

LETTER

Individual diet specialisation in sparrows is driven by phenotypic plasticity in traits related to trade-offs in animal performance

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Abstract

Individual diet specialisation (IS) is frequent in many animal taxa and affects population and community dynamics. The niche variation hypothesis (NVH) predicts that broader population niches should exhibit greater IS than populations with narrower niches, and most studies that examine the ecological factors driving IS focus on intraspecific competition. We show that phenotypic plasticity of traits associated with functional trade-offs is an important, but unrecognised mechanism that promotes and maintains IS. We measured nitrogen isotope ($\delta^{15}\text{N}$) and digestive enzyme plasticity in four populations of sparrows (*Zonotrichia capensis*) to explore the relationship between IS and digestive plasticity. Our results show that phenotypic plasticity associated with functional trade-offs is related in a nonlinear fashion with the degree of IS and positively with population niche width. These findings are opposite to the NVH and suggest that among individual differences in diet can be maintained via acclimatisation and not necessarily require a genetic component.

Keywords

Assimilation efficiency, diet variation, digestive efficiency, digestive enzymes, individual diet specialisation, niche breadth, niche variation hypothesis, niche width, phenotypic plasticity, stable isotopes, trade-offs, trophic niche.

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INTRODUCTION

An appreciation for individual-level ecological variation over the past two decades has shown that individual diet specialisation (IS) is a frequent phenomenon in many animal taxa and communities (Bolnick *et al.* 2003; Araújo *et al.* 2011; Maldonado *et al.* 2017). Variation in resource use among individuals is likely essential for producing and maintaining frequency-dependent ecological interactions that influence population dynamics and ultimately drive evolutionary diversification (Svanbäck & Bolnick 2007; Araújo *et al.* 2011). Understanding how species have evolved a particular niche width has been the principal motivation for studies on IS, and it has been suggested that the size of a population's niche width is the combined result of two opposing phenomenon. First, the diversifying effect of intraspecific competition, which through an increase in among-individual variation results in an expansion of population niche width. Second, the effect of interspecific competition that acts to prevent niche expansion (Araújo *et al.* 2011; Kernaléguen *et al.* 2015). These

predictions are articulated in the niche variation hypothesis (Van Valen 1965; NVH), which proposes that an increase in population niche width is related to higher incidences of IS. Thus, it would be expected that populations with broader niches should exhibit greater among-individual variation in resource use in comparison to populations with narrower niches (Bolnick *et al.* 2007). Several studies have found support for this prediction, and most of them have focused on the effects of intraspecific and interspecific competition as the primary ecological forces that drives these patterns (Bolnick 2001; Svanbäck & Bolnick 2005, 2007; Newsome *et al.* 2015). Nevertheless, the observed or realised degree of IS is a product of both ecological factors (e.g. competition) and individuals' intrinsic (e.g. physiological) traits that define the fundamental IS (Hutchinson 1957; Bolnick *et al.* 2003). Thus, to understand the effects of temporal variation in resource quality or quantity on IS, consideration of mechanisms that promote both the fundamental and realised IS are required (Bolnick *et al.* 2003).

It has also been suggested that individual niche width is maintained by morphological, behavioural, and/or

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physiological functional trade-offs (Bolnick *et al.* 2003; Svanbäck & Eklöv 2003; Tinker *et al.* 2008; Araújo *et al.* 2009). Functional trade-offs constrain individual niche width because individuals incur costs in exploitation efficiency when they use alternative resources. This implies that different phenotypes cannot use the same spectrum of resources with equal performance, thus promoting IS. While studies that have observed significant levels of IS allude to the existence of trade-offs (Araújo & Gonzaga 2007; Araújo *et al.* 2009), only a few have found that among individual diet variation is associated with trait variation that is directly related to IS (e.g. Hsu *et al.* 2014). For example, Tinker *et al.* (2012) showed that variation in prey preference rankings among individuals reflected differences in individual's prey-specific handling efficiency. We suggest that one reason why most studies have failed to find a correlation between trait variation and IS is because they focus on morphological traits (e.g. Araújo *et al.* 2007, 2009), which are known to be less responsive to short-term environmental changes than behavioural or physiological traits (Ricklefs & Miles 1994) that have largely been ignored in studies of IS.

Traits underlying functional trade-offs are subject to evolution, thus they may differ among environments and ultimately influence the level of fundamental IS within populations. For example, many species living in seasonal environments have evolved plastic phenotypic traits that enable individuals to maintain performance and maximise fitness under variable conditions (Pigliucci 1996; Cavieres & Sabat 2008). Thus, individual niche widths are maintained to avoid performance costs when individuals switch diets, but those costs are predicted to decline as the degree of phenotypic plasticity increases (Van Tienderen 1997; Sabat & Bozinovic 2008). Following this rationale, maximum levels of phenotypic plasticity in traits underlying functional trade-offs, remove efficiency cost to exploit alternative resources, which would produce high population total niche width and low degrees of IS, contrary to predictions of the NVH (Fig. 1).

Digestion provides the functional link between energy intake and the utilisable energy needed for survival, growth, and reproduction (Naya *et al.* 2008) and is responsive to short-term shifts in diet composition (Karasov *et al.* 2011; Rott *et al.* 2017). Consumption of a wide range of resources that differ in quality result in physiological trade-offs associated with resource digestion; e.g. different diets require different digestive conditions, enzymes, or detoxification mechanisms for efficient assimilation (Afik & Karasov 1995; Karasov *et al.* 2011; Kohl & Dearing 2011; Barceló *et al.* 2016). Thus, nutritional differences in the energy and macromolecular content among diet items may impact digestive performance, which could affect energy budgets when animal are faced with the prospect of switching diets. We previously showed that the rufous-collared sparrow (*Zonotrichia capensis*) has a large degree of individual variation in the activity of a digestive enzyme related to protein consumption (aminopeptidase-N), which in turn was associated with the percentage of insects in individual diets in the field (Sabat *et al.* 1998). In this context, an increase in the activity of aminopeptidase-N probably results in beneficial digestive conditions for the consumption of high protein diets, enabling a bird to maximise its rate of energy gain when eating insects (Karasov 1996).

Thus, an individual's ability to reversibly adjust their phenotype to enhance performance should influence whether (or not) its niche is maintained over time, thereby affecting the degree of IS in populations. Despite, the apparent relationship between phenotypic plasticity and IS, it has not been explored. Here we test whether levels of IS, total population niche width, and their within and between individual components are related to the magnitude of population digestive phenotypic plasticity among four populations of a widely distributed songbird that occur across a steep environmental gradient. We hypothesised that phenotypic plasticity in digestive traits is related to the degree of IS in a population, but not in a (positive) linear fashion (Fig. 1). Instead, we predicted that populations with high degrees of digestive plasticity exhibit a wider population niche and are composed of more individual generalists, and thus show lower degrees of IS; note this prediction is opposite to that proposed by the NVH. Likewise, a population composed of individuals with no digestive plasticity would have constrained individual and population dietary niches and by extension would have low degrees of IS. In contrast, populations composed of individuals with moderate levels of digestive plasticity would show the highest degrees of IS, because individuals can afford to specialise on a subset of the population niche width, rather than incur performance costs associated with shifts in diet composition (Fig. 1). We use a unique combination of isotope-based estimates of dietary variation and enzyme assays (maltase, sucrose and aminopeptidase-N) to quantify digestive plasticity. Stable isotope analysis has become an effective tool to examine IS through the estimation of individual and population isotopic variance using animals' tissues that represent diet over different timescales (Matich *et al.* 2011; Hanson *et al.* 2015; Bond *et al.* 2016; Maldonado *et al.* 2017). Nitrogen isotope ($\delta^{15}\text{N}$) values have been widely used to characterise trophic level and food-chain length (Post 2002) and here we measured $\delta^{15}\text{N}$ values of whole blood and feathers collected from each individual as a proxy of dietary variation to quantify individual and population trophic niches (Bearhop *et al.* 2004; Vander Zanden *et al.* 2010).

We also evaluated the presence of digestive costs in relation to diet shifts through laboratory experiments of apparent assimilation efficiency (AE) in birds captured from one of our study sites. Our data suggest that the magnitude of phenotypic plasticity of traits related to functional digestive trade-offs is a relevant factor for the maintenance of IS within populations. This study is the first to examine the relationship between plasticity in physiological (phenotypic) traits and IS among populations of a widespread species, and our results focus attention on the importance of environmental conditions in which phenotypic traits related to functional trade-offs have evolved.

METHODS

Field collection and study sites

Rufous-collared sparrow (*Zonotrichia capensis*) is distributed between southeast Mexico and Cape Horn in southern South America (Goodall *et al.* 1951). The subspecies studied here

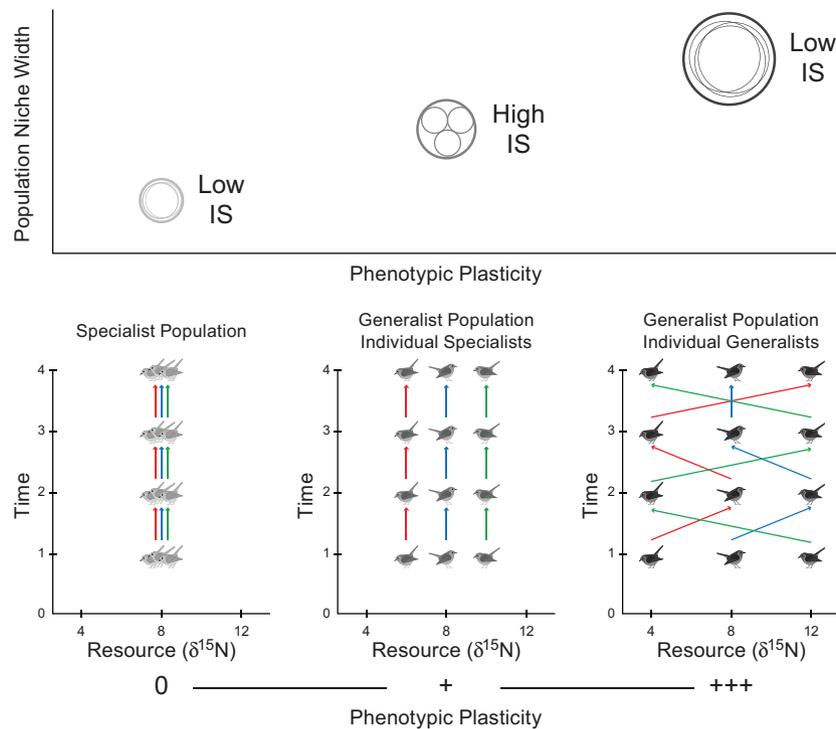


Figure 1 Conceptual diagram of how differences in the magnitude of phenotypic plasticity in traits related to trade-offs in resource use affects both individual and population niche widths and the degree of fundamental individual specialisation (IS) in natural populations. The lower panel describes how individuals use dietary resources over time. Populations comprised of individuals that lack phenotypic plasticity in traits related to trade-offs in resource use (0) utilise a narrow range of dietary resources. In the population with moderate levels of plasticity (+), each individual maintains a consistent individual niche width through time to avoid costs in digestive performance. In contrast, populations composed of individuals with high levels of phenotypic plasticity (+++) are able to use a wide range of resources without incurring a performance cost associated with switching diets. The upper panel, depicts how different patterns of resource utilisation in relation with phenotypic plasticity (outlined in the lower panel) affect the population niche width and the degree of fundamental IS. Population niche width (bold circles) is positively correlated with the magnitude of phenotypic plasticity in traits related to trade-offs, but the highest degrees of fundamental IS are observed intermediate levels of plasticity. Populations composed of individuals that lack phenotypic plasticity (0) exhibit both low intraindividual diet variation and population niche width, and consequently have low degrees of fundamental IS. In the population with moderate levels of plasticity (+), individuals consume a narrow portion of a relatively higher population niche width, and thus have higher levels of fundamental IS. Populations in which individuals have high levels phenotypic plasticity (+++) are composed of generalist individuals in which intraindividual diet variation is similar to the population niche width, and thus have lower levels of fundamental IS. Note that this conceptual diagram primarily deals with the influence of phenotypic plasticity on the fundamental IS, however, the observed (realised) IS is also affected by ecological factors such as the strength of intra and interspecific competition (Araújo *et al.* 2011). The lower panel of this figure was modified from Vander Zanden *et al.* (2010).

(*Zonotrichia capensis chilensis*) is non-migratory (Chapman 1940) and omnivorous, consuming both seeds and insects (Lopez-Calleja 1995; Sabat *et al.* 1998; Ramirez-Otarola *et al.* 2011). Males are territorial and socially monogamous, and both males and females contribute to parental care (Chapman 1940; Miller & Miller 1968). This species exhibits no testosterone modulation in response to social stimuli (Addis *et al.* 2011). Lastly, our previous work shows that populations of *Z. capensis* in Chile exhibit plasticity in phenotypic traits such as metabolic rate and digestive enzymes associated with environmental seasonality (Cavieres & Sabat 2008; Maldonado *et al.* 2011). Non-reproductive adult birds were captured with mist nets at four sites in Chile that differ in climatic conditions: Desert (Copiapó, 27°18' S 70°25' W), Semi-Desert (Vicuña, 30°01' S 70°42' W), Mediterranean (Santiago, 33°31' S 70°50' W) and Cold-Temperate (Llanquihue, 41°16' S 73°00' W) (Fig. 2c; see Table S1 in Supporting Information). Captures were conducted in March (austral autumn) of 2013 at the Desert, Mediterranean and Cold-Temperate sites and in

March of 2014 at the Semi-Desert site. After collecting tissue samples for isotope analysis, sparrows were released at the site of capture, with the exception of the Semi-Desert population that were transported to the laboratory at Universidad de Chile (Santiago, Chile) to perform experiments that quantified apparent assimilation efficiency (AE) and digestive enzyme plasticity (see below). All capture and experimental activities were covered by an Institutional Animal Care and Use permit issued by Universidad de Chile and governmental collection permit (No. 8172) issued by Servicio Agrícola y Ganadero (SAG) to K.M.

Stable isotope analysis

Immediately after capture, we obtained a *c.* 5 cm section from the tip of each of two primary feathers (p1 and p9) and took a *c.* 50 μL blood sample from the brachial vein using heparinised microhematocrit capillary tubes. Because feathers are a metabolically inert tissue, the isotopic signature reflects diet at

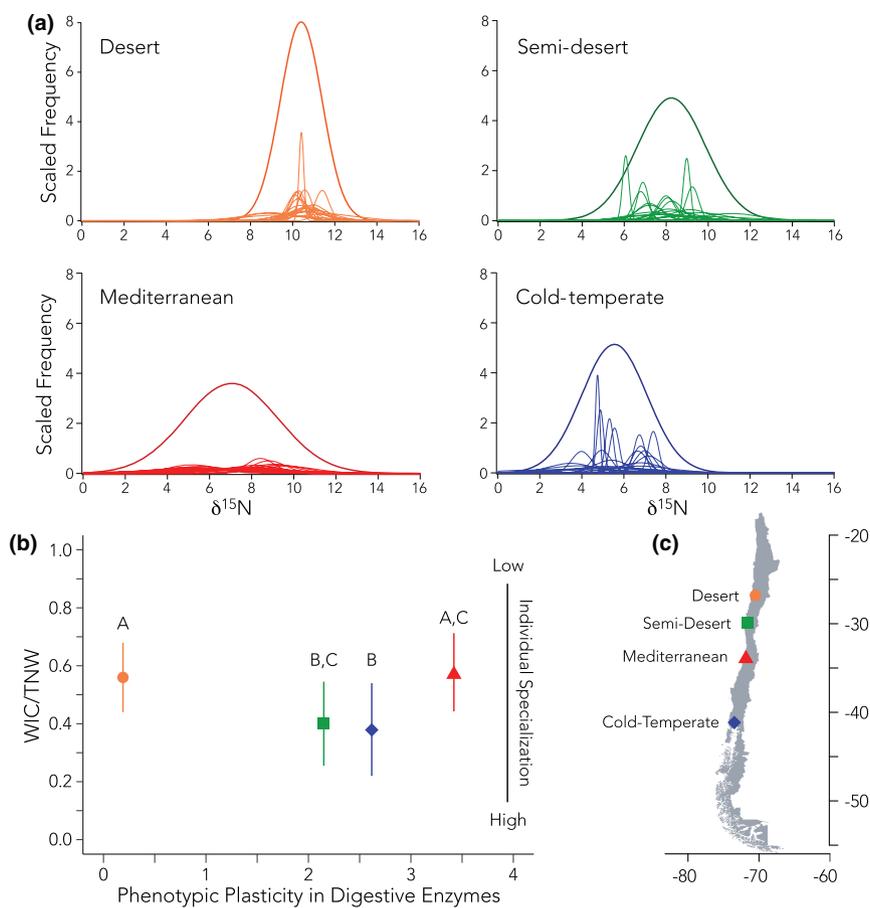


Figure 2 (a) Lighter curves correspond to normal functions $N(\mu_t, \sigma_t)$ with an unitary area, where μ_t and σ_t are the mean and standard deviations of feather and blood $\delta^{15}\text{N}$ values that represents the individual niches. Bold curves correspond to the normal function $N(\mu_t, \sigma_t)$, where μ_t , σ_t are the mean and standard deviations of feather and blood $\delta^{15}\text{N}$ values of all individuals of the population, which represents the population isotopic niche width (TNW). For visual clarity, population (bold) curves were equally scaled for the four populations, with an area equal to 20. (b) Individual specialisation (IS) and phenotypic digestive plasticity of *Zonotrichia capensis* from the four study sites. Study sites are denoted with coloured symbols: Desert (orange circles), Semi-Desert (green squares), Mediterranean (red triangle); Cold-Temperate (blue diamonds). (c) Map of Chile showing the approximate location of the study sites.

time of moult (Bearhop *et al.* 2004), which occurs from October to December in the austral spring (Davis 1971). Since feather growth is sequential (Campbell & Lack 1985), we collected P1 and P9 to be ensure that they reflect diet at the beginning and end of the *c.* 2-month moulting period (Davis 1971; King 1972). In contrast, the isotopic composition of whole blood reflects resources assimilated *c.* 30 days prior to sample collection (Hobson & Clark 1992; Martínez del Rio *et al.* 2009; Vander Zanden *et al.* 2015). Since birds were captured in March during the austral fall, a comparison of the isotopic composition of feathers to whole blood provides a multi-month longitudinal record of dietary variation for each individual.

To characterise baseline isotopic variation in primary producers available to each population, we collected samples of the 16 most abundant plant species at each of the four study sites for isotope analysis. Feather samples were cleaned with a 2 : 1 chloroform : methanol solvent (Hobson & Bairlein 2003) and all bird and plant samples were dried at 80 °C. Samples were then homogenised and *c.* 0.5–0.6 mg of each sparrow tissue or *c.* 2–3 mg of plants were loaded into tin capsules for isotope analysis. Nitrogen isotope ($\delta^{15}\text{N}$) values of sparrow tissues and plants were measured on a Costech 4010 elemental

analyser coupled to a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer at the University of Wyoming Stable Isotope Facility. Isotope data are expressed in delta (δ) notation using the equation: $\delta^{15}\text{N}_{\text{standard}} = [R(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - R(^{15}\text{N}/^{14}\text{N})_{\text{standard}}] / R(^{15}\text{N}/^{14}\text{N})_{\text{standard}}$. The internationally accepted standard for $\delta^{15}\text{N}$ values is the atmospheric nitrogen and units are parts per thousand, or per mil (‰). Within-run analytical precision (\pm SD) was estimated to be $\pm 0.2\text{‰}$ via repeated analysis of two internal proteinaceous reference materials.

Digestive performance

Birds from the Semi-desert population were habituated to laboratory conditions for 1 week before AE experiments. After the habituation period, a group of nine sparrows were first fed a high-carbohydrate and low-protein diet consisting of *Phalaris canariensis* seeds for 6 days and then fed a high-protein and low-carbohydrate diet consisting of *Tenebrio molitor* larvae for eight additional days (Table S2). We calculated the AE of each individual every 2 days by measuring the total dry mass and energy content of food consumed and excreta following

methods described in McConnachie & Alexander (2004); see Appendix S1 for additional details about AE experiments.

In a previous study, we compared the magnitude of dietary acclimation on digestive enzyme activities (maltase, sucrose and aminopeptidase-N) using the standardised effect size (SES) as a measure of phenotypic (digestive) plasticity of *Z. capensis* populations from the Desert, Mediterranean and Cold-Temperate sites (Maldonado *et al.* 2011). This study expands upon our previous work by adding data from the Semi-Desert population. To measure the degree of digestive enzyme plasticity in this population, we performed diet acclimation experiments. Individuals ($n = 18$) were randomly separated into two groups, which were acclimated to an insect-based (*Tenebrio molitor*) or seed-based (*Phalaris canariensis*) diet that were provided *ad libitum* for 7 weeks. After the acclimation period, birds were euthanised, weighed (± 0.1 g), and the small intestine was removed. Small intestine were weighed (± 0.001 g) and then washed in a 0.9% NaCl solution and stored at -80 °C (Sabat *et al.* 1998). Digestive enzyme plasticity was assessed via standardised protocols to measure enzymatic activities (Maldonado *et al.* 2011); additional details are provided in Appendix S1.

Data analysis

To estimate the degree of individual specialisation (IS) of each population we used the approach outlined in Roughgarden 1974; which defines two components of the niche that when summed are equal to the population or total niche width (TNW), the within-individual component (WIC) that reflects the average of resource variability utilised by individuals and the between-individual component (BIC) that represents the between-individual variation in average resource use. Thus, we estimate TNW as the variance in $\delta^{15}\text{N}$ values among whole blood and feathers (P1 and P9) of all individuals that comprise the population; WIC as the mean of the variance of $\delta^{15}\text{N}$ values of whole blood, P1 and P9 feather from each individual, and BIC as the variance of the mean of $\delta^{15}\text{N}$ values of whole blood, P1 and P9 feathers of each individual. The degree of IS is reflected in the WIC/TNW ratio. Relatively low WIC/TNW ratios closer to zero indicate greater prevalence of IS in a population, and as ratios approach one individuals become more generalist and consume a higher proportion of the resources utilised by the entire population. In contrast to other metrics used to measure niche breadth, the WIC/TNW ratio uses the diet of the entire population as a proxy for resource availability; individuals' diets are compared with those from the population instead of with the environment, and thus, estimates of the abundance of resources available in the environment are not required (Bolnick *et al.* 2002). Calculations of TNW, WIC, BIC and IS were carried out in RInSp package in R (Zaccarelli *et al.* 2013).

We estimated the mean of TNW, WIC, BIC, the degree of IS and the associated 95% confidence intervals using 10 000 nonparametric bootstrap resamplings. The resampled population was constructed by randomly sampling (with replacement) the same number individuals in each population for which we had empirical data. We then estimate niche measures for each resampling as suggested above. Differences in TNW, niche components (WIC and BIC), and the degree of IS among

populations were evaluated with a Monte-Carlo permutation procedure; to obtain *P*-values, the observed difference in the means of two populations was compared to the differences of randomly assembled pairs of populations generated through 10 000 permutations. To test the statistical significance of IS within a population against a null model, we used a nonparametric Monte Carlo procedure of 10 000 replicates to obtain *P*-values for IS; note the null model represents a population composed of generalists individuals that sample randomly from the population's TNW (Bolnick *et al.* 2002). The Monte Carlo procedure is included in the RInSp package in R (Zaccarelli *et al.* 2013).

To assess whether the TNW of sparrow populations was not a simple reflection of the isotopic variation in local primary producers, we first calculated the isotopic variance in plant $\delta^{15}\text{N}$ using the same procedure as we did to estimate sparrow population TNW and calculating the 95% confidence intervals of plant $\delta^{15}\text{N}$ at each site. We also performed a Monte-Carlo permutation procedure to test for significant differences in plant $\delta^{15}\text{N}$ variance among populations. *P*-values were obtained through the comparison of the observed difference in the means of two populations with the differences of randomly assembled pairs of populations generated through 10 000 permutations. Finally, we compared plant variance with sparrow population TNW (Fig. S1).

We used two different approaches to test the effect of diet switching on sparrows' apparent assimilation efficiency. We used a mixed model in which diet type (seeds or insects) was implicitly considered and AE was the dependent variable, the random effect was the individual subjects, and the fixed effect was the experimental day. This model was compared with a GLM model (without the random effect) using the AKAIKE Information Criterion. To detect statistical differences between feeding trails we performed post hoc Tukey test for multiple comparisons. We also consider a model design that explicitly tests the hypotheses that individuals reduce their AE after switching diets, but then return to baseline values. To do so we fit generalised linear mixed models (GLMM) using the glmmTMB R package (Brooks *et al.*, 2017) in which AE depended on the nonlinear effect (second-order polynomial) of the continuous variable representing time in relation to when the diet switch occurred. Because assimilation efficiency ranges from 0 to 100%, we assumed a beta error distribution with a log-link function and the individual ID was assigned as a random effect that included random intercepts to account for the variability between subjects. This model was compared with a GLMM in which the effect of time in relation to when the diet switch occurred was a linear variable. We used the deviance and chi-square test to provide estimates of statistical significance of model selection.

The effect of the diet acclimation on digestive enzyme activity was evaluated using a MANCOVA analysis. The analysis considered the total activities of three digestive enzymes (maltase, sucrose and aminopeptidase-N) as dependent variables and the body mass of individuals as covariate. The magnitude of the digestive enzyme plasticity of each population was estimated by the standardised effect size (SES), which was calculated using the unbiased estimator of Hedge (*g*) and its

asymptotic SE. The estimation of SES was obtained using the adjusted least square mean from MANCOVA and the MANCOVA residual error as variance estimator (Kline 2004; Kampenes *et al.* 2007; Maldonado *et al.* 2011). When appropriate, assumptions of normality and homoscedasticity in residuals were examined with Q–Q plots and plot of residuals against fitted values respectively (Zuur *et al.* 2010). All statistical analyses were performed using the R platform (v3.4.1; R Core Team, 2016).

RESULTS

Zonotrichia capensis sample sizes, total niche widths (TNW), within-individual (WIC) and between-individual (BIC) components of niche width and the degree of individual specialisation (IS) from the four sites are reported in Table 1 and Fig. 2; note this table also includes results of statistical tests for each variable. The exact *P*-values obtained for pairwise comparisons of *Zonotrichia capensis* populations are also reported in Table S3. TNW significantly varied among populations, and was highest at the Mediterranean site and lowest at the Desert and Cold-temperate sites; TNW did not differ between the Semi-Desert and Cold-Temperate sites. The WIC of niche width was significantly higher at the Mediterranean site in comparison to the other populations, whereas WIC of the Desert, Semi-Desert and Cold-Temperate sites did not significantly differ from one another. The BIC of niche width was similar among all populations. The degree of IS was significantly higher at the Cold-Temperate site in comparison to the Desert and Mediterranean sites. The degree of IS at the Semi-Desert site was significantly higher than in the Desert population, but marginally insignificant ($P = 0.057$) in comparison to IS in the Mediterranean population. The Monte Carlo resampling procedure that we used to assess the existence of IS relative to a null model (population of generalist individuals) revealed that sparrow populations from the Semi-Desert ($P < 0.01$) and Cold-Temperate ($P < 0.01$) sites are comprised of more individual specialists, whereas the Mediterranean ($P = 0.07$) and Desert ($P = 0.06$) sites are comprised of more generalist individuals (Fig. 2).

There were no significant differences in the degree of variation in plant $\delta^{15}\text{N}$ values among sites, showing that baseline isotopic effects were not responsible for the significant variation in sparrow TNW and degree of IS among populations (Fig. S1).

Birds experienced a significant reduction in AE after switching from a diet of seeds to one of insects, but then returned within 4 days to AE values similar to those observed before

the diet switch (Fig. 3; Tukey test, $P < 0.05$). Likewise, we found an effect of time since diet switch on AE ($P = 0.02$), which shows that switching diets results in a reduction in AE, followed by a response to baseline values after the birds acclimate to their new diet. MANCOVA results that assessed differences in digestive enzyme activities between acclimation diets revealed that Desert sparrows did not change their overall enzyme activities in response to acclimation to either an insect or seed diet (Wilks's = 0.58, $F_{3,11} = 2.62$, $P = 0.1$). In contrast, diet induced changes in digestive enzyme activities in the Semi-Desert (Wilks's = 0.10, $F_{3,8} = 21.11$, $P < 0.001$), Mediterranean (Wilks's = 0.15, $F_{3,9} = 16.19$, $P < 0.001$) and Cold-Temperate populations (Wilks's = 0.36, $F_{3,11} = 6.57$, $P = 0.008$). Consequently, the magnitude of digestive enzyme plasticity (estimated via SES) was highest in the Mediterranean population (3.42), intermediate at the Cold-Temperate (2.62) and Semi-Desert (2.15) populations, and much lower in the Desert (0.19) population.

There was a positive association between TNW and the degree of phenotypic plasticity in digestive enzymes (Fig. 4). The Desert population had the lowest digestive plasticity and the lowest TNW, whereas the Mediterranean population had the highest phenotypic plasticity and the highest TNW. Semi-Desert and Cold-Temperate populations shared a moderate magnitude of digestive plasticity had intermediate levels of TNW. The populations with the lowest (Desert) and highest (Mediterranean) degrees of phenotypic plasticity in digestive enzymes had the lowest degrees of individual specialisation, whereas populations shared a moderate magnitude of digestive plasticity (Semi-Desert and Cold-Temperate) had the highest level of IS (Fig. 2b).

DISCUSSION

Our results contradict the NVH, as the sparrow population with the broadest niche width was comprised of individuals that were dietary generalist and had a high level of (phenotypic) plasticity in digestive enzymes. In general, phenotypic plasticity has an adaptive value in organisms that experience temporal variation in environmental conditions (Cavieser & Sabat 2008). In relation to diet, animals that consume resources that vary in macromolecular composition can maintain digestive performance by varying gut size, nutrient transport activities and the hydrolysis rates of tissue-specific enzymes (Karasov *et al.* 2011). Some have suggested that reversible regulation of phenotypic traits such as intestinal digestive enzymes should be present in generalist but absent in specialist species (Buddington *et al.* 1991; Karasov 1996). The approach often used to test this

Table 1 Samples sizes (*n*), within individual component (WIC), between individual component (BIC), total isotopic niche width (TNW) and individual specialisation index (IS) of *Zonotrichia capensis* from the four sites examined in this study. Units for WIC, BIC and TNW are in ‰²

Population	<i>n</i>	WIC	BIC	TNW	IS
Desert (Copiapó)	20	0.63 ^a (0.35–0.97)	0.50 ^a (0.21–0.81)	1.13 ^a (0.58–1.69)	0.56 ^a (0.44–0.68)
Semi-Desert (Vicuña)	22	1.04 ^a (0.52–0.18)	1.52 ^a (0.72–2.44)	2.57 ^b (1.44–3.96)	0.40 ^{b,c} (0.26–0.55)
Mediterranean (Santiago)	23	2.71 ^b (1.87–3.58)	2.07 ^a (1.37–2.84)	4.78 ^c (3.88–5.72)	0.57 ^{a,c} (0.42–0.70)
Cold-Temperate (Llanquihue)	22	0.90 ^a (0.4–1.53)	1.41 ^a (0.82–1.95)	2.31 ^{a,b} (1.46–3.19)	0.38 ^b (0.22–0.54)

Numbers in parentheses below mean values are 95% bootstrap confidence intervals of the mean. Differences for WIC, BIC, TNW or IS among populations were evaluated with a Monte-Carlo permutation procedure. Different superscript letters denote significant differences ($P < 0.05$) among populations.

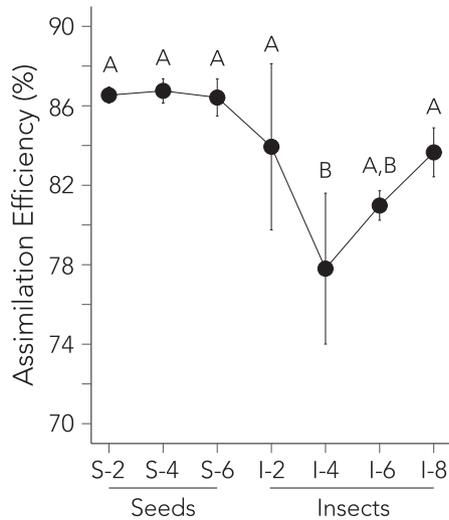


Figure 3 Apparent assimilation efficiency (AE) of *Zonotrichia capensis* collected from the Semi-Desert site after being fed with a high-carbohydrate and low-protein seed (S) diet and then switched to a high-protein and low-carbohydrate insect (I) diet. Birds were exposed to the seed diet for a total period of 6 days (S2–S6) and then abruptly switched to the insect diet for a total period of 8 days (I2–I8). AE was assessed every 2 days from the beginning of the experiment.

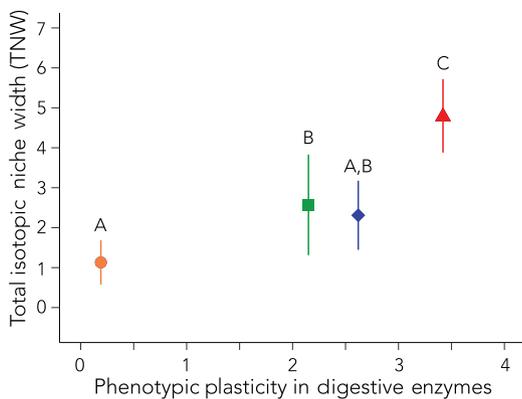


Figure 4 Population isotopic niche width (TNW) and phenotypic digestive plasticity of *Zonotrichia capensis* from the Desert (orange circles), Semi-Desert (green squares), Mediterranean (red triangle) and Cold-Temperate (blue diamond) sites.

hypothesis first coarsely categorises species as dietary generalists or specialists based on their natural history and then measures the plasticity of a particular digestive trait (Buddington *et al.* 1991; Sabat *et al.* 1998, 1999). The conclusions of these studies are controversial, because it's difficult to classify species as dietary specialist or generalist in the absence of quantitative measures of population dietary breadth, and the ability to modulate digestive enzymes may differ among species, which appears to have a phylogenetic rather than ecological basis in some contexts (Martínez del Río 1990; Gatica *et al.* 2006). No study has tested whether phenotypic plasticity is related to dietary flexibility in which niche width was assessed directly at the population or individual level. Our study shows an association between digestive enzyme flexibility and dietary niche width,

which suggests that higher phenotypic plasticity enables individuals in a population to exploit a broader spectrum of resources (Fig. 4).

In theory, dietary IS is maintained within populations because traits associated with capture and processing of a particular resource result in decreased procurement performance of another resource (Bolnick *et al.* 2003). Previously, we showed that *Zonotrichia capensis* individuals that consume a higher proportion of insects exhibited increased activity of a digestive enzyme related to enhanced protein consumption (Sabat *et al.* 1998). Here we found that *Zonotrichia capensis* from the Semi-Desert site immediately decreased their digestive performance during a switch from a granivorous (carbohydrate-rich) to an insectivorous (protein-rich) diet, which was associated with a decrease in energy acquisition, despite AE eventually returning to baseline values (Fig. 3). Such physiological trade-offs may promote IS when omnivorous species like sparrows primarily consume carbohydrate- or protein-rich prey, which prevents individuals from consuming resources that span the entire population dietary niche width without incurring a cost in digestive performance.

We found that the degree of IS was lower in the sparrow population from the Mediterranean site; this population had both the highest digestive plasticity and TNW of the four populations we examined (Figs 2 and 4). This result suggests that a diverse, generalist diet did not significantly impact the digestive performance of individuals at the Mediterranean site. The sparrow population at the Desert site also had a (low) level of IS similar to that observed at the Mediterranean site, however, they showed no digestive plasticity and a lower TNW in comparison to all of the other sites (Figs 2 and 4). These patterns show that the sparrow population at the Desert site is comprised of individuals that specialise on similar types of resources; i.e. a specialist population. In contrast, sparrows from the Semi-Desert and Cold-Temperate sites had intermediate levels of digestive plasticity and TNW and high degrees of IS in comparison with the other two sites (Figs 2 and 4). These patterns show that sparrows from the Semi-Desert and Cold-Temperate sites are generalist populations comprised of more individual specialists than the other two populations. When combined with results from our laboratory experiments, these patterns suggest that individuals from the Semi-Desert and Cold-Temperate sites likely maintain their individual dietary niches to avoid the (digestive) costs of switching diets.

Individual variation within populations should result from adaptive genetic differences (Van Valen 1965; Grant *et al.* 1976; Smith & Skúlason 1996; Wilson 1998; Bell 2007) to facilitate selective processes which ultimately could yield speciation and facilitate adaptive radiation (Araújo *et al.* 2011). However, there is little empirical data that robustly links adaptive genetic variation and phenotypic variation in ecological traits (Jaenike & Holt 1991; Leibold *et al.* 1994; Tessier & Leibold 1997). The few studies that have tested the relationship between interindividual ecological vs. genetic variation within populations have failed to generate strong support for this expectation (Grant *et al.* 1976; Agashe & Bolnick 2010; De León *et al.* 2012). Our study suggests that among individual dietary differences can be driven by acclimatisation to a specific subset of resources in a populations' dietary niche. In

such a scenario, genetic differences are not needed to maintain IS. If differences among individuals are due to acclimatisation rather than genetic variation, it is likely that the degree of IS within a given population could vary over short (seasonal) timescales, a phenomenon that has been reported in several studies (e.g. Araújo & Gonzaga 2007; Kernaléguen *et al.* 2015; Dehnhard *et al.* 2016). Even though our study shows that digestive characteristics may be modified by acclimation, we cannot rule out the possibility that the observed individual variation within the populations we examined could be due to genetic differences, and we emphasise that the relationship between ecological variation and genetic diversity needs further exploration.

The realised IS is affected by ecological factors such as resource availability, predation, and the strength of intra and interspecific competition (Araújo *et al.* 2011). Theory and a growing amount of empirical data show that intraspecific competition increases IS (Svanbäck & Bolnick 2007; Tinker *et al.* 2008, 2012; Newsome *et al.* 2015). In contrast, theory predicts that higher interspecific competition should reduce the prevalence of IS, but empirical support for this mechanism is lacking (Bolnick *et al.* 2010; Araújo *et al.* 2011). Information about the mechanisms that influence the fundamental IS should generate predictions for how population niche expansion could be achieved via changes in WIC and/or BIC. For example a temporal reduction in available resources may enable individuals from highly flexible populations to incorporate other low-value resources without abandoning previously used resources, and hence maintain similar degrees of IS. In contrast, individuals from moderately flexible populations would not add novel medium or low-value resources to their diets but instead continue to use previously preferred resources, which would ultimately result in the maintenance of individual niche breadth and a corresponding increase in the degree of IS (Araújo *et al.* 2008). Finally, when a high-value resource is highly abundant, all individuals in a population may switch to primarily foraging on this resource, leading to a decrease in the degree of IS, even in a population with a moderate level of digestive plasticity.

Our findings contrast with the NVH, and we suggest that this hypothesis may be more applicable to predicting dietary patterns at the interspecific level (Maldonado *et al.* 2017). The NVH may not adequately address phenomenon that occur among individuals of the same species that are the target of the microevolutionary processes that shape the functional traits of species. We also must consider that the NVH primarily explores the effects of inter and intraspecific competition and thus its predictions focus on the realised IS that is shaped by a combination of abiotic and biotic factors (Bolnick *et al.* 2003). Overall, our results suggest that phenotypic plasticity in traits related to physiological, behavioural, and/or morphological trade-offs in resource use is likely an important mechanism that affects the degree of fundamental IS. Nevertheless, a note of caution is necessary since our comparative field-based approach is not able to assess causality between IS and phenotypic plasticity. Future laboratory experiments are needed to better constrain the multiple variables that could affect the relationships observed here. More data on variation in intrinsic physiological traits among individuals, which help shape the fundamental IS,

is needed to identify additional mechanisms that promote and maintain IS in natural populations.

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AUTHORSHIP

KM designed the study, collected and analysed the data and drafted the manuscript; SDN contributed to the design of the study, data analysis and edited the manuscript; PR-B contributed to advised on conceptual and data analysis; JMR performed diet experiments and collected data, GP collected and prepared isotopes samples and performed diet experiments; PS contributed to the design of the study, advised on analyses, and edited the manuscript; all authors contributed substantially to revisions.

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