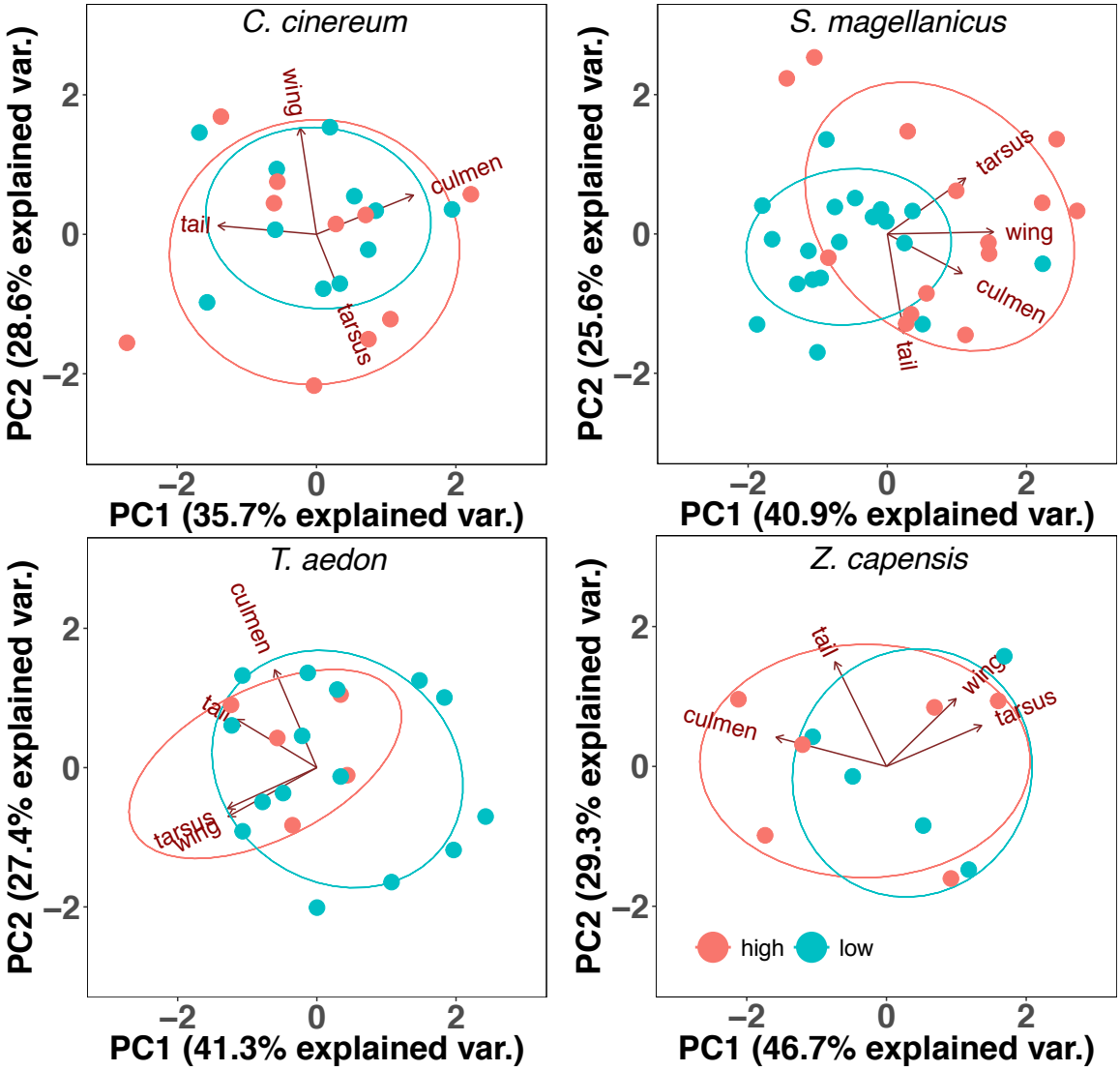
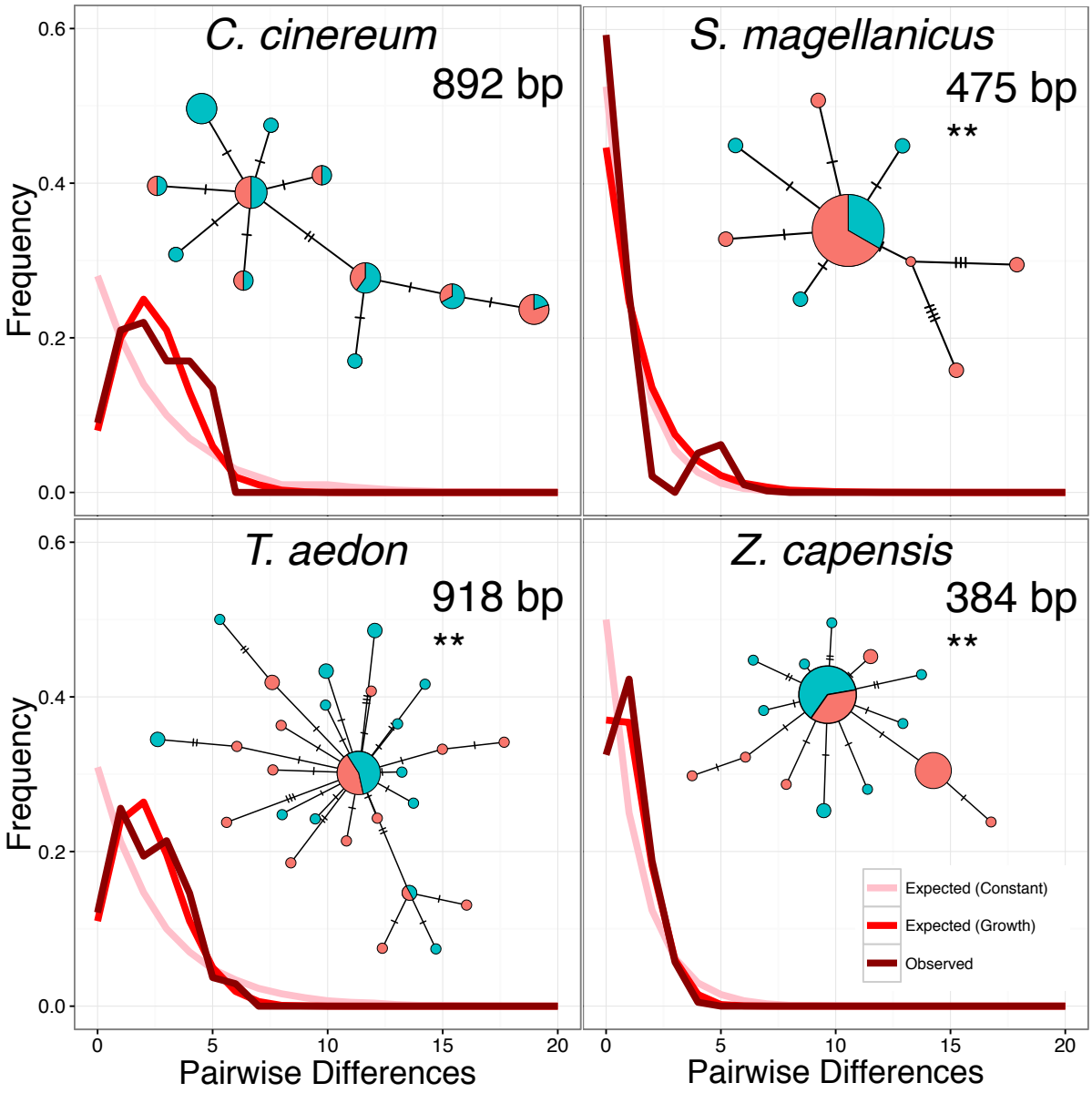
Figure 3.


C. cinereum
wing
culmen
tail
tarsus
PC2 (28.6% explained var.)
PC1 (35.7% explained var.)
S. magellanicus
tarsus
wing
culmen
tail
PC2 (25.6% explained var.)
PC1 (40.9% explained var.)
T. aedon
culmen
tail
tarsus
wing
PC2 (27.4% explained var.)
PC1 (41.3% explained var.)
Z. capensis
tail
wing
tarsus
culmen
high
low
PC2 (29.3% explained var.)
PC1 (46.7% explained var.)

827 **Figure 4.**



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