Trophic niche width increases with bill-size variation in a generalist passerine: a test of niche variation hypothesis

Yu-Cheng Hsu[1], Pei-Jen Shaner[2]*, Chun-I Chang[2], Linhua Ke[2] and Shuh-Ji Kao[3]

[1]*Department of Natural Resources and Environmental Studies, National Dong Hwa University, Hualien, Taiwan;* [2]*Department of Life Science, National Taiwan Normal University, Taipei, Taiwan; and* [3]*Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan*

Summary

1. The niche variation hypothesis (NVH) predicts that populations with wider niches are phenotypically more variable than populations with narrower niches, which is frequently used to explain diversifying processes such as ecological release. However, not all empirical evidence supports the NVH. Furthermore, a relationship between population phenotypic variation and niche width can be caused by sexual selection or environmental gradients, which should be carefully considered along with competition in explaining niche variation.

2. In this study, we used eight populations of a generalist passerine species, *Paradoxornis webbianus* (vinous-throated parrotbill), to test the NVH. We assessed evidence of ecological sexual dimorphism and environmental gradients in bill morphology of *P. webbianus*. A total of 170 *P. webbianus* from eight sites ranging 24–2668 m in altitude were included in this study. We used two principal components to quantify bill morphology: one describes bill size and the other describes bill slenderness. We used stable carbon and nitrogen isotope values of bird feathers to quantify trophic positions, and we estimated population trophic niche width using Bayesian standardized ellipse area.

3. *Paradoxornis webbianus* with larger and more slender bills fed at higher trophic levels and population trophic niche width tended to increase with bill-size variation, supporting the NVH. The males had larger bills and marginally higher nitrogen isotope values than the females, suggesting ecological sexual dimorphism. Despite a positive correlation between bill size and wing length indicating sexual selection for larger male size, only three of the eight populations showed both male-biased bill size and male-biased wing length. Sexual dimorphism explained 13%–64% of bill-size variation across sites, suggesting its role in niche variation could vary greatly among populations. The variation in bill slenderness in *P. webbianus* increased with elevation. However, neither bill-size variation nor trophic niche width changed with elevation. Therefore, environmental gradients that could be reflected in the elevation are not likely to drive the observed morphological and niche variation.

4. This study provides an empirical case for the NVH and highlights the importance of investigating sexual dimorphism and environmental gradients in studies of niche dynamics.

Key-words: ecological specialization, elevational gradients, *Paradoxornis webbianus*, stable isotope, trophic polymorphism

Introduction

Ecological niche reflects biotic and abiotic interactions at local scales (Leibold 1995) and delineates species range at geographic scales (Soberón 2007). Therefore, understanding niche dynamics is of critical importance to both evolutionary ecology and conservation biology. The niche variation hypothesis (NVH) predicts that populations with wider niches are phenotypically more variable than populations with narrower niches (Van Valen 1965). The premise of the

*Correspondence author. E-mail: pshaner@ntnu.edu.tw

NVH is that individuals experience strong trade-offs between niche width and competitive ability, which facilitates disruptive selection and individual specialization (Ackermann & Doebeli 2004). Therefore, populations comprised of individual specialists are more efficient in exploiting wider niches than populations comprised of individual generalists. The NVH has been used to explain important eco-evolutionary processes such as ecological release (e.g. Costa *et al.* 2008; Bolnick *et al.* 2010) and maintenance of polymorphism (e.g. Galeotti & Rubolini 2004; Lavm & Nevo 2008). However, empirical evidence is equivocal, showing support for the NVH in several cases where individual specialization was directly measured in terms of niche width yet showing lack of support in others where individual specialization was indirectly measured with morphological proxies (Bolnick *et al.* 2007 and references therein).

One challenge of using morphological proxies for individual specialization lies in the uncertainty of how traits might be constrained by and/or correlated with other traits. Even though morphology of feeding apparatus such as jaws or bills is often assumed to reflect individual diets, previous studies have shown that it can be constrained by growth (e.g. Lister & McMurtrie 1976) or correlated with other traits under sexual selection (e.g. Price 1984). Furthermore, feeding apparatus may also serve other functions such as thermoregulation (e.g. Greenberg *et al.* 2012) or parasite defence (e.g. Clayton *et al.* 2005). As a result, links between morphological variation in feeding apparatus and trophic niche width may not always be strong. Nevertheless, testing the NVH with morphological traits is still appealing because they can often be measured precisely even in wild populations. Trophic morphology is of particular interests to ecologists because of its broad implications in predator–prey interactions, food web processes and nutrient cycling (Cohen 1977; Layman *et al.* 2007). Following the NVH, populations comprised of individual diet specialists should occupy wider trophic niches. If trophic morphology reflects individual diets, populations with larger variation in trophic morphology should also occupy wider trophic niches.

Sexual dimorphism is an important source of intrapopulation morphological variation. When sexual dimorphism is largely maintained by intersex competition and niche specialization (ecological sexual dimorphism), the resulting morphological variation and niche expansion can be viewed as a form of the NVH. However, when sexual dimorphism is largely maintained by sexual selection (Shine 1989), the interpretation of the evidence for the NVH becomes more complicated. For example, sexual selection for larger body size in male *Geospiza fortis* (Darwin's medium ground finch) led to sexual dimorphism in bill size (Price 1984). Assuming sexual selection is stronger when resource abundance is low, an apparent relationship between morphological variation (strong sexual selection increases phenotypic variation) and population niche width (low resource abundance increases individual diet specialization) may emerge without any of the underlying

processes responsible for the NVH, such as trade-offs between niche width and competitive ability, and disruptive selection. Sexual dimorphism in trophic morphology has been reported in many bird species, some of which were shown to have an ecological cause (e.g. Temeles *et al.* 2000; Stein *et al.* 2008; Temeles, Miller & Rifkin 2010). Therefore, when testing the NVH in bird species, it is helpful to also assess the support for ecological sexual dimorphism. Although disentangling the effects of natural and sexual selection is difficult, sex differences in both diet niche and trophic morphology are frequently taken as evidence for ecological sexual dimorphism (Temeles *et al.* 2000; Temeles, Miller & Rifkin 2010).

Environmental gradients can have profound influence on patterns of niche variation. Systematic changes in trophic niche width of a consumer species along an environmental gradient may reveal an underlying gradient in food availability. For example, *Lutra lutra* (Eurasian otter) increased diet niche width at higher altitudes due to reduced prey availability (Remonti, Balestrieri & Prigioni 2009). Prey diversity and richness of *Tyto alba* (barn owl) increased in latitude in central Argentina, which was likely driven by the geographic distribution of their primary prey (Leveau *et al.* 2006). On the other hand, trophic morphology, such as bill surface area, has been found to vary along climate gradients: *Melospiza melodia* (song sparrow) in coastal areas had larger bill surface area than those in mesic habitats, a potential adaption for heat dissipation in drier, costal climate (Greenberg *et al.* 2012). If both food availability and climate conditions vary along environmental gradients, with the former facilitating wider trophic niche width and the latter larger variation in trophic morphology, a relationship between niche and morphological variation could also emerge. Therefore, understanding how niche and morphological variation respond to environmental gradients may help us interpret large-scale patterns in niche variation.

Stable carbon and nitrogen isotopic compositions (δ^{13}C ‰, δ^{15}N ‰) of animal tissues reflect the isotope values of their diets (Post 2002). When background stable isotope values (i.e. isotope values of primary producers in a food web) are adjusted for by subtracting them from consumers' values, stable isotope values could be used to delineate animals' trophic positions and compare trophic niches among populations (Bearhop *et al.* 2004). Furthermore, δ^{15}N values can be used to determine a consumer's trophic level with the application of a trophic enrichment factor (e.g. 3‰ per trophic level; Vanderklift & Ponsard 2003). Differences in δ^{13}C values, on the other hand, suggest individuals are feeding on foods with different carbon sources, such as C_3 vs. C_4 plants in a terrestrial food web. Finally, population trophic niche width can be defined as the area enclosed by the trophic positions of all individuals in the δ^{13}C-δ^{15}N dual isotope space (Jackson *et al.* 2011). Bird feather tissues have been shown to reflect dietary isotope values over a period of several weeks (e.g. *c.* 50 days in *Catharacta skua*; Bearhop *et al.* 2002).

Therefore, feather samples are likely to provide information on individuals' longer-term diets.

In this study, we used eight populations of a generalist passerine, *Paradoxornis webbianus* (vinous-throated parrotbill), to test the NVH. In addition, we assessed evidence of ecological sexual dimorphism and environmental gradients in their bill morphology. As a feeding apparatus, the avian bill has been studied extensively in the context of divergent selection, disruptive selection, niche partitioning and ecological sexual dimorphism (e.g. Grant 1986; Smith 1987; Benkman 1993; Temeles *et al.* 2000; Marquiss & Rae 2002). *Paradoxornis webbianus* is a diet and habitat generalist widely distributed in East Asia (Robson 2007). They are non-migratory, which makes it easier to delineate their trophic niche with stable isotopes. In addition, *P. webbianus* in Taiwan has a wide elevational distribution from sea level to over 2600 m, providing opportunities to investigate patterns of morphological and niche variation along environmental gradients. Specifically, we ask the following questions: (i) Is bill morphology correlated with individual trophic position? – testing the validity of using bill morphological variation as a proxy for degree of individual diet specialization; (ii) Does population trophic niche width increases with intrapopulation variation in bill morphology? – testing the NVH; (iii) Is there evidence for ecological sexual dimorphism? (iv) Do trophic niche and bill morphology change along elevational gradients?

Materials and methods

STUDY SITE AND BIRD SAMPLING

This study included eight sites in Taiwan (Table 1), ranging from 24–2668 m in altitude and covering several habitat types for *P. webbianus* (i.e. forests, old-fields, farmlands). The shortest distance between sites is 1·8 km and the longest 68·8 km. Because *P. webbianus* has high site fidelity (Lee, Lee & Hatchwell 2010a; Lee *et al.* 2010b) and small home ranges (Severinghaus 1987) with individuals frequently recaptured within 100-m radius (Yu-Cheng Hsu, unpublished data), each site was treated as a separate population in this study. We define a 'population' as all individuals captured from a site. It should be noted that *P. webbianus* is highly gregarious, with limited natal dispersal (Lee, Lee & Hatchwell 2010a). Therefore, if there are strong within-flock genetic structures or if different flocks at a site have distinct ecological backgrounds, a population would be more appropriately represented by multiple-flock samples. Although we could not discern flock membership of the individuals used in this study, within-flock genetic structures in *P. webbianus* were found to be seasonally unstable and were not strong enough to create inbreeding (Lee *et al.* 2009, 2010b). In addition, home ranges of neighbouring *P. webbianus* flocks could overlap extensively with membership changes under some circumstances (e.g. urban campus; Severinghaus 1987). Given the unstable within-flock genetic structures, overlapping home ranges and potential membership changes, it is unlikely that strong flock-specific bill morphology or trophic niche exists in *P. webbianus*. Mist-netting was conducted between May of 2009

and October of 2012 (Table 1). Each captured bird was banded, and recaptured individuals were excluded from the analyses. Upon first capture, morphological traits of the birds were measured, and a few feathers from their chest region were collected for stable isotope analysis. Approximately 20 µL of blood was obtained by venipuncture from the brachial vein and stored in 100% ethanol for molecular sexing. All birds were released at the locations they were captured immediately after processing.

MORPHOLOGICAL MEASUREMENTS AND MOLECULAR SEXING

A digital calliper (Mitutoyo, Kanagawa, Japan) and a ruler with a zero-stop were used to measure four morphological traits to the nearest mm: (i) bill length, which is the distance from the proximal edge of the nostril to the tip of the bill; (ii) bill width, which is the width between the proximal edges of the nostril; (iii) bill depth, which is the depth at the proximal edge of the nostril (Eck *et al.* 2012); and (iv) maximum wing length, a proxy for body size (e.g. Hamilton 1961; James 1970). Five technicians performed morphological measurements with two to four technicians per site. Technician identity was random regarding the site or sex of the birds and was incorporated as a random factor in subsequent statistical analyses. In addition, because bill morphology could change seasonally (e.g. Matthysen1989), we also included season as a random factor in subsequent analyses. Due to logistic constraint and extreme difficulties to capture *P. webbianus* at some sites that likely represent this species' range limit (for instance, one banding trip every 2–3 months for 4 years from 2009 to 2013 at site HEH resulted in a total of 16 birds captured, which were all included in current study), we did not have sufficient samples from every month for a detailed test of seasonal effect. Instead, we grouped all samples into two major seasons: breeding (April to August) and non-breeding season (September to March) (Robson 2007).

Because *P. webbianus* is sexually monomorphic in plumage, molecular sexing was performed. Genomic DNA from blood was extracted using the methods in Gemmell & Akiyama (1996). Fragments of chromo-helicase-DNA binding protein (CHD) gene from the sex chromosomes were amplified by polymerase chain reaction (PCR), using the primers 2550F and 2718R (Fridolfsson & Ellegren 1999). The PCR protocol followed Fridolfsson & Ellegren (1999) with slight modifications (see Yang, Chiang & Hsu 2012 for details).

STABLE ISOTOPE ANALYSES

Foliar samples from 10 to 12 plant species at six of the eight sites were collected to estimate baseline stable isotope values (Table S1, Supporting Information). At each site, we selected the commoner plant species to be included in the baseline values. *Paradoxornis webbianus* is frequently found along forest edges and open habitats (Yu-Cheng Hsu, personal observation) where common plant species can be easily determined through a visual inspection. For the two sites where plant samples were not available (sites ZHA and LUO), mean isotope values of the plants from the two nearest sites were used (Table S1, Supporting Information). These site-specific baseline values were used to adjust feather isotope values ($\delta^{13}C_{adj} = \delta^{13}C_{feather} - \delta^{13}C_{plants}$; $\delta^{15}N_{adj} = \delta^{15}N_{feather} - \delta^{15}N_{plants}$) such that large differences in

Table 1. The elevation, temperature, sampling time and sample size of the study sites. Daily temperature data of 2010 were obtained from Data Bank for Atmospheric Research at Taiwan Typhoon and Flood Research Institute and Taroko National Park, which were used to calculate mean monthly temperatures, and mean temperatures of the coldest and warmest months. The range of sampling time varied among sites, from the first sampling in May of 2009 to the last in October of 2012. Whether the sampling at a site occurred during breeding season, non-breeding seasons or both was noted with 'B', 'NB' or 'Both' in the parenthesis following the sampling time (breeding season for *Paradoxornis webbianus* is between April and August; Robson 2007)

Site	Elevation (m)	Monthly temperature (of the coldest and warmest months) (°C)	Sampling time (B, NB, Both)	Sample size (males, females)
Chongde (CHO) 121°39′30·00″E, 24°09′09·00″N	24	23 (18, 28)[a]	2010 March & April, 2011 July (Both)	21 (10, 11)
Donghwa (DON) 121°32′47·50″E, 23°56′59·90″N	41	23 (18, 28)[b]	2009 September & October, 2011 July & September, 2012 March (Both)	18 (9, 9)
Zhaofeng (ZHA) 121°27′59·93″E, 23°47′18·88″N	102	23 (17, 28)[c]	2011 November (NB)	24 (17, 7)
Xibao (XIB) 121°29′04·00″E, 24°12′21·00″N	963	16 (10, 22)[d]	2009 December, 2010 January & March & May & September, 2011 June, 2012 May (Both)	21 (10, 11)
Lianhua (LIA) 121°29′49·00″E, 24°13′02·00″N	1129	16 (10, 22)[d]	2009 May & June (B)	20 (10, 10)
Luoshao (LUO) 121°27′03·24″E, 24°12′24·84″N	1140	16 (10, 22)[d]	2011 December (NB)	30 (14, 16)
Wuling (WUL) 121°18′18·36″E, 24°23′30·48″N	1927	12 (5, 17)[e]	2011 April (B)	20 (10, 10)
Hehuan (HEH) 121°17′56·00″E, 24°10′14·00″N	2668	10 (5, 14)[f]	2009 August, 2010 April, 2011 October, 2012 June & October (Both)	16 (6, 10)

[a]weather station C0T9C0; [b]weather station C0T841; [c]weather station C0T9F0, [d]weather station H1; [e]weather station C0U730; [f]weather station C0T790. Data for weather stations C0T9C0, C0T841, C0T9F0, C0U730 and C0T790 were from Data Bank for Atmospheric Research at Taiwan Typhoon and Flood Research Institute (http://www.ttfri.narl.org.tw/eng/index.html); data for weather station H1 were from Taroko National Park, Taiwan (Yu-Joe Hsia, personal communication).

background values among sites would not mislead our interpretation of the relative trophic position of the birds. Specifically, site WUL had intense fertilizer use in recent years; therefore, its $\delta^{15}N_{plants}$ are strongly enriched compared with other sites (Table S1, Supporting Information). Without baseline adjustment, *P. webbianus* at WUL would appear to be feeding at a much higher trophic level than a typical omnivorous bird. Although these baseline values are crude and may contain random errors, our estimation of population trophic niche width is independent of the baselines because it only measures relative positions of individuals within a site. In addition, site was included as a factor when testing sex difference in isotope values (see Statistical Analyses below). Therefore, any random errors in baseline values would be accounted for as part of the site effect. We also tested sex difference in unadjusted feather isotope values, and the results were qualitatively similar (Table S2, Supporting Information), indicating the baseline adjustment did not affect our conclusion on ecological sexual dimorphism.

Prior to isotope analysis, shafts were carefully removed from feather tissues using surgical scissors, and the remaining tissues were lipid-extracted, oven-dried at 60 °C for 24 h (Hobson 2008) and loaded into a tin cup. Plant foliar tissues were oven-dried at 60 °C for 48–72 h, ground into powder and loaded into a tin cup. Approximately 0·5–1 mg of feather or foliar tissue was used in each sample. The isotopic compositions of the samples were measured on a ThermoFinnigan Delta Plus Advantage Isotope Ratio Mass Spectrometer (Bremen, Germany).

STATISTICAL ANALYSES

The principal component analysis (PCA) was performed on the length, width and depth of the bills. The relationships between bill PC scores and wing length were tested using Pearson correlations. Sex, site and their interaction effects on PC scores and feather isotope values (square-root transformed $\delta^{13}C_{adj}$ and $\delta^{15}N_{adj}$) were tested using analysis of variance (ANOVA), followed by contrast comparisons between sexes for each site. Technician identity and season (i.e. breeding versus non-breeding seasons) were included as random factors when analysing morphological variables. Season was also included as a random factor when analysing isotope values. The variance component of sex at each site was used to quantify relative contribution of sexual dimorphism to intrapopulation variation in bill PC scores. Feather isotope values were pooled across sites and regressed against PC scores to test the relationships between individual trophic position and bill morphology (square-root transformed $\delta^{13}C_{adj}$ or $\delta^{15}N_{adj} = \alpha + \beta_{PC1} \times PC1 + \beta_{PC2} \times PC2$; α is the intercept, and β_{PC1} and β_{PC2} are the slopes of the first and second PCs, respectively). Sex-specific β_{PC1} and β_{PC2} were tested first: if they are significant, regressions were run for males and females separately; if they are not significant, regressions were run without sex-specific slopes. Population trophic niche width was calculated using Bayesian standardized ellipse area (SIAR package in R; http://cran.r-project.org/web/packages/siar/index.html; Jackson *et al.* 2011). Spearman correlations were used to test the relationships

between intrapopulation variation in PC scores and median standardized ellipse area, as well as elevational trends in mean PC scores, intrapopulation variation in PC scores, mean feather isotope values and median standardized ellipse area. Because samples taken from a longer time period are likely to produce both larger morphological variation and wider trophic niche width, we also tested correlations between the range of sampling time (i.e. the number of months between first and last sampling at a site; Table 1) and variation in PC scores or median ellipse area.

Results

BILL MORPHOLOGY AND WING LENGTH

A total of 170 *P. webbianus* were included in this study (for sampling time and sample size at each site; see Table 1). All three bill morphological traits showed trends of male-biased sexual dimorphism (mean ± SE: bill length, male = 6·53 ± 0·06 mm, female = 6·30 ± 0·04 mm; bill width, male = 4·86 ± 0·03 mm, female = 4·72 ± 0·02 mm; bill depth, male = 6·23 ± 0·03 mm, female = 5·92 ± 0·03 mm; n = 86 for males and 84 for females; Fig. 1). The first two principal components explained 83% of total variance in bill morphology and were retained for subsequent analyses. Higher values of the first principal component (PC1) represent longer, wider and thicker bills, whereas higher values of the second principal component (PC2) represent longer and narrower bills (Table 2).

Neither technician identify nor season affected PC1 scores, but they each explained a significant amount of variation in PC2 scores (Table 3). There were no sex-by-site interactions, and *P. webbianus* at different sites had different PC scores (Table 3). The males had consistently larger bills than females, whereas both had similar bill shape (Table 3; Fig. 2a,b). Sex on average explained 33% of intrapopulation variation in PC1 scores (ranging from 13 to 64% across sites) and 1% of intrapopulation variation in PC2 scores (ranging from 0 to 8% across sites).

Technician identify, but not season, explained a significant amount of variation in wing length (Table 3). There was a significant sex-by-site interaction (Table 3), and contrast comparisons suggest that the males had larger wing length than females at four of the eight sites (Fig. 2c). Wing length was positively correlated with PC1 scores (r = 0·16, P = 0·03, n = 170) but not PC2 scores (P = 0·88, n = 170), suggesting an individual with a larger body size also had a larger bill.

TROPHIC NICHE

Feather isotope values, whether unadjusted ($\delta^{13}C_{feather}$ and $\delta^{15}N_{feather}$) or adjusted ($\delta^{13}C_{adj}$ and $\delta^{15}N_{adj}$), produced qualitatively similar results (Table S2, Supporting Information). Season did not affect $\delta^{13}C_{adj}$ or $\delta^{15}N_{adj}$ values, and there were no sex-by-site interactions (Table 4). The males and females had similar $\delta^{13}C_{adj}$ values except for site LIA (Table 4; Fig. 3a). The males had
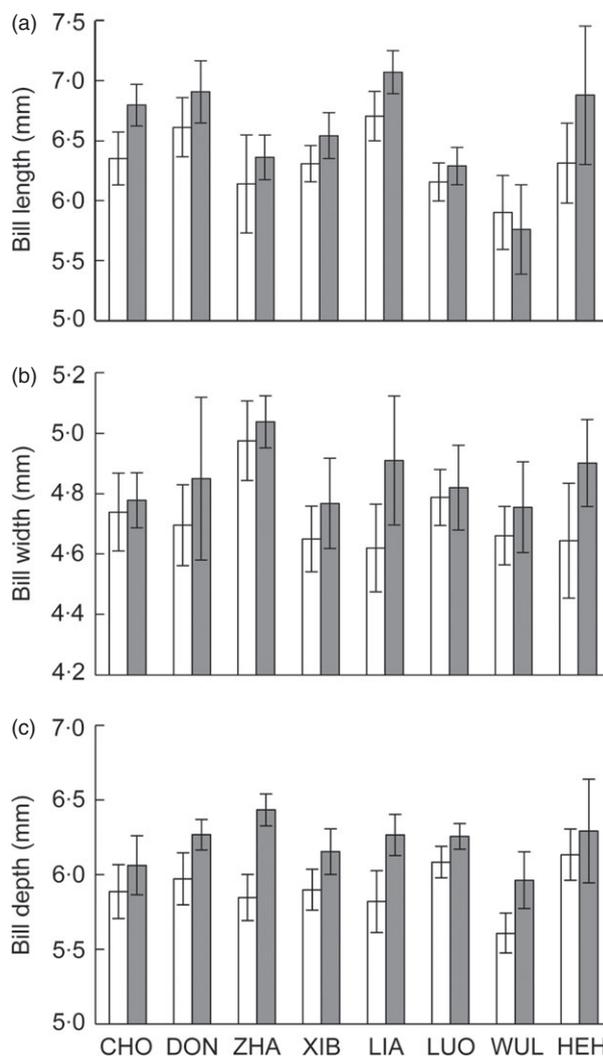
Fig. 1. Bill length (a), bill width (b) and bill depth (c) of male (filled) and female (unfilled) *Paradoxornis webbianus* across the eight sites. The error bars are ± 95% confidence limits. Sites on the horizontal axis are arranged from left to right in ascending order of elevation: Chongde (CHO), Donghwa (DON), Zhaofeng (ZHA), Xibao (XIB), Lianhua (LIA), Luoshao (LUO), Wuling (WUL) and Hehuan (HEH) (Table 1).

Table 2. Principal component axis loadings of bill morphology in *Paradoxornis webbianus* based on 170 adults across eight sites

Trait	PC1	PC2
Bill length	0·4931	0·7946
Bill width	0·5695	−0·6026
Bill depth	0·6577	−0·0740

marginally higher $\delta^{15}N_{adj}$ values, which was mainly driven by sites DON and HEH (Table 4; Fig. 3b). *Paradoxornis webbianus* at different sites had different $\delta^{13}C_{adj}$ and $\delta^{15}N_{adj}$ values (Table 4), which could be partly a result of our baseline adjustment and/or their generalist diets.

Table 3. The ANOVA results of bill morphology and wing length in *Paradoxornis webbianus*. Bill morphology of *P. webbianus* is measured with two principal components, PC1 and PC2; the former reflects bill size and the latter bill slenderness

Source	Numerator DF	Denominator DF	PC1		PC2		Wing length	
			F	P	F	P	F	P
Sex	1	149	58·73	<0·0001	0·01	0·92	35·57	<0·0001
Site	7	149	3·2	0·004	4·28	0·0002	3·66	0·001
Sex*Site	7	149	1·27	0·27	0·84	0·55	3·37	0·002
Season	1	149	0·53	0·47	4·29	0·04	0·03	0·87
Technician	4	149	1·66	0·16	5·87	0·0002	2·70	0·03
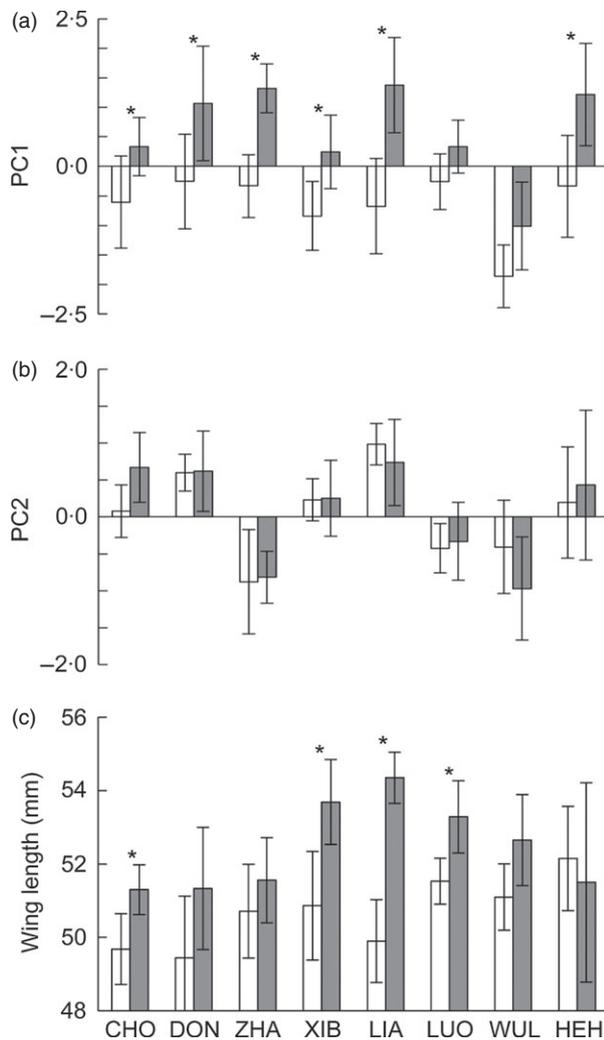
Fig. 2. The first (a) and second (b) principal component (PC1 and PC2) scores of bill morphology and wing length (c) of male (filled) and female (unfilled) *Paradoxornis webbianus* across the eight sites. PC1 scores are positively correlated with bill sizes, and PC2 scores are positively correlated with bill slenderness (Table 2). The error bars are ± 95% confidence limits. The asterisks above the bars denote significant differences between sexes in contrast comparisons ($P < 0.05$). Sites on the horizontal axis are arranged from left to right in ascending order of elevation: Chongde (CHO), Donghwa (DON), Zhaofeng (ZHA), Xibao (XIB), Lianhua (LIA), Luoshao (LUO), Wuling (WUL) and and Hehuan (HEH) (Table 1).

BILL MORPHOLOGY AND TROPHIC NICHE

Individual $\delta^{13}C_{adj}$ values of *P. webbianus* did not change with PC1 scores ($P = 0.29$), and there was no sex-specific slopes of PC1 scores ($P = 0.24$). However, sex-specific slopes of PC2 scores on $\delta^{13}C_{adj}$ values were significant ($P = 0.01$). When sexes were analysed separately, $\delta^{13}C_{adj}$ values of females decreased with increasing PC2 scores ($\beta_{female-pc2}$: $P = 0.008$; Fig. 4a) but not that of males ($\beta_{male-pc2}$: $P = 0.68$). There were no sex-specific slopes of either PC1 or PC2 scores on $\delta^{15}N_{adj}$ values (sex-specific β_{pc1}: $P = 0.88$; sex-specific β_{pc2}: $P = 0.46$). After removing sex-specific slopes from the regression model, $\delta^{15}N_{adj}$ values were found to increase with both PC scores (β_{pc1}: $P = 0.04$; β_{pc2}: $P = 0.008$; Fig. 4b).

The populations with higher variations in PC1 scores showed marginally larger standard ellipse areas ($r_s = 0.69$, $P = 0.058$, $n = 8$; Fig. 5), indicating a positive relationship between intrapopulation variation in bill size and population trophic niche width. The correlation was not driven by the one site that had an extremely large ellipse area (i.e. site HEH) because the trend persisted after this site was removed ($r_s = 0.71$, $P = 0.071$, $n = 7$). The variations in PC2 scores, on the other hand, were not correlated with median ellipse areas ($P = 0.82$, $n = 8$). The range of sampling time (i.e. the number of months between first and last sampling at a site) did not correlate with variations in PC scores nor median ellipse areas ($P > 0.3$, $n = 8$), indicating the varying lengths of sampling time across sites are not likely to produce a spurious correlation between bill-size variation and trophic niche width.

ELEVATIONAL TRENDS

The mean PC scores and isotope values did not correlate with elevations ($P > 0.4$, $n = 8$), indicating bill morphology and trophic niche of *P. webbianus* did not change systematically along elevational gradients. The variations in PC2 scores increased with elevations ($r_s = 0.74$, $P = 0.04$, $n = 8$). However, neither variations in PC1 scores nor median ellipse areas changed with elevations ($P > 0.6$, $n = 8$).

Table 4. The ANOVA results of trophic position in *Paradoxornis webbianus*. Trophic position of *P. webbianus* is measured with stable isotope values, $\delta^{13}C_{adj}$ and $\delta^{15}N_{adj}$; the former reflects dietary carbon source and the latter trophic level

Source	Numerator DF	Denominator DF	$\delta^{13}C_{adj}$		$\delta^{15}N_{adj}$	
			F	P	F	P
Sex	1	153	1·02	0·31	3·25	0·073
Site	7	153	25·35	<0·0001	48·6	<0·0001
Sex*Site	7	153	1·54	0·16	0·88	0·52
Season	1	153	0·61	0·44	0·39	0·53
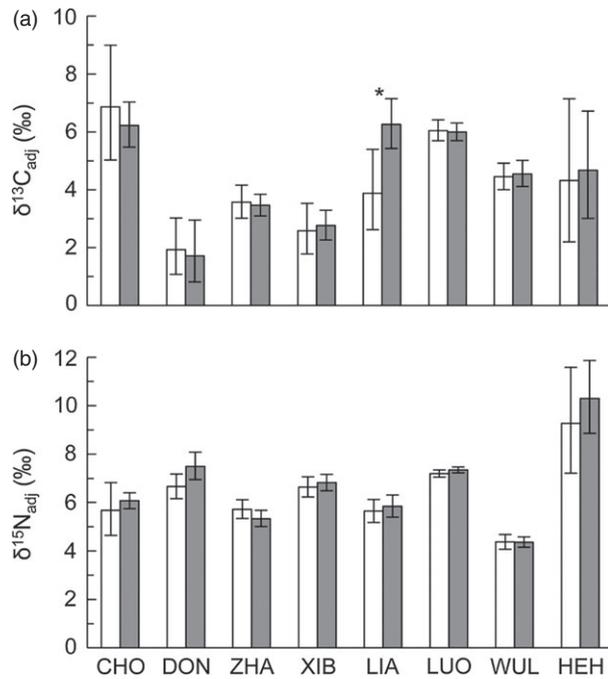
Fig. 3. The $\delta^{13}C_{adj}$ (a) and $\delta^{15}N_{adj}$ (b) values of male (filled) and female (unfilled) *Paradoxornis webbianus* across the eight sites. The $\delta^{13}C_{adj}$ and $\delta^{15}N_{adj}$ values were adjusted for site-specific baseline isotope values (i.e. mean $\delta^{13}C$ and $\delta^{15}N$ values of 10–12 plant species; Table S1, Supporting Information). The error bars are ± 95% confidence limits. The asterisks above the bars denote significant differences between sexes in contrast comparisons ($P < 0.05$). Sites on the horizontal axis are arranged from left to right in ascending order of elevation: Chongde (CHO), Donghwa (DON), Zhaofeng (ZHA), Xibao (XIB), Lianhua (LIA), Luoshao (LUO), Wuling (WUL) and Hehuan (HEH) (Table 1).

Discussion

In this study, we demonstrated that *P. webbianus* with larger and more slender bills fed at higher trophic levels, and for females, individuals with different bill slenderness varied in diets. Furthermore, *P. webbianus* populations that were more variable in bill size tended to have broader trophic niche width. These results suggest that bill morphology is an appropriate proxy for individuals' diets in this generalist passerine and provide empirical support for the niche variation hypothesis (Van Valen 1965).

We found some evidence for ecological sexual dimorphism in bill size. Male *P. webbianus* had marginally higher $\delta^{15}N_{adj}$ values than females, which in combination
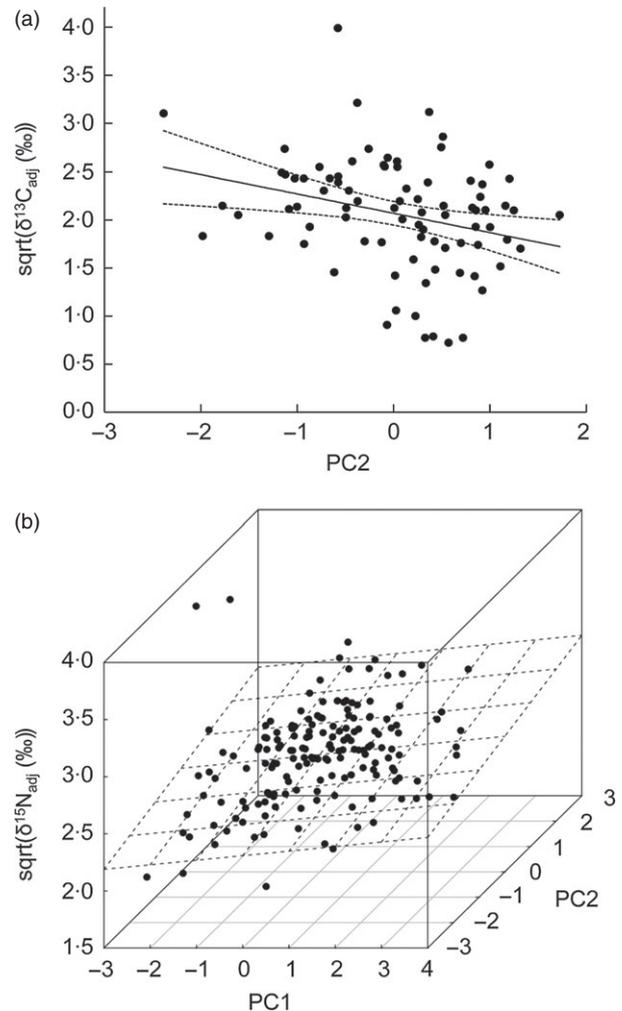
Fig. 4. The relationships between $\delta^{13}C_{adj}$ values of female *Paradoxornis webbianus* and PC2 scores (a), and between $\delta^{15}N_{adj}$ values of *P. webbianus* and both PC1 and PC2 scores (b). The $\delta^{13}C_{adj}$ and $\delta^{15}N_{adj}$ values were adjusted for site-specific baseline isotope values (i.e. mean $\delta^{13}C$ and $\delta^{15}N$ values of 10–12 plant species; Table S1, Supporting Information). Each dot denotes an individual *P. webbianus*. The regression line (solid line) with 95% confidence intervals (dashed lines) plotted in (a) is based on the model: square-root ($\delta^{13}C_{adj}$) = $2.07 \pm 0.06 - 0.20 \pm 0.07 \times$ PC2, and the regression plane plotted in (b) is based on the model: square-root ($\delta^{15}N_{adj}$) = $2.53 \pm 0.02 + 0.04 \pm 0.02 \times$ PC1 + $0.07 \pm 0.03 \times$ PC2.

with male-biased bill size and the tendency for individuals with larger bills to feed at higher trophic levels, supports ecological sexual dimorphism. Although bill size and wing
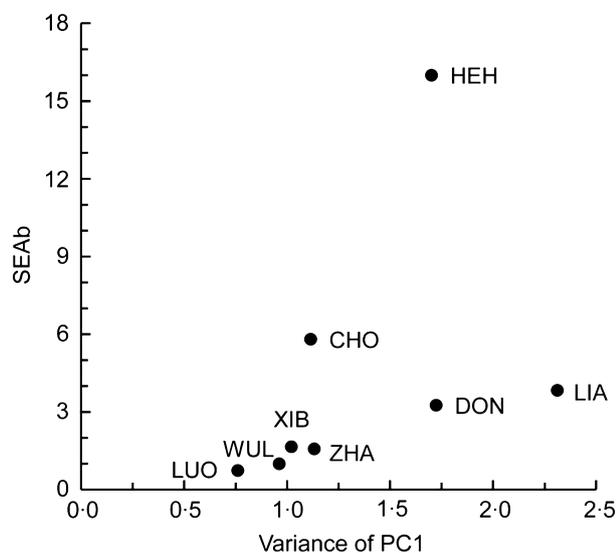
Fig. 5. The correlation between intrapopulation variance of PC1 scores and Bayesian standardized eclipse areas (SEA$_b$). The median SEA$_b$ were used. Each dot denotes a population of *Paradoxornis webbianus*. Site names (Table 1) are labelled next to the circles.

length (a proxy for body size) were positively correlated at the individual level, suggesting sexual selection for larger males could play a role in male-biased bill size, the support for sexual selection is relatively weak when we examined patterns at the population level. Among the eight populations, only three exhibited both male-biased bill size and wing length (i.e. CHO, XIB and LIA; Fig. 2a,c). Taken together, these patterns suggest that the relative influences of sexual and natural selection on *P. webbianus* morphology could vary among populations. Finally, due to the low resolution of stable isotope values, we were not able to detect fine-scale diet partitioning between sexes. For example, if males and females partition diets based on different sizes of the same foods, their isotope values would not be different.

Sexual dimorphism on average explained 33% of intra-population variation in bill size, which differed greatly from site to site (13%–64%). There are several possible explanations. First, sex ratio may be different among sites, and a skewed sex ratio is likely to increase relative contribution of sexual dimorphism to morphological variation. We speculate that the large contribution of sexual dimorphism to bill-size variation at site ZHA (64%) was related to its skewed sex ratio (males/females: 2·4, n = 24). However, to determine the effect of sex ratio, we would need a more consistent sampling design and larger sample sizes. Alternatively, sexual dimorphism may be larger for those populations facing stronger sexual and/or natural selection. For example, site LIA, where sexual dimorphism explained 59% of bill-size variation, had a sex ratio of 1 but was the only site that showed significant sex difference in $\delta^{13}C_{adj}$ values, suggesting a role of ecological sexual dimorphism in this population. Coincidentally, vegetation composition at LIA was more diverse

compared with other sites, with a well-developed successional gradient from old-field to secondary growth forest, which is more likely to provide different food resources with distinct $\delta^{13}C$ values for *P. webbianus*.

The positive relationship between $\delta^{15}N_{adj}$ values and both PC scores suggests that *P. webbianus* with larger and more slender bills fed at higher trophic levels. Furthermore, the female-only, negative relationship between $\delta^{13}C_{adj}$ values and bill slenderness suggests that females with more slender bills might not only feed at higher trophic levels but also use different carbon sources from other females. Trophic partitioning along carbon isotope values can be achieved through several different processes. For example, if these females specialize on a subset of prey that feed primarily on plants or plant parts with lower $\delta^{13}C$ values, they may simultaneously increase their $\delta^{15}N_{adj}$ values and decrease their $\delta^{13}C_{adj}$ values. Feather $\delta^{13}C$ and $\delta^{15}N$ values have been found to increase with carbon and nitrogen elemental concentrations of the diets in another omnivorous bird species (*Dendroica coronate*; Pearson *et al.* 2003). Therefore, it is also possible that these females specialize on a subset of foods with higher nitrogen concentrations and lower carbon concentrations. Even though males and females had similar bill shape and shared the same 'bill shape-nitrogen isotope' relationship, only females exhibited this additional niche partitioning along carbon isotope values, suggesting potentially stronger food competition among females than among males in *P. webbianus*.

Population niche width expansion through individual specialization has been documented under increased intra-specific competition (e.g. Smith 1990; Svanbäck & Bolnick 2005, 2007), reduced interspecific competition (e.g. Bolnick *et al.* 2010) and increased environmental variability (e.g. Grant *et al.* 1976; Darimont, Paquet & Reimchen 2008). Among our study sites, HEH is at the highest elevation with the lowest temperatures (Table 1) and potentially represents this species' range limit. Interestingly, *P. webbianus* at this site also had a larger variation in bill size (1·70, comparing to the mean ± SE = 1·34 ± 0·18 across the eight sites) and a broader trophic niche width (16·00, comparing to the mean ± SE = 4·23 ± 1·79 across the eight sites). It is likely that the shorter growing season, stronger seasonality and physiologically demanding climate at HEH provide the ecological conditions favouring niche expansion through individual specialization. In fact, bill shape variation in *P. webbianus* increased with elevation. Nevertheless, we did not find elevational trends in bill-size variation or trophic niche width. Therefore, it is not yet clear whether niche expansion through individual specialization in *P. webbianus* is more likely at higher elevations. Ding *et al.* (2005) reported that while net primary productivity decreased linearly with elevation from 1400 to 3700 m in Taiwan, bird species richness did not begin to decrease with elevation until above 2000 m. Given that our study sites were all below 2000 m except for site HEH at 2668 m (Table 1), some of the ecological

conditions such as reduced interspecific competition may only exist at site HEH.

Other processes unrelated to biological interactions could also explain the correlation between bill-size variation and trophic niche width in *P. webbianus*. First, the avian bill can have thermoregulatory functions; therefore, bill morphology might change along climate gradients (e.g. Greenberg *et al.* 2012). Although we did not find correlations between mean bill size or bill-size variation and elevation, we caution that elevation may not be a good proxy for the microclimatic conditions that could influence a species' thermoregulation. Therefore, we cannot yet rule out the possibility that bill morphology and trophic niche of *P. webbianus* are linked through some form of underlying environmental gradients. Secondly, unequal sample size across sites may produce large variations in multiple variables for sites with smaller sample sizes and small variations in the same variables for sites with larger sample sizes. While we tried to keep the sample size consistent across sites, some sites, such as HEH, were extremely difficult to sample due to their naturally small population size. However, when we compared bill-size variation and trophic niche width at site HEH calculated using a smaller sample size ($n = 11$) and a larger sample size ($n = 16$), we did not find these variables to be very sensitive to the 45% increase in sample size (bill-size variation changed from 2·00 at $n = 11$–1·70 at $n = 16$; trophic niche width changed from 17·40 at $n = 11$–16·00 at $n = 16$). In addition, population trophic niche width calculated using Bayesian ellipse area is unbiased with respect to sample size (Jackson *et al.* 2011), suggesting median ellipse areas of *P. webbianus* populations are not likely biased by different sample sizes across sites.

This study demonstrated that populations with more variable bill size tended to have wider trophic niche in a generalist passerine with a broad geographic and elevational distribution in East Asia. Evidence for ecological sexual dimorphism in bill size was found in some *P. webbianus* populations. Sexual dimorphism in bill size, regardless of its origin (i.e. sexual selection or natural selection), can significantly contribute to intrapopulation bill-size variation and potentially facilitate population niche expansion; however, its relative importance may vary among populations. Finally, in the light of recent studies showing multiple functions of feeding apparatus and the ubiquitous environmental gradients in large-scale studies, we caution that abiotic factors should be carefully considered along with biological interactions in explaining observed variations in trophic morphology and trophic niche width.

Data accessibility

Data on morphological measurements, stable isotope values, sex, band number and sampling site and date of the 170 *Paradoxornis webbianus* can be found at: DRYAD entry doi: 10.5061/dryad.40jj0.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Plant stable carbon and nitrogen isotope values ($\delta^{13}C$ and $\delta^{15}N$) at the study sites. All samples were collected between 2010 and 2011.

Table S2. The ANOVA results of sex differences in feather $\delta^{13}C$ and $\delta^{15}N$ values for *Paradoxornis webbianus*.