Sexual dimorphism in breast stripe width and beak eco-morphology in Himalayan Green-backed Tits (*Parus monticolus*)

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ABSTRACT

Quantitative field data on sexual dimorphism is scant for most bird species. In this first field study of a western Himalayan population of Green-backed Tits (*Parus monticolus*), we demonstrate that breast stripe width is a dimorphic trait that is a reliable measure to sex this species in the wild. Based on our ability to sex the birds in hand, we describe the sexual dimorphism in eco-morphological variables in our study population. Sexual dimorphism in beak morphology changed from winter to summer due to males having shorter beaks (4.5%) in summer; however, there was little change in female beak measurements. Our study on this Himalayan congener of a widely studied European species can be used to inform studies of the evolutionary ecology of morphological traits in closely-related species inhabiting vastly different habitats.

Keywords: beak length, breast stripe, Green-backed Tit, Himalayas, sexual dimorphism

1. INTRODUCTION

Divergent selection pressures on males and females drive sexual dimorphism in birds which can lead to ecological and behavioural differences among the sexes. Research on the mechanisms driving sexual dimorphism (in morphology and plumage) has been crucial in the development of significant ecological and evolutionary theory (Owens and Hartley, 1998). However, due to the lack of research, quantitative field data on sexual dimorphism remains scant for most passerines in the wild. Research on dimorphism in hitherto under-studied species can help in better understanding their ecology and the generality of the results of previous dimorphism studies. Among passerines, the Great Tit (*Parus major*, family Paridae) has been used as a model species to investigate a suite of eco-evolutionary and behavioural hypotheses (Dhondt, 2012). There is extensive research on breast stripe sexual dimorphism in Great Tits showing that breast stripes might indicate variation in male quality, social dominance and sex recognition (Järvi and Bakken, 1984; Slagsvold, 1993; Quesada and Senar, 2007; Remeš and Matysiokova, 2013) but other studies have shown this to be erroneous (Senar *et al*., 2003) or contextual (Senar *et al*., 2014). The Green-backed Tit (*Parus monticolus*) is the sister species to the Great Tit species complex (Johansson *et al*., 2013). It is a montane specialist of the Sino-Himalayan region and thus offers the unique opportunity to study trait evolution in sister taxa that inhabit distinct habitats. Like Great Tits, Green-backed Tits exhibit a characteristic dark breast stripe that runs vertically from their throat down. Hofmann *et al*. (2007) showed that Green-backed Tits are dimorphic in their plumage under UV light. Sexes are known to differ in their breast stripes, however, there is no data on size and function of this trait in the wild (Gosler and Clement, 2016).

Here, we present results from the first morphometric and molecular-based study on Green-backed Tits that quantifies intersexual differences in breast stripe width in this species. We also demonstrate seasonal variation in sexual dimorphism of eco-morphological traits in a western Himalayan population of this under-studied species.

2. METHODS

2.1 Study site, sample collection and preparation

Birds were trapped using 6–9 m mistnets at several locations within the Mandal Valley (30.44685°N; 79.27328°E; ~1550 m above sea level), Kedarnath Wildlife Division in Uttarakhand, India. Each bird was sexed based on breast stripe width and colour. In the hand, birds with black and broad breast stripes were recorded as males and birds with thin and ashy breast stripes as females. For 48 individuals caught in 2015 we measured breast stripe width at the clavicle (following Figuerola

 Paper 1700789 https://doi.org/10.3184/175815617X15063340995374

and Senar, 2000) using a Standard Wing60/Universal Flush Wing Rule. In addition, 20–40 μL of blood from the sub-brachial wing vein (never exceeding 1% of the individual's body mass) was collected and stored on FTA cards (Whatman®). Subsequently, molecular sexing of 48 birds was done blindly following Griffiths *et al.* (1998) to determine our ability to sex each bird accurately in the hand. Based on our ability to successfully sex Green-backed Tits in the hand, we quantified sexual dimorphism in eco-morphological variables. 65 males and 48 females were measured in winter (January–March 2014/2015) and 31 males and 18 females in the spring/early summer (April–May 2014/2015). We measured beak length, width and depth, and tarsus length using Fowler UltraCal Mark IV electronic calipers. Beak shape was calculated as the ratio between beak length and beak depth: higher values represent relatively more pointed beaks. Wing length (mm) was measured using a Standard Wing60/Universal Flush Wing Rule and mass using a Pesola 93010 Micro 30 g × 0.25 g spring scale. We were not able to age birds accurately in the field hence age data is not used in our analysis. All birds were ringed and released at the capture site after processing.

2.2 Statistical analysis

2.2.1 Breast stripe width

We performed a logistic regression to test whether breast stripe width is a significant predictor of sex (male or female). To test if breast stripes were relatively wider in larger males, we regressed breast stripes against tarsus length.

2.2.2 Sexual dimorphism and the eco-morphology of beaks

Based on our ability to sex birds in the hand (see Section 3), we explored sexual dimorphism in our study population using standard eco-morphological variables. We first quantified intersexual differences in tarsus length (which does not change across years) and wing length (which does not change within seasons) using ANOVA. Since beak measurements are plastic across seasons (Gosler, 1987a), intrasexual variation in beak morphometrics was compared between winter and summer. In beak measurements that *did* change across seasons, we assessed if one sex changed significantly more using an interaction term between sex and season. In each test, we controlled for body size differences by dividing the beak morphology variables by the tarsus length of the individual (Gosler, 1987a). We also compared mass (in winter and summer separately) between males and females. All birds were measured using the same equipment by the same researcher (S.B.). Recaptured birds were not used in the analysis (see S1 in ESI). All statistical analyses were done in the R programming language (R Development Core Team, 2015).

3. RESULTS

Our comparison of breast stripe width of Green-backed Tits with our results from molecular sexing confirmed that 97.9% (47/48) of the individuals were sexed correctly in the hand. Only one bird identified as a male in the hand was a female based on the molecular sexing. Breast stripe width was a significant predictor of sex (pseudo $R^2 = 0.804$, $\beta = -0.9506$, SE = 0.2883, $P = 0.0039$) (Figures 1 and 2). However, male breast stripe width was not correlated with body size of the male (tarsus length) ($r = 0.09$, $P = 0.58$).

Males were significantly larger than females in tarsus (4.2%) and in wing length (4.7%). Males were 6% heavier than females in winter, however no significant difference in mass was recorded in summer (Table 1). In winter, male beaks were 6% longer ($P < 0.01$) and 4.6% deeper ($P < 0.05$) than female beaks (Table 1). Male beaks also had a different shape being more pointed (ratio of beak length over beak depth) than females ($P < 0.001$) (Table 1). In summer, there were no significant differences in beak length and beak shape among the sexes. However male beaks were 4.3% deeper than female beaks ($P < 0.001$) (Table 1).

In females, the beak measurements were not significantly different between winter and summer. Male beaks, however, differed between seasons. Male beaks were 4.5% shorter and 20% less pointed in summer compared to winter (Table 1). The significant interaction terms for beak length ($F = 5.608$, $P = 0.019$) and beak shape ($F = 2.81$, $P = 0.001$) (Figure 3), confirm that the change in the magnitude of difference in beak morphology among the sexes across seasons was due to the altered beak morphology of the males.

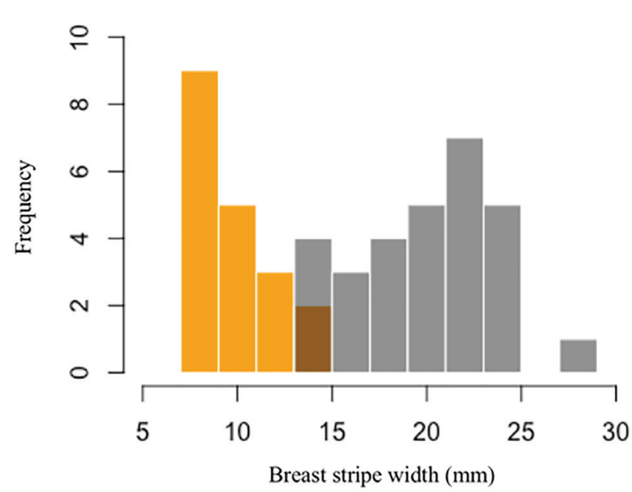

Figure 1 Frequency distribution of breast stripe width (mm) in female (orange) and male (dark grey) Green-backed Tits ($n = 48$) measured during 2015.

Figure 2 Sexual dimorphism in size and colour of Green-backed Tit breast stripe. Top panel: Males have broad and black breast stripe; Bottom panel: females have thin ashy breast stripes. Males had a significantly broader breast stripe (mean ± SD, 18.82 ± 4.06 mm) compared to females (10.66 ± 2.53 mm) ($F_{1,46}$ = 47.61, P < 0.001).

Table 1 Morphological dimorphism in Green-backed Tit (*Parus monticolus*). All measures are in mm. Asterisks indicate significant difference with * = P < 0.05, ** = P < 0.01 and *** = P < .001. Numbers in parentheses are SD. The significance of differences does not change when variables are controlled for size differences

Measure (mm)	Male (n = 96)	Female (n = 66)	Magnitude of Difference
Tarsus	17.52 (0.96)	16.8 (0.84)	$F_{1,160}$ = 4.11***, 4.2% larger
Wing	66.01 (0.8)	63.78 (0.92)	$F_{1,160}$ = 27.84***, 4.7% longer
	Winter		
	(n = 65)	(n = 48)	
Mass (g)	13.73	13.0	$F_{1,111}$ = 6.89**, 6% more
Beak Length	9.36 (0.57)	8.80 (0.29)	$F_{1,111}$ = 5.63**, 6% larger
Beak Width	4.31 (0.34)	4.40 (0.298)	$F_{1,111}$ = 0.89, P = 0.367
Beak Height	4.03 (0.21)	3.85 (0.17)	$F_{1,111}$ = 2.86*, 4.6% deeper
Beak Shape	2.32 (0.167)	2.182 (0.11)	$F_{1,111}$ = 8.83***
	Summer		
	(n = 31)	(n = 18)	
Mass	12.87 (0.81)	13.30 (0.22)	$F_{1,47}$ = 1.78, P = 0.193
Beak Length	8.94 (0.37)	8.78 (0.55)	$F_{1,47}$ = 0.58, P = 0.33
Beak Width	4.64 (0.32)	4.44 (0.40)	$F_{1,47}$ = 0.42, P = 0.67
Beak Height	4.16 (0.20)	3.99 (0.13)	$F_{1,47}$ = 7.76***, 4.3% deeper
Beak Shape	1.87 (0.12)	1.96 (0.22)	$F_{1,47}$ = 1.5, P = 0.14

4. DISCUSSION

Using morphometrics and molecular sexing, we confirm that Green-backed Tit males have a significantly broader breast stripe than females. Breast stripe width can thus be used as a reliable trait to sex this species in the wild (Figure 2). Despite being smaller in size and mass than Great Tits in Europe (Gosler, 1987a) and measured using

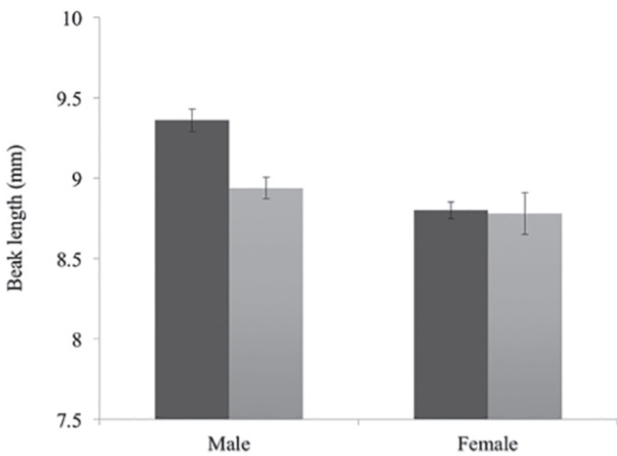

Figure 3 Change in male and female beak length (mm) across seasons in Green-backed Tits. Male beaks shortened in length significantly more than female beaks from winter (dark grey) to summer (light grey) (season*sex interaction term, $F_{1,94} = 5.608$, $P < 0.02$). Female beak length did not change across seasons. Error bars indicate standard errors.

the same technique, breast stripes in Green-backed Tits were about twice as wide as Great Tits in Europe (Järvi and Bakken, 1984; Senar *et al*., 2014). The breast stripe width of European Great Tit males is more comparable to that of females in Himalayan Green-backed Tits. While breast stripe width was not correlated with body size in males, a more detailed study of reproductive success would shed more light on whether breast stripe width is a signal of male quality in Green-backed Tits. Given the exaggerated difference in breast stripe width in two phylogenetically closely related species, Green-backed Tits represent an excellent model system to study the parallel evolution of an easily measurable trait as a signal of quality and social status.

In Green-backed Tits, tarsus length (a measure for skeletal size) and wing length is ~5% larger in males than in females. These differences were comparable to dimorphism reported in studies of Great Tits (Dhondt *et al*., 1979; Gosler, 1987a). Furthermore, beak morphology also differs between the sexes and between seasons but not in the same way as in west European Great Tits (Gosler, 1987b) (Table 1). While we also found a significant season-by-sex interaction, this came about in a different way in our study. Gosler (1987b) observed that in winter, male beaks are longer than in females and the reverse is true in summer. In our study, average female beak length did not change with season, while male beaks were on an average 4.5 % shorter in summer (Table 1, Figure 3). This reduction in male beak length in summer is attributed to the disproportionately high wear caused by males provisioning females while incubating and brooding nestlings. Because females are courtship fed by their partner their beak increases in length, thereby reversing the size difference between the sexes (Matthysen *et al*., 1991). In Green-back Tits, female beaks did not increase in length from winter to summer suggesting that they might forage for food relatively more during incubation and brooding than European Great Tits. Beak dimensions in Great Tits change across seasons based on primary food resources consumed (Dhondt, 2012), where longer beaks predict a more insectivorous diet (Gosler, 1987b). We show that male Green-backed Tits had longer beaks in winter suggesting that their diets might be more insectivorous than the females.

The ecology of Himalayan birds is poorly understood and few studies have gone beyond studying the biogeography of species. Several Himalayan parids are closely related to well-studied species in Europe. This literature on European congeners can be used to inform studies of the evolutionary ecology of morphological traits in closely-related Himalayan species inhabiting vastly different habitats.

5. ACKNOWLEDGEMENTS

We thank the Wildlife Institute of India, the Uttarakhand State Forest Department, Viral Joshi, Shailee Shah, Manon Munoz, Vijay Ramesh, Harish Maithani and many volunteers and technicians who helped during fieldwork and in the lab. This research was supported by funds from the Athena Fund at the Cornell Lab of Ornithology, the Explorer's Club and by the Wellcome Trust/DBT India Alliance. All applicable institutional permits were obtained and national guidelines for the care and use of animals were followed.

6. ELECTRONIC SUPPLEMENTARY INFORMATION

The ESI is available through: http://ingentaconnect.com/content/stl/abr/2017/00000010/00000004.

Published online: 17 October 2017

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