

Sex bias in a wintering population of Dunlin *Calidris alpina* in central Taiwan

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The sexes of many migratory shorebird species differ in timing and distance of their migrations, but this phenomenon has been little studied for birds in the East Asian–Australasian Flyway (EAAF). Using molecular sexing techniques (but also taking morphometric data), we compared the sex ratio of Dunlin *Calidris alpina* sampled on the west coast of Taiwan between September 2007 and May 2008. We found an overall sex bias (59.2%) toward females, including juveniles. A discriminant function deduced from three morphological measurements correctly identified 89.0% of Dunlin, but performed better in identifying males than females. Since Taiwan is at the southern end of the Dunlin's East Asian winter range, our results support the 'arrival-time' hypothesis whereby the sex that arrives earlier on the breeding grounds winters closer to them. To maintain stable populations, conservation must take account of this differential use of wintering grounds by the two sexes.

INTRODUCTION

In many migratory bird species, individuals of different sex or age differ in their routes and timing of annual migration (Cristol *et al.* 1999). Several hypotheses have been proposed to explain these differential migrations, focusing on differences in territorial behaviour, body size and behavioural dominance. The arrival time hypothesis proposes that certain individuals winter closer to the breeding grounds because competition for mates or territory favours early arrival to the breeding grounds (see Morbey & Ydenberg 2001). The body size hypothesis proposes that the larger-bodied sex can winter at higher latitudes due to larger fuel reserves and better thermal efficiency (see Catry *et al.* 2005). The dominance hypothesis suggests that the dominant sex will out-compete the less dominant one for resources, forcing the less dominant sex to migrate longer distances or winter in inferior habitats (see Marra 2000). Testing these hypotheses is difficult because they often are not mutually exclusive. An early test of these hypotheses by Myers (1981), using data from several shorebird species, found that the sex that arrived earlier at the breeding grounds was the sex that wintered at higher latitudes. He proposed that, regardless of size and behavioural dominance, difference in arrival time alone is sufficient to predict a latitudinal cline in wintering shorebirds.

Dunlin *Calidris alpina* is a long-distance migratory shorebird, breeding along the Arctic rim in Europe, Iceland, Scandinavia, Siberia, North America and Greenland (Piersma *et al.* 1996) and wintering in temperate and tropical regions throughout the Northern Hemisphere (Wenink & Baker 1996). Studies on wintering Dunlin in Hong Kong have found two distinct mtDNA haplotypes, Siberian and Alaskan (Wenink & Baker 1996). Significant differences exist in culmen length and mtDNA haplotypes among Dunlin of different breeding populations (Wenink & Baker 1996, Wenink *et al.* 1996). Dunlin ringed in Taiwan have been recaptured on breeding grounds in both Alaska and Siberia, and in Taiwan we have re-sighted Dunlin which were ringed in Alaska and Sakhalin Island (Lanctot *et al.* 2009, Fernández *et al.* 2010, CC pers. obs.). During the non-breeding season, significant numbers migrate from breeding grounds to winter in eastern China, Japan, Korea, Hong Kong and Taiwan (Bamford *et al.* 2008). Around 950,000 Dunlin use this flyway every year, making the East Asian–Australasian Flyway (EAAF) one of the five major flyways for this species (Bamford *et al.* 2008).

Female Dunlin are larger than males, but it is the males that arrive earlier at their breeding grounds and defend territories each spring (Holmes 1966). According to the arrival time hypothesis,

males should winter nearer the breeding grounds than females, whereas the dominance and body size hypotheses predict the opposite.

The sex of wintering Dunlin is difficult to identify in the field, even in the hand (Prater *et al.* 1977). In many studies, sex is assigned by difference in bill length (e.g. Chao *et al.* 1991, Warnock *et al.* 1997, Shepherd *et al.* 2001, Shepherd & Lank 2004). However, bill length overlaps to some extent among the sexes (Brennan *et al.* 1984, Shepherd *et al.* 2001, Meissner 2005) and geographically distinct groups of Dunlin also vary in bill length (Wenink & Baker 1996). Instead, by amplifying fragments of sex-specific DNA sequences, the sex of all individuals of monomorphic bird species like Dunlin can be accurately determined (e.g. Baker & Piersma 1999, Fridolfsson & Ellegren 1999).

Differential migration of Dunlin has been reported in North America (Shepherd *et al.* 2001), but their migration patterns have not been explored in the EAAF. In this study, we present data on the sex ratio of Dunlin wintering in central Taiwan, one of their southernmost wintering areas (Wei *et al.* 2009). In addition, using the results of molecular sexing, we derive a discriminant function and examine its accuracy in distinguishing the sex of Dunlin in Taiwan.

METHODS

Field methods

Our study area is Han Pao wetland, Changhua County, Taiwan (23.95°N 120.36°E). Each year, more than 10,000 Dunlin migrate through or winter here (Hu 2005). This area is one of the largest intertidal mudflats in Taiwan (c.38 km²). It has a long history of shellfish and oyster farming, and shorebirds often rest and feed in these habitats (Hu 2005).

We mist-netted Dunlin from September 2007 to May 2008, three to four times every month, at night from evening to high tide. Most ringing sites were on agricultural lands with some shallow water. We defined birds netted from September to November 2007 as the autumn population, December 2007 to February 2008 as the winter population, and March to May 2008 as the spring population. We ringed and weighed each Dunlin captured and, for most, measured (to the nearest mm): maximum wing length, tarsus length, total head length and bill length. We then extracted 20–40 µl of blood by venipuncture from the brachial vein and placed the blood in SET buffer (0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH = 8.0). We aged the Dunlin based on the presence of either buffy-edged inner median coverts (first-years) or white-edged inner median coverts (after first year) (Choi *et al.* 2010). We could

identify birds as first-years before February, but coverts became worn and buffy fringes disappeared from March.

Molecular sexing

We extracted DNA from blood using standard methods (Gemmell & Akiyama 1996). Fragments of the 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 reaction volume was 20 μ l and contained c.10 ng DNA, 0.8 μ l dNTP (2.5 mM of each nucleotide), 0.4 μ l 2550F primer (10 mM), 0.4 μ l 2718R primer (10 mM), 2.0 μ l 10 \times PCR buffer, 15.92 μ l dH₂O and 0.4 U μ l Taq DNA polymerase (Protech). The PCR included 3 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 40 seconds at 46°C and 50 seconds at 72°C, and a final extension reaction at 72°C for 5 minutes. The PCR products were electrophoresed in 1% agarose gel, using a Bio-100™ DNA Ladder (Protech) as size reference. We then dyed the gels in ethidium bromide and examined them under ultra-violet light. We identified the sex of birds by the presence or absence of the female-specific DNA fragment of the CHD-W gene.

Statistical analysis

We used a chi-square goodness-of-fit test to determine if the sex ratio significantly deviated from 1:1 for both adult and juvenile Dunlin. We also compared the difference of sex ratios within each season with the same test. Mean bill length, total head length, maximum wing length and tarsus length were compared between sexes using the Student's *t*-test. We used three measurements (maximum wing length, tarsus length and bill length) to derive a discriminant function using SPSS 14.0 to assign sex.

RESULTS

From September 2007 to May 2008, we caught and sexed 748 Dunlin in the Han Pao wetland (Figure 1). Excluding recaptured individuals, 693 birds were ringed (Table 1). The sex ratio was significantly different from 1:1 ($\chi^2 = 23.27$, $P < 0.001$): 410 individuals were female (59.2%) and 283 individuals were male (40.8%). We identified 138 Dunlin as juvenile (<20%), but failed to age some individuals because the buffy-edged inner median

Table 1. Number of Dunlin ringed from September 2007 to May 2008 at Han Pao Wetland, Taiwan.

	Females	Males	Total
Number of birds ringed	410	283	693
Adult	316	239	555
First-year young	94	44	138

Table 2. Measurements (mean \pm SE, with ranges below in parentheses) of Dunlins captured from September 2007 to May 2008 at Han Pao Wetland, Taiwan.

Measurement	Females <i>N</i> =325	Males <i>N</i> =240	<i>t</i> -test (two-tailed)
Weight (g)	57.92 \pm 5.79(41.70–79.90)	53.36 \pm 5.91(33.6–75.30)	$t_{563} = 9.15$, $p < 0.001$
Bill (mm)	37.61 \pm 1.81 (31.81–37.61)	33.69 \pm 1.58 (28.89–38.26)	$t_{563} = 26.83$, $p < 0.001$
Head (mm)	62.33 \pm 1.93 (55.97–68.38)	58.09 \pm 1.81 (50.57–63.45)	$t_{563} = 26.50$, $p < 0.001$
Tarsus (mm)	26.95 \pm 1.06 (23.80–31.33)	25.87 \pm 1.09 (21.14–29.00)	$t_{563} = 11.75$, $p < 0.001$
Wing (mm)	127.09 \pm 2.77 (119.00–134.00)	123.89 \pm 3.14 (114.00–134.00)	$t_{563} = 12.81$, $p < 0.001$

coverts were unclear after midwinter. Among juveniles, 94 were female (68.1%) and 44 were male (31.9%), significantly female-biased ($\chi^2 = 18.1$, $P < 0.001$). Combining data from all captures, the sex ratio did not significantly deviate from 1:1 in autumn (73 females, 61 males, $\chi^2 = 1.07$, $P = 0.3$). However, in winter and spring, the sex ratio was significantly female-biased (winter: 238 females, 163 males, $\chi^2 = 14.03$, $P < 0.001$, spring: 137 females, 76 males, $\chi^2 = 17.47$, $P < 0.001$ respectively).

Of Dunlin that were both sexed molecularly and measured ($n = 565$ individuals), females were significantly larger than males. However, these measurements showed overlap between the sexes (Table 2). For discriminant analysis, we excluded total head length in the analysis because it was not independent from bill length and both were highly correlated (Pearson's correlation coefficient $r = 0.92$, $P < 0.001$).

The best discriminant function included bill length (BL), tarsus length (TL) and wing length (WL). The resulting function was:

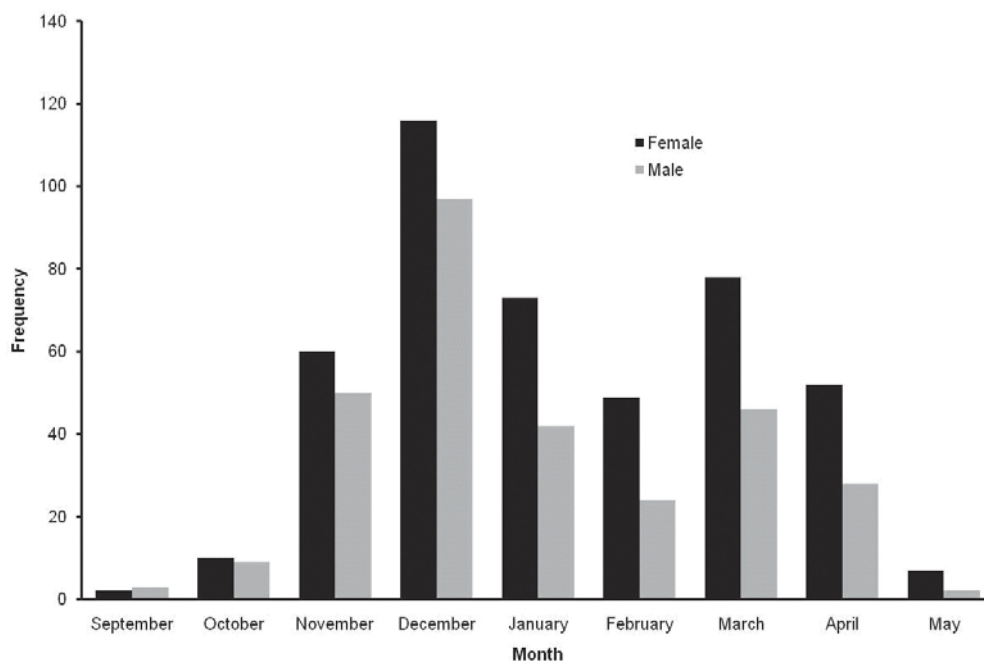


Figure 1. Number of female and male Dunlin captured from September 2007 to May 2008 at Han Pao Wetland, Taiwan.

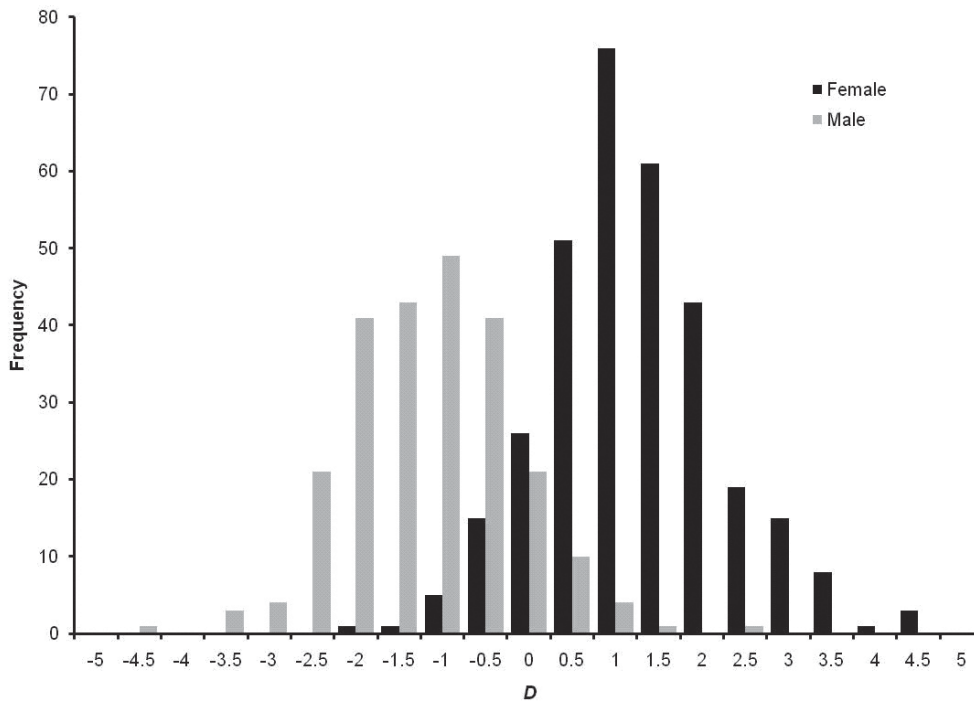


Figure 2. Distribution of the D values of the discriminant function in male and female Dunlin captured from September 2007 to May 2008 at Han Pao Wetland, Taiwan.

$D = 0.515 \times BL + 0.05 \times TL + 0.105 \times WL - 33.018$ (Wilks' lambda = 0.413).

The value of $D < 0$ was identified as male and $D > 0$ was identified as female. This function correctly identified the sex of 89.03% of Dunlin, with 92.9% of males and 86.2% of females respectively (Figure 2). This function performed better in identifying males than in females ($\chi^2 = 6.46, P = 0.011$).

DISCUSSION

The wintering sites of Dunlin in East Asia range from the eastern coast of Liaoning province, China (45°N), Japan, South Korea to Hong Kong (22°N). Few Dunlin are found wintering farther south than Hong Kong (Bamford *et al.* 2008). As our study site (Han Pao wetland) is located at 23.95°N, we assume it is one of the southernmost wintering sites. Numbers of Dunlin reached their peak from December to February, indicating that most Dunlin used the Han Pao wetland as a wintering ground, instead of as a stopover site.

Within the EAAF, at least three species of shorebirds show sex-biased migration: Curlew Sandpiper *Calidris ferruginea* males winter farther south; and Eastern Curlew *Numenius madagascariensis* and Bar-tailed Godwit *Limosa lapponica* males winter farther north (Nebel 2007). We found that the overall sex ratio of Dunlin in central Taiwan is significantly female-biased. Both the body size hypothesis and the dominance hypothesis predict that females, which are larger and therefore presumably more dominant than males, would winter at northern sites. Our data thus support the arrival time hypothesis, which states that the sex that arrives later at the breeding grounds will winter farther south (Morbey & Ydenberg 2001). The arrival time hypothesis also predicts more male Dunlin in the northern wintering grounds, such as Japan, Korea or northern China. With further studies from these regions, this hypothesis can be further tested.

In North America, Shepherd *et al.* (2001) found male-biased populations of Dunlin at higher latitude wintering areas and suggested that there is a latitudinal cline in the sex ratio of wintering Dunlin populations. Our data are consistent with this. Another possibility for the female-biased sex ratio in our study site comes from local differences in habitat preference between sexes. Shepherd & Lank (2004) reported that male Dunlin preferred agricultural

land and females preferred mudflats on wintering grounds in British Columbia. All Dunlin we sampled were caught on agricultural land, but we found more females, suggesting that the findings of Shepherd & Lank (2004) are not generally applicable. Our sampling, however, was conducted during high tides when all Dunlin came into agricultural lands, thus minimising such habitat-based sex bias.

We used the results of molecular sexing to develop a discriminant function to identify Dunlin sex by morphological measurements. For sex-size dimorphic species, discriminant function analysis of morphometric data is a common technique to sex birds that are monomorphic in plumage (Brennan *et al.* 1984, Iko *et al.* 2004). Previous discriminant function and maximum likelihood function analyses have correctly identified the sex of over 90% of non-breeding Dunlin (Brennan *et al.* 1984, Shepherd *et al.* 2001, Meissner 2005), but were all conducted on a single subspecies. In Taiwan, wintering Dunlin come from several geographically distinct subspecies (Lanctot *et al.* 2009), which differ in body size (Wenink & Baker 1996, Bamford *et al.* 2008). The mix of subspecies in our study site may result in the lower discriminant power of the function, or might be a result of differing morphological measurements among ringers in our study (Barrett *et al.* 1989).

CONCLUSION

We found a significant female-biased wintering Dunlin population in central Taiwan. This is the first study to reveal a differential sex ratio in this species in the EAAF. East Asia has one of the highest human population densities in the world and the fastest population and economic growth, especially in coastal areas (Liu 2009). Shorebird habitats are therefore being rapidly altered (Ge *et al.* 2007). The loss of Dunlin habitats used heavily by either sex could result in disproportionate population declines (Steiffetten & Dale 2006). Identification of sites that are preferred by members of one sex is crucial for conservation (Nebel 2007). Our data alone are not sufficient to test hypotheses for the differential migration of Dunlin in this region. More long-term data from different latitudes in the EAAF, such as eastern China, Korea, Japan and other parts of Taiwan, will help the understanding of the migration ecology of this species.

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