BEHAVIORAL ECOLOGY – ORIGINAL RESEARCH

Intraspecific variation in exploratory behavior and elevational affinity in a widely distributed songbird

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Abstract
Populations of the same species can vary substantially in their behavioral and morphometric traits when they are subject to different environmental pressures, which may lead to the development of different adaptive strategies. We quantified variation in exploratory behavior and morphometric traits among two rufous-collared sparrow populations that occur at low and high elevations in central Chile. Moreover, we used census and δ²H values of feather and blood to evaluate migration. We found that individual sparrows inhabiting high elevations were larger and showed more intense exploratory behavior in comparison with those that were captured at lower elevation. Moreover, we observed a steady decline in sparrow abundance during the winter and similar δ²H values for blood collected in the winter and summer at this site, which were significantly lower than blood δ²H values observed at low elevation. This pattern suggests that individuals do not move long distances during winter, and likely they remain at similar elevations in refuge habitats. As predicted, our results support the existent of different adaptive strategies among populations of the same species, and suggest that the combination of behavioral, morphometric, and stable isotope data is a novel and robust integrative approach to assess differences in adaptation across environmental gradients.

Keywords Exploratory behavior · Migration · Zonotrichia capensis · δ²H

Introduction
When species have large distributional ranges, it is likely that local populations are subject to different selective pressures, which can lead to the evolution of different adaptive strategies based on local environmental conditions. For birds, such ecophenotypic variation may include inter-population differences in morphology (e.g., wing length; Chapman 1940; Handford 1985) and physiology (testosterone levels; Hudson and Kimzey 1966; Broggi et al. 2004, Cavieres and Sabat 2008, Addis et al. 2011). From a behavioral perspective, populations may also develop different strategies to address common challenges that manifest as behavioral differences in food hoarding (Quispe et al. 2009), tool use (Whiten and van Schaik 2007), social behavior (Chapman and Rothman 2009), and mating tactics (Kolluru et al. 2007).

One behavior that is likely to vary among populations is exploratory behavior, because it allows individuals to obtain information about food items, feeding sites, territory quality, refuges, escape routes, and potential mates, among others (see Dingemanse et al. 2002; van Dongen et al. 2010; van Oers and Naguib 2013). It is unlikely that

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Our research proposes a robust and integrative approach to assess ecological and evolutionary variation across environmental gradients, applicable to bird species difficult to monitor and recapture.
these environmental characteristics are consistent across the range of local habitats occupied by a species with a wide geographic distribution (e.g., Beauchamp 1999; Van Oers et al. 2004; Garamszegi et al. 2008; Minderman et al. 2009). Some studies have shown that exploration is repeatable, heritable and has consequences for fitness (Dingemanse et al. 2002; Drent et al. 2003; van Dongen et al. 2010). There are very few studies, however, that report variability in exploratory behavior among populations of the same species (e.g., van Dongen et al. 2010; Maldonado et al. 2012).

Another behavior that exhibits intraspecific variation is migration. This behavior has been associated with changes in habitat conditions, such as food availability or suitable breeding sites (Chernetsov 2012). Variation in migratory behavior results in the existence of partial migratory populations composed of residents that remain in specific areas throughout the year, groups engaged in small-scale (e.g., nomadic) movements, and/or even migratory individuals that make seasonal latitudinal or elevational movements (Newton and Dale 1996). Comparative analyses of birds have shown that migratory species are more exploratory than non-migratory species (Metteke-Hofmann et al. 2002).

Moreover, several studies have shown that some morphometric measurements, such as wing size, increase with the distance covered during migration (Pennycook 1975; Leisler and Winkler 2003; Fiedler 2005).

The rufous-collared sparrow (Zonotrichia capensis) is an interesting species to examine inter-population variation in behavioral and morphological traits, because it is widely distributed in a broad range of habitats from southern Mexico (10°N) to the southern tip of South America (55°S), and from sea level to > 4000 m of elevation (Chapman 1940; Lougheed et al. 2013). Although there are more than 20 described subspecies based on morphological variation (Chapman 1940; Handford 1983, 1985; Lijtmaer and Tubaro 2007), recent research has identified only three distinct lineages (Lougheed et al. 2013). Genetic analysis has also found evidence of population structure among groups that inhabit different elevations (Lougheed and Handford 1992; Cheviron and Brumfield. 2009). Population-level differences in migratory behavior have also been reported. For example, individuals who inhabit higher elevations behave as migrants, while those inhabiting lower elevations are residents (Ortiz and Capllonch 2011). This pattern is expected given the adverse winter conditions of high elevation habitats, especially in the southern portion of its range in southern South America. Therefore, it is likely that individuals that inhabit high elevations show enhanced exploratory behavior, because this would allow them to obtain more information about both abiotic and biotic conditions that impact resource availability and habitat quality in these challenging and dynamic environments.

Studying migratory behavior of small birds is difficult with the traditional methods such as observation and banding, because recapture rates are very low (Hobson 2005). Hydrogen isotope (δ2H) analysis of bird tissues has rapidly become an effective tool for characterizing bird migration (Hobson 2011), including elevational movement (Hobson et al. 2003; Hardesty and Fraser 2010; Newsome et al. 2015).

Most studies compare tissue (typically feather) δ2H values to those of isoscapes depicting geographic variation in precipitation δ2H (Bowen et al. 2005). However, feathers are molted and regrown during short periods (e.g., weeks) and thus cannot capture seasonal movements. Comparison of feather δ2H data with that from metabolically active tissues (e.g., blood or liver) or continuously growing metabolically inert tissues (e.g., claws) provides a way to examine movement across seasons (Martinez Del Rio et al. 2009; Dalerum and Angerbjörn 2005; Mazeroille and Hobson 2005; Newsome et al. 2015; Villegas et al. 2016). Furthermore, a multi-tissue approach also allows one to assess partial migration in populations that contain both resident and migratory individuals.

In this study, we quantified intraspecific variation in behavior, morphological traits, and habitat between two populations of Zonotrichia capensis that occur at different elevations in central Chile. Specifically, our novel approach coupled field experimental observations, morphological measurements, and a δ2H-based assessment of elevational affinity to link exploratory behavior with habitat preferences at the population level. Such detailed analyses of animal behavior may yield important insights into how animals adapt to different environmental challenges, and how such pressures produce and maintain variation between populations of the same species.

Materials and methods

Study sites

Rufous-collared sparrows were captured using mist nets between April and September in 2013 from two different elevation sites in central Chile: Rinconada de Maipu (33°31′S, 70°50′W, ~ 500 m) and Farellones (33°21′S, 70°17′W, 2300–2700 m) with the purpose of contrasting exploratory behavior, morphometric traits, and isotope values between populations (see below). In addition, we captured rufous-collared sparrows at two intermediate elevation sites: Yerba Loca (33°20′S, 70°18′W, ~ 1500 m) and El Arrayan (33°15′S, 70°25′W, ~ 1000 m), in an attempt to recapture individuals previously banded in Farellones and detect assess seasonal elevational movements via tissue hydrogen isotope analysis. All sites are characterized by a Mediterranean climate and dominated by Andean xeric scrubland vegetation (Thrower and Bradbury 1977), with a
gradient in mean annual precipitation from 300 to 400 mm at the low elevation sites increasing to 500–900 mm at the high elevation sites (di Castri and Hajek 1976; Dirección meteorológica de Chile 2016).

Field study

We quantified exploratory behavior via novel environment experiments in a large field-portable cage [270 cm (length) × 150 cm (width) × 150 cm (height)] made of PVC poles and semitransparent black shading cloth (Fig. 1a). Four wooden perches (80 cm long, 2 cm in diameter) were hung throughout the cage at varying heights between 50 and 110 cm off the ground. One perch was placed diagonally at the opposite corner at the far end of the cage, and the remaining three were spaced at regular intervals (70 cm apart) along the long axis of the cage. The floor was divided into nine similar quadrants that when added to the four perches, four walls, the top and the central pole division yielded 19 possible perching locations (see van Dongen et al. 2010). Thirty-eight non-reproductive adult birds were captured and introduced into the small acclimatization cage (30 cm length, 25 cm width, and 39 cm height) in a corner of the experimental cage and covered with a cloth during a 5-min acclimatization period. At the beginning of the experimental period, the cloth was removed, then, the door of the holding cage was opened, and finally, an observer dictated the number and destination of all movements made by each subject onto a digital voice recorder over a period of 10 min.

We then measured to the nearest 0.1 cm the bill and tarsus length with digital calipers, the wings, and tail length with both wing and tail rules, and mass in grams (± 0.1 g) with a 60 g Pesola (± 0.1 g). A blood sample was obtained from the brachial vein, and several drops were dried on two glass microscope slides. The remaining blood was stored on FTA cards for later genetic sexing. Finally, a primary feather (p9) was collected from the left wing and the bird was released.

Counts

We conducted weekly census counts from January 2013 to December 2013. We designated a ~ 10-km monitoring area that spanned from 2000- to 3000-m elevation. This area included five ~ 1 km² plots, which contained 5 points separated by ~ 250 m, where all sparrows that occurred within 50 m around each point were counted for 5 min from 6:30 AM to 12:00 AM (Ralph et al. 1996).

Genetic sexing

Sex could not be determined from morphological measurements during the pre-breeding stage. We extracted DNA from the collected blood that was stored on FTA cards using a QIAGEN DNA extraction kit (Valencia, CA, USA). The gene fragments CHD-W (unique to females) and CHD-Z (found in both males and females) were amplified using polymerase chain reactions (PCR) with primers P2 and P8. The PCR product was run on a 3% agarose gel to separate from the gene fragments. Sex was determined by visualization of the gel, with males exhibiting bands only of CHD-Z and females having bands of both CHD-W and CHD-Z. This protocol was adapted from Griffiths et al. (1998).

Exploratory behavior

Audio recordings were analyzed using JWatcher 1.0 to obtain the duration and frequency of behaviors and location of the sparrows in the experimental cage. With this information, we calculated richness and diversity metrics of exploratory behavior using a richness index and Shannon’s diversity index. The richness index is equal to $S = n/N$, where $n$
represents the total number of locations occupied during the experiment and N represents the total number of locations in the experimental cage. Diversity in exploratory behavior was quantified via Shannon’s diversity index using the equation $H = - \sum_i p_i \ln p_i$, where $p$ represents the total number of times a perch $i$ was visited, expressed as a proportion of the total number of perch visits throughout the trial (see van Dongen et al. 2010 for further details).

### Stable isotope analysis

Primary feathers were treated with a 2:1 chloroform:methanol solvent mixture to remove surface contaminants and bars were cut into small (< 1 mm) pieces with scissors and air-dried. Dried whole blood samples were scraped off from the glass microscope slides into microcentrifuge tubes and homogenized by mixing. δ²H values were measured with a Thermo Scientific high-temperature conversion elemental analyzer (TCEA) coupled to a Thermo Scientific Delta V isotope ratio mass spectrometer at the Center for Stable Isotopes at the University of New Mexico (Albuquerque, N.M., USA). Isotopic results are expressed as δ values, $\delta^2H = 1000 \times \left[ \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \right]$, where $R_{\text{sample}}$ and $R_{\text{standard}}$ are $^2H/^1H$ of the sample and standard, respectively. Vienna Standard Mean Ocean Water (V-SMOW) is the internationally accepted standard for δ²H analysis and the units are parts per thousand or per mil (‰) (IAEA/WMO 2011). Precision for ($\delta^2H$) was determined by analysis of the three internal reference materials for each tissue (blood or feather); within-run variation (SD) in δ²H values of these reference materials was 3–4‰.

### Data analysis

To compare the elevation of capture with δ²H based estimates of elevation, we applied an elevational lapse rate of 8–10‰ per 500 m (see Poage and Chamberlain 2001; Hardesty and Fraser 2010) to precipitation δ²H data collected from Rinconada de Maipu, where sparrows are year-round residents. All statistical analyses were performed in STATISTICA 7.0 (StatSoft, 2004). All data were tested for normality and homoscedasticity via the Shapiro–Wilk test and Levene’s test, respectively. We used ANOVA and Kolmogorov–Smirnov tests to examine the effects of location and sex on exploratory behavior and body measurements. We used a repeated measures ANOVA to test for differences between feather and blood δ²H values among populations.

### Results

#### Exploratory behavior and morphology

We found significant differences in richness and diversity of exploratory behavior among populations, as both indices were higher for birds from Farellones than those from Rinconada de Maipu (richness index: $F = 9.58, P = 0.003$; diversity index: $F = 7.45, P = 0.009$; see Fig. 1b and c). Furthermore, we found that sparrows from the high elevation site (Farellones) had significantly greater mass, tarsus, wing, tail, and total length (Table 1; mass: $F = 20.72, P = 0.001$; tarsus length: $F = 19.23, P = 0.001$; tail length: $F = 7.26, P = 0.011$; wing length: $F = 25.40, P = 0.001$; and total length: $F = 7.90, P = 0.008$). We did not find significant differences in behavior and morphometric traits between males and females in either population (diversity index: $F = 1.60, P = 0.21$; richness index: $F = 0.78, P = 0.38$; mass: $F = 0.49, P = 0.48$; bill length: $F = 0.20, P = 0.65$; tarsus length: $F = 1.33, P = 0.25$; tail length: $F = 7.24, P = 0.65$; wing length: $F = 0.28, P = 0.10$; and total length: $F = 0.70, P = 0.40$).

### Counts

Rufous-collared sparrows were observed during the entire year at all three low elevation sites, while at Farellones we observed a decline in abundance from May to July (Fig. 2). We observed a decline in abundance of sparrows at Farellones, which remained high during the summer months (Fig. 2). Additionally, we found that sparrows from the Farellones site had significantly greater mass, tarsus, wing, tail, and total length (Table 1; mass: $F = 20.72, P = 0.001$; tarsus length: $F = 19.23, P = 0.001$; tail length: $F = 7.26, P = 0.011$; wing length: $F = 25.40, P = 0.001$; and total length: $F = 7.90, P = 0.008$). We did not find significant differences in behavior and morphometric traits between males and females in either population (diversity index: $F = 1.60, P = 0.21$; richness index: $F = 0.78, P = 0.38$; mass: $F = 0.49, P = 0.48$; bill length: $F = 0.20, P = 0.65$; tarsus length: $F = 1.33, P = 0.25$; tail length: $F = 7.24, P = 0.65$; wing length: $F = 0.28, P = 0.10$; and total length: $F = 0.70, P = 0.40$).

#### Feather and blood δ²H

Feather δ²H values across all sites were negatively and significantly associated with elevation ($\beta = -0.48, R^2 = 0.55$, $P < 0.001$).

### Table 1: Morphological traits (mean ± SE) in Z. capensis, from Farellones ($n = 15$) and Rinconada de Maipu ($n = 19$)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Rinconada</th>
<th>Farellones</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>20.6 ± 0.2</td>
<td>22.0 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tarsus length (mm)</td>
<td>20.7 ± 0.1</td>
<td>21.7 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Wing length (mm)</td>
<td>73.3 ± 0.6</td>
<td>78.0 ± 0.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tail length (mm)</td>
<td>63.2 ± 0.6</td>
<td>66.1 ± 0.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>143.0 ± 1.3</td>
<td>144.7 ± 3.3</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Significance is denoted by $P$ values
The Farellones population had mean (± SD) feather δ²H values of −86 ± 3‰ that were not significantly different from the Yerba Loca population (−79 ± 2‰) (F = 4.08, P = 0.08). However, δ²H values of feathers collected from sparrows at Farellones were significantly lower than feathers collected at the lower elevation sites of El Arrayan (−56 ± 4‰) (F = 40.7, P < 0.001) and Rinconada de Maipu (−58 ± 4‰) (F = 15.5, P < 0.001). Feathers collected from Yerba Loca also had significantly lower δ²H values than those from El Arrayan (F = 31.09, P < 0.001) and Rinconada de Maipu (F = 9.12, P = 0.01). Finally, we did not find significant differences in feather δ²H values between El Arrayan and Rinconada de Maipu populations (F = 0.08, P = 0.77). Based on these results, we segregated these sites into two groups: low elevation (Rinconada de Maipu and El Arrayan) and high elevation (Yerba Loca and Farellones).

The high elevation population had mean (−83 ± 2‰) feather δ²H value that were significantly lower than that of the low elevation site (−57 ± 3‰) (Table 2; F = 35.4, P = 0.001). Likewise, the mean δ²H value of blood representing winter collected from Farellones (−111 ± 2.1‰) was significantly lower than that collected from the low elevation site (−95 ± 3‰) (Fig. 3; F = 9.01, P = 0.01). In addition, the offset in δ²H values between metabolically inert feathers representing summer and metabolically active blood representing winter (Δ²Hfeather–blood) was similar at high and low elevation populations (F = 0.74, P = 0.4). There were no significant differences in the Δδ²H values of blood or feathers collected in the summer or winter at the high elevation site Farellones (Fig. 3 δ²Hfeather: F = 4.57, P = 0.07; δ²Hblood: F = 0.61, P = 0.45). Finally, we did not find significant differences between capture elevation and estimated elevation among populations based on elevational lapse rates in precipitation δ²H values; see “Materials and Methods” (Table 3).

### Discussion

Our understanding of behavioral variation has been subject to remarkable shifts in recent years. For decades, it was assumed that individual behavior was very plastic or even noise, that could rapidly change in accordance with the behavior of other conspecifics, and/or to
changing environmental conditions (see Sih et al. 2004; Réale et al. 2007; Dingemanse et al. 2010). Despite the presumed advantages of behavioral plasticity, it has been found that specific behavioral patterns exist and can be different among and/or within populations (Sih et al. 2004; Laland and Janik 2006; Bell 2007). Our study found differences in exploratory behavior among populations; specifically, exploration was higher in birds inhabiting higher elevations (> 1500 m) relative to those occurring at lower elevations (< 1000 m). This result was predicted because exploratory behavior is an important mechanism to obtain information about environmental conditions and quality (see Dingemanse et al. 2002; Careau et al. 2009), particularly in extremely seasonal environments such as those that occur at high elevations. For example, in the Farellones site (~ 2300 m), environmental conditions are harsh, particularly in winter, with limited primary production and decreased food availability for rufous-collared sparrows relative to populations that occur at lower elevations (Körner 2007). Therefore, the ability to rapidly obtain environmental information via exploration is likely important for finding food or shelter under these conditions (see Mettke-Hofmann 2006). In addition to differences in exploratory behavior, we also found population-level differences in morphological traits, as all body measurements were significantly larger in birds inhabiting higher elevation sites year-round. This result was also expected because body size should increase in response to colder conditions in accordance with Bergmann’s rule (Meiri and Dayan 2003). Overall, these behavioral and morphological patterns suggest that different environmental pressures lead to the development of different adaptive strategies in this species. Cheviron and Brunfield (2009) found significantly reduced mitochondrial gene flow along elevational transects in Z. capensis in the Peruvian Andes and concluded that divergent selection pressures along environmental gradients can drive differentiation among populations. The highly seasonal climatic conditions present at higher elevations, however, likely promotes elevational migration (Newton and Dale 1996) such that morphometric adaptations may be related to the distance that birds migrate (Leisler and Winkler 2003). In such a scenario, exploratory behavior could be important for deciding when its best to migrate.

Descriptive studies show that some populations of Z. capensis in Chile and Argentina undertake latitudinal and elevational migrations (Chapman 1926; Olrog 1979; Narosky and Di Giacomo 1993; Ortiz and Capllonch 2011). These patterns have been gleaned from seasonal fluctuations in abundance of birds at different elevations throughout the year and occasionally from recapture of banded individuals (see Stiles 1985; Loiselle and Blake 1991; Johnson and Maclean 1994; Winker et al. 1997; Burgess and Minga 2000; Wright 2005; Brown 2006). Most studies that have recaptured marked individuals, however, have not directly assessed seasonal migration, since they only note presence or absence during a single period of the annual life cycle. To our knowledge, there are no data that show the Farellones population of rufous-collared sparrow are elevational migrants. Even though we observed birds at Farellones (2300 m) all year-round, our data showed that they diminished strongly during winter (Fig. 2). Furthermore, we did not recapture individuals banded at our highest elevation site at other localities.

Fortunately, isotope analysis revealed that blood representing the winter months collected from high elevation had lower mean δ2H values than blood collected at the same time from sparrows at low elevation (Fig. 3). The magnitude of the δ2H offset in blood among high and low elevation sites is similar to that in feathers representing the summer months (Fig. 3), suggesting that these populations are residents and remain at their respective elevations year-round. Furthermore, the δ2H values of blood collected from high elevation sites during the winter and summer months were similar (Fig. 3). These patterns show that δ2H analysis is a faithful recorder of elevation of origin in Z. capensis chilensis and that the high elevation population does not migrate downslope during the winter. Finally, the difference in observed δ2H values for either blood or feathers between sparrows that occur in low and high elevation sites conforms to expectations based on the application of published elevational lapse rates in precipitation δ2H for the Andes (Table 3).

The differences found in exploratory behavior, morphological traits, and lack of elevational movement show the existence of ecological differentiation between high and low elevation Z. capensis chilensis populations. Those results support the idea that different adaptive strategies could evolve in response to the steep gradient in the environmental conditions present across elevation in the Andes. Finally, we show that our combination of behavioral, morphometric, and isotope data is an informative and novel approach to assess ecological and evolutionary differences among populations, especially in species difficult to monitor with traditional methods.

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Author contribution statement YP conceived the ideas, led the analysis, and wrote the manuscript. VG assisted with the field work and contributed to the development of the ideas. VC assisted with the field work.
work and edited the manuscript. SDN contributed to the ideas, provided assistance with the analysis and editing of the manuscript. PS contributed to the ideas, provided assistance with the analysis, and edited the manuscript, and RAV contributed to the development of the ideas and helped editing the manuscript.

**Compliance with ethical standards**

**Conflict of interest** The authors have no conflict of interest to declare.

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