Investigations of sexual dimorphism in live Kittlitz’s Plover Charadrius pecuarius from inland South Africa

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Charadrius plovers generally show little sexual or seasonal dimorphism in size and coloration, but most published measurements come from museum specimens. We aimed to determine sexual size dimorphism in live Kittlitz’s Plovers Charadrius pecuarius, based on measurements of 96 males and 112 females ringed at Barberspan Bird Sanctuary (North West province, South Africa) between February 2008 and October 2009 and sexed by DNA analysis. The females were significantly heavier than the males in September–October, but their mass decreased significantly from September to the end of March (β = –0.10, t = 3.82, P = 0.0002), likely because of egg laying. Body mass has limited utility for sexing, because heavy birds with high fat scores of both sexes, possibly itinerants, occurred in all months. Tarsus-and-toe length differed between sexes (P = 0.966). Wing length of birds with old primaries decreased in September–October (ANCOVA, F1,113 = 8.84, P = 0.003), but did not differ between the sexes (ANCOVA, F1,113 = 0.23, P = 0.626). Wing length for birds with fresh feathers, total head length, bill length, tarsus length and height of the white forehead patch did not differ between sexes. We attribute this lack of any clear sexual dimorphism to the species’ monogamous mating system and shared parental care, and to its simple terrestrial displays, which would likely result in weak intersexual selection.

Introduction

Few plovers of the family Charadriidae show sexual dimorphism in size or coloration in contrast with other families of waders. Small Charadrius plovers in particular show only small sexual or seasonal dimorphism in plumage (Urban 1986, Piersma et al. 1996). The sexes in some species differ slightly in their head or breast patterns, with males usually having more contrasting colours than females, but these differences are often noticeable only in the breeding plumage within a pair (Prater et al. 1977, Hayman et al. 1987, Turpie and Tree 2005). Because of these difficulties with visual sexing, few sources provide measurements for males and females of any Charadrius plovers, and those come mostly from museum specimens (Prater et al. 1977, Cramp and Simmons 1983). However, DNA techniques of bird sexing using small birds’ blood or feather samples (Griffith et al. 1998, Fridolfsson and Ellergren 1999) now allow researchers to determine the extent of any sexual size dimorphism based on measurements collected from live individuals in the field and to relate any difference to the behavioural patterns of certain geographic populations.

The Kittlitz’s Plover Charadrius pecuarius is a common species in all sub-Saharan Africa, avoiding only dry, mountainous and forested regions (Urban 1986, Turpie and Tree 2005). Populations from most of the range are largely migratory or nomadic. Their movement patterns are usually irregular and dependent on rainfall, but populations from the east of South Africa, Swaziland and southern Mozambique are considered sedentary. Thus in many areas local residents temporarily mix with migratory or nomadic populations (Tree 1997, Underhill et al. 1999, Tree 2001, Turpie and Tree 2005, Dodman and Parker 2009). Sexes differ slightly in the coloration of the head and the breast, and some individuals, supposedly adult males, do not go into non-breeding dress, but sexing by these features is extremely difficult (Tree 1974, Prater et al. 1977, Hayman et al. 1987). Birds start to attain breeding plumage between March and June, when they are about 21 months old (Tree 1974, Turpie and Tree 2005). Evidence of the Kittlitz’s Plover’s possible sexual size dimorphism is limited. Measurements of male and female Kittlitz’s Plover of the sedentary Madagascan population, which have been considered a separate subspecies (Hayman et al. 1987), have been presented (Zefania et al. 2010). However, for the partially migratory east and southern African populations, only the wing length is provided for males and females, based on a small sample of museums specimens. These measurements do not differ significantly. To date no other sex-related morphological measurements have been provided (Prater et al. 1977, Cramp and Simmons 1983). Several sources (Urban 1986, Tree 1974, Turpie and Tree 2005) present mean values of other measurements based on larger but unsexed samples.

This paper aims to determine any dimorphism in size and in the head patterns of adult male and female Kittlitz’s Plover based on measurements taken in the field from birds of the South African inland population.
Materials and methods

The data analysed in this study comes from Kittlitz’s Plovers that we ringed at Barberspan Bird Sanctuary in the North West province, South Africa (26°34’ S, 25°36’ E) between February 2008 and October 2009. Waders were caught using mist nets and walk-in traps at five locations up to 8 km apart in the reserve on expeditions from 24 February to 3 March 2008, 18–21 December 2008, 27–28 March 2009, followed by daily ringing from 4 September to 29 October 2009. Data from all the ringing sites at Barberspan and from both years were combined in our analyses.

We measured the Kittlitz’s Plovers’ total head length, bill length and tarsus length using callipers accurate to 0.1 mm, and the wing length and tarsus-and-toe length using a stopped ruler accurate to 1 mm (Meissner 2008). Before measuring the wing length we checked if the longest, outermost, primary was old (worn), new (freshly moulted) or growing; if still growing we did not take this measurement. We assessed the amount of subcutaneous fat below two apertia: the interclavicular depression (furcular depot) and the axillary region (apteria lateralis) according to the fat score scale proposed by Meissner (2009). Additionally, on 58 of these Kittlitz’s Plovers we measured the height of the frontal white patch (which we call the diadem height) from the base of the bill to the lower edge of the black frontal stripe above the white patch with callipers accurate to 0.1 mm. The measurements were taken by all three authors, using the same techniques that were cross-checked among themselves to ensure that results were identical. We were unable to take this full set of measurements from every bird, so sample sizes are not equal in different analyses. We used only the measurements taken at the first capture of each individual to avoid bias by recaptured birds. From Kittlitz’s Plovers aged as adults and in full breeding plumage we collected samples of 50–100 μl blood stored in 95% ethanol for later DNA analysis. Genomic DNA was extracted from the blood samples using a Blood Mini Kit (A&A Biotechnologie, Gdynia, Poland). The specific amplification of the chromo-helicase-DNA binding (CHD) gene was performed with the 2550F/2718R primer pair (Fridolfsson and Ellegren 1999). The PCR reaction was conducted with 2 μl DNA template in 15 μl volumes in the REDTaqReadyMix PCR Reaction Mix with 1.5 mM MgCl₂ (Sigma-Aldrich). The reaction profile was an initial denaturing step of 94 °C for 2 min, followed by 40 cycles at 50 °C for 30 s, 94 °C for 30 s, 72 °C for 2 min, with a final elongation at 72 °C for 5 min. PCR products were separated on 3% agarose gels and visualised in ultraviolet light by ethidium bromide staining. The samples that produced two bands on the gel, representing the CHD-W and CHD-Z alleles, were sexed as females, and those that produced one band (the CHD-Z allele) were sexed as males (Fridolfsson and Ellegren 1999).

We successfully sexed 204 Kittlitz’s Plovers (97.7% of the collected samples) by this method. Five females were identified by the presence of an egg in the cloaca or by a large cloacal protuberance; for one female this method was confirmed by DNA sexing. Thus we finally analysed the measurements of 208 birds (96 males and 112 females). Most of these birds (79%) were trapped between 4 September and 29 October 2009, so some analyses were conducted using only this sample. In the remaining calculations data from all the catching periods were combined and we set the beginning of the breeding season at 1 September, following the literature suggesting a May–July break in Kittlitz’s Plover breeding at Barberspan (Tarboton 1987) and our observations of the first nesting attempts in September 2009. We analysed the wing length separately for two groups of birds: individuals with old worn primaries and birds with freshly moulted primaries. Because the wing length and the body mass might change during the season, for these traits one-way analysis of covariance (ANCOVA) was performed with age as the factor and the number of the pentade (five-day period) in the year, according to the standard pentade scheme by Berthold (1973), as a covariate.

To provide a measure of the sexual dimorphism, the Storer’s index (DI; Storer 1968) was calculated according to the formula DI = 100(f – m)/(0.5(f + m)), where f and m are the mean values of the analysed measurement in females and males, respectively. Any negative value of this index indicates that the females’ measurements were greater than those of the males. Statistical analyses were conducted using STATISTICA 9.0 software (StatSoft, Tulsa).

Results

For birds with old primaries the wing length decreased in subsequent pentades between 4 September and 29 October 2009 (ANCOVA; F₁,153 = 8.84, P = 0.003). In this period the mean wing length did not differ significantly between the sexes (ANCOVA; F₁,153 = 0.23, P = 0.626). The wing length (WL; both sexes combined) decreased by 0.17 mm per pentade, according to the linear regression equation: WL = 110.64 − 0.17*PENTADE (F₁,153 = 6.95, P = 0.009). This means that during these two months (12 pentades) the birds’ wings become on average 2 mm shorter.

Body mass might vary over the season, so for this analysis we used only data from birds caught between 4 September and 29 October 2009. In this period the body mass differed significantly between the sexes (ANCOVA; F₁,162 = 9.32, P = 0.003), but it did not differ in the subsequent pentades (ANCOVA; F₁,162 = 0.002, P = 0.959). In September–October 2009 females were on average heavier than males and this trait showed the highest degree of sexual dimorphism (Table 1). However, the body mass varied greatly within this period both in males and females (Figure 1). Females showed a significant decrease in body mass between the beginning of September and the end of March, calculated from the data from 2008 and 2009 combined (regression coefficient b = –0.10, t = 3.82, P = 0.0002). Two females with an egg in the cloaca were caught in October 2009 (Figure 2). In males the decreasing trend was not significant (regression coefficient b = –0.50, t = 1.75, P = 0.083). The fat score and the body mass were significantly correlated both in males (Spearman rank coefficient rₛ = 0.44, t = 4.42, P < 0.0001) and in females (rₛ = 0.39, t = 3.96, P = 0.0001) (Figure 2).

Of the other measurements only the tarsus-and-toe length differed significantly between the sexes (P = 0.066), the males’ being longer (Table 1). The wing length of males and females with new primaries did not differ significantly. Apart from the body mass, the index of sex dimorphism showed...
Table 1: Measurements for adult male and female Kittlitz’s Plover caught between September and March in 2008–2009 at Barberspan Bird Sanctuary in North West province, South Africa. Mean and range (in parentheses), standard deviation (SD) and sample size (N), and results of comparisons between the sexes by the t-test or Cochran-Cox test, and the Storer’s dimorphism index (DI), are provided.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Males</th>
<th>Females</th>
<th>t-test or Cochran-Cox test (t')</th>
<th>Storer’s dimorphism index (DI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total head length (mm)</td>
<td>Mean (range) = 41.26 (39.3–43.3)</td>
<td>Mean (range) = 41.08 (38.3–43.0)</td>
<td>t = 1.49, P = 0.137</td>
<td>0.44</td>
</tr>
<tr>
<td>Bill length (mm)</td>
<td>Mean = 16.39 (14.6–18.1)</td>
<td>Mean = 16.25 (13.9–18.2)</td>
<td>t = 1.13, P = 0.261</td>
<td>0.86</td>
</tr>
<tr>
<td>Tarsus length (mm)</td>
<td>Mean = 31.82 (29.0–34.3)</td>
<td>Mean = 31.59 (28.1–34.9)</td>
<td>t = 1.36, P = 0.177</td>
<td>0.73</td>
</tr>
<tr>
<td>Tarsus-and-toe length (mm)</td>
<td>Mean = 52.2 (48.0–56.0)</td>
<td>Mean = 51.8 (47.0–56.0)</td>
<td>t = 1.85, P = 0.066</td>
<td>0.77</td>
</tr>
<tr>
<td>Diadem height (mm)</td>
<td>Mean = 5.79 (4.9–7.4)</td>
<td>Mean = 5.93 (3.1–8.1)</td>
<td>t = 0.51, P = 0.612</td>
<td>−2.39</td>
</tr>
<tr>
<td>Wing length (fresh primaries) (mm)</td>
<td>Mean = 108.1 (104–112)</td>
<td>Mean = 107.2 (100–112)</td>
<td>t = 0.75, P = 0.464</td>
<td>0.84</td>
</tr>
<tr>
<td>Body mass (3 Sep–1 Nov) (g)</td>
<td>Mean = 35.1 (27–48)</td>
<td>Mean = 37.0 (30–51)</td>
<td>t = 3.06, P = 0.003</td>
<td>−5.27</td>
</tr>
</tbody>
</table>

Figure 1: Body mass of males (black circles; n = 112) and females (white circles; n = 97) of adult Kittlitz’s Plover caught in subsequent pentades between September and March in 2008–2009 at Barberspan Bird Sanctuary in North West province, South Africa. The line shows the significant regression between the body mass and the number of the pentade for females. The middle dates of the pentades are shown.

Discussion

The samples analysed in this study come mostly from the resident population of Kittlitz’s Plover breeding at Barberspan. This is suggested by the fact that 25% of the individuals examined in this study were recaptured in the reserve, some multiple times over 2006–2010, and the presence of females laying eggs among the birds examined in October. In 2008–2010 we observed Kittlitz’s Plovers nesting in all months between September and March, with most broods between September and January (Lipshutz et al. 2011 and authors’ unpublished data). Tree (1973) stated that Kittlitz’s Plovers caught during the breeding season that weigh more than 41 g should almost certainly be taken as female, which is not confirmed in our study (Figure 2). Waders that permanently reside in tropical regions might deposit fat before breeding and in preparation for the dry season, when food might be scarce (McNeil 1971). Some individuals in our study accumulated substantial fat stores, which could be used as a buffer against any temporary decrease in food availability (Witter and Cuthill 1993, Tree 2001).
periods of heavy rain and cold. Irregular influxes of Kittlitz’s Plovers occur at Barberspan, mostly in the winter months between April and July (Milstein 1975, Lipshutz et al. 2011), but in some years also in November and December (Farkas 1962, Tree 2001). Tree (2001) suggests that Barberspan Bird Sanctuary lies on the migration route of birds moving both southwards and northwards, in relation to the summer rains or the drying of smaller wetlands afterwards. Thus we cannot exclude the possibility that some of the Kittlitz’s Plovers we examined were itinerants (Lipshutz et al. 2011). Kittlitz’s Plovers from the migratory populations store fat reserves of up to 30–40% of their lean mass (Tree 2001). The wide variation in the amount of accumulated fat therefore reduces the suitability of body mass as a metric for sexing Kittlitz’s Plovers. Furthermore, even within each fat score the ranges of male and female body mass are practically the same (Figure 2). The only exception were females with a fat score of 0 and a high body mass, which were about to lay eggs (Figure 2). Other measurements showed negligible, statistically non-significant differences between males and females that are probably also not biologically relevant. This corresponds well with the lack of sexual size dimorphism in wing length, tarsus length and body mass found in Kittlitz’s Plovers of the Madagascan population (Zefania et al. 2010). Though our results from testing the differences between the means of tarsus-and-toe length suggested that this trait might be useful in sex identification, with the tarsus-and-toe being longer in males, the dimorphism index for this measurement was low. The difference in the tarsus-and-toe length could be an effect of the different sexes’ roles in the scraping ceremony during courtship. Usually the male prepares a scrape for a nest, placing his breast on the ground and kicking sand backwards. The female might join him, but usually only fits herself in the scrape and makes the final choice of the nest site (Urban 1986, Turpie and Tree 2005). In Kittlitz’s Plover, as in many Charadrius plovers, sexes differ slightly in their head and breast pattern (Cramp and Simmons 1983, del Hoyo et al. 1996). Thus we expected that the size of the diadem might also differ between the sexes, as in the Ringed Plover Charadrius hiaticula breeding in Central Europe, where males’ diadems are slightly larger than those of females (P Chylarecki pers. comm.). However, the height of the diadem in the Kittlitz’s Plover was the most variable measurement of all, with standard deviations of 13% and 20% of mean values for males and females, respectively. This might reflect difficulties in taking this measurement because of the flexible edges of the feather line at the base of bill and the border between the white diadem and the black frontal stripe at the crown. Wear of these feathers might also have influenced the results we obtained. The wing length decreased significantly during the season, most likely because of the wear of the longest primary, hence this measurement should be used with caution. The decrease of 2 mm over two months (about 2% of mean wing length) that we determined is large when compared with data on feather wear in long-distance migrants (Pienkowski and Minton 1973, Branson and Minton 1976, Summers et al. 1987). Serra et al. (2001) showed that in one species, the Grey Plover Pluvialis squatarola, populations migrating over longer distances produced more durable primary feathers than the shorter-distance migrants. Thus sedentary Kittlitz’s Plovers might produce primaries of lower quality that are less resistant to abrasion than those produced by migrants. Kittlitz’s Plover might also start breeding when less than a year old, with prenuptial moult of only the contour feathers (Turpie and Tree 2005). The first, juvenile, set of primaries is usually of worse quality than those of adults (Ginn and Melville 1983). The presence of first-year birds, whose primaries wear faster than those of older birds, in our sample of Kittlitz’s Plovers in breeding plumage might explain the apparent rapid shortening of wing length we noted.

Though our results showed that the sexing of Kittlitz’s Plover using external measurements or the body mass is practically impossible, observers have reported that slight differences in coloration combined with the birds’ behaviour has allowed them to identify and follow the sexes within a breeding pair (Clark 1982, Turpie and Tree 2005). So size differences could also occur within a breeding pair, but only in a disassortative mating system, which is uncommon among waders (Jehl 1970, Jónsson 1987). To our best knowledge no detailed study on the mating system of this species has yet been published.

In waders sexual dimorphism in size is the effect of sexual selection that would affect the males’ mating success and the females’ fecundity. Factors considered to be crucial for the evolution of sexual size dimorphism in this group are: (1) the mating system, with polygyny in favour of larger males and polyandry in favour of larger females, and (2) the agility of the male’s displays, with acrobatic aerial displays favouring smaller males with more rounded wings than males that perform simple displays on the ground (Figueurola 1999, Székely et al. 2000, 2004, 2006). The Kittlitz’s Plover is socially monogamous and territorial. Displays and territorial behaviour take place mostly on the ground and no aerial displays have been observed (Cramp and Simmons 1983, Urban 1986, Turpie and Tree 2005). Male and female Kittlitz’s Plover also share parental care throughout incubation and chick rearing (Tree 1974, Clark 1982, Urban 1986). Thus rather weak intersexual selection would be expected in this species, which might account for the lack of differences between the sexes in the measurements that we analysed.

Kittlitz’s Plover shows clinal geographical variation in size with equatorial populations generally smaller than the populations in higher latitudes, including southern Africa (Tree 1974, Cramp and Simmons 1983, Hayman et al. 1997). The longest documented movement in the eastern and southern African population of this species was only 332 km (Underhill et al. 1999, Parker and Dodman 2009). Therefore our results probably apply mainly to the population of Kittlitz’s Plover that inhabits the north-western part of South Africa and adjacent areas.

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