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EVALUATING THE USE OF MORPHOMETRIC MEASUREMENTS FROM MUSEUM SPECIMENS FOR SEX DETERMINATION IN MOUNTAIN PLOVERS (*CHARADRIUS MONTANUS*)

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ABSTRACT.—The Mountain Plover (*Charadrius montanus*) is a shorebird species endemic to the dry, terrestrial ecosystems of the Great Plains and southwestern United States. Breeding Bird Survey data suggest that Mountain Plover populations have declined by >60% in the last 30 years. A better understanding of the population dynamics of the Mountain Plover is important in determining future management goals for this species. However, this effort is hampered by the inability to determine the sex of Mountain Plovers accurately under field conditions. In an effort to develop a simple method for sexing plovers in the hand, we measured external morphometric characteristics from 190 museum specimens of adult Mountain Plovers in alternate (breeding) plumage. Logistic regression and discriminant function analyses were performed on 10 external morphometric measurements (lengths of unflattened wing chord, 10th primary, central rectrix, outer rectrix, total head length, exposed culmen, culmen, bill depth, bill width, and tarsus). The results of these analyses indicated that Mountain Plover sexes were similar for all measures except culmen length. However, further analysis determined that culmen length accurately predicted sex in less than two-thirds of the specimens, suggesting that this measure is a poor predictor of sex in Mountain Plovers. Structurally, Mountain Plovers appear to be nearly identical between the sexes, and other methods of sexing birds (e.g., plumage characteristics, behavioral observations, or molecular markers) should be further assessed for devising a simple method for sexing Mountain Plovers under field conditions.

Key words: Mountain Plover, Charadrius montanus, *sex determination, morphometric measurements, museum specimens.*

The Mountain Plover (*Charadrius montanus*) is a shorebird species endemic to the dry, terrestrial ecosystems of the Great Plains and the southwestern United States (Knopf 1996). A species of the shortgrass prairie ecosystem, plovers use open, relatively flat, arid environments and prefer intensively grazed grassland habitats (Knopf and Miller 1994, Knopf 1996, Knopf and Rupert 1999). During the last 3 decades, breeding populations of the Mountain Plover have become increasingly isolated as its native shortgrass prairie habitat has been converted to agriculture and urban development (Samson and Knopf 1996, Knopf and Rupert 1999). Recent analyses of North American Breeding Bird Survey data indicate that Mountain Plovers have declined by 63% in the last 30 years (U.S. Department of the Interior 2002). Population declines of the Mountain Plover have made it a species of concern throughout its current range and proposed for listing as a threatened species under the U.S. Endangered Species Act (U.S. Department of the Interior 2002).

Despite concern over its declining populations, little is known about the population dynamics of this species, particularly its mating system (Graul 1973a, Jehl and Murray 1986). Earlier studies describing breeding behavior of Mountain Plovers indicated that this species is monogamous, with the female laying an initial clutch for the male to incubate and then a 2nd clutch to incubate herself (Graul 1973a, Graul 1976, McCaffery et al. 1984). However, sex determination in these past studies has relied on direct observations of courtship behavior and copulations (Graul 1973a), egg laying by females (Graul 1973a, SJD personal observation), or internal examination of collected plovers (FLK unpublished data). Compilation of detailed population data, such as sex ratios of breeding populations and differential mortality or migration among the sexes, has been hampered by the inability to determine the sex

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of breeding Mountain Plovers accurately in the field. As an initial step in addressing this issue, we evaluated the use of external morphometric measures from museum specimens to develop a simple technique for determining the sex of Mountain Plovers in the field.

METHODS

The goal of this study was to develop a simple mathematical equation for sexing Mountain Plovers in the hand using linear morphometric measurements. However, because of the difficulty in capturing and collecting such measurements from live Mountain Plovers (WMI personal observation), we chose first to evaluate a morphometric sexing criteria using museum specimens. After an extensive search of museum collections, we located 611 Mountain Plover specimens, which we believe represent nearly all of the Mountain Plover specimens in North America (see Acknowledgments). We chose to limit our analysis to specimens in adult breeding (alternate) plumage where sex was identified by the specimen preparator, because of our uncertainty of age-related differences in soft tissue measurements and the potential misidentification of sex by museum preparators due to atrophy of the gonadal tissues during the nonbreeding season. For the purpose of this analysis, we have assumed that the sex indicated on the museum labels of the remaining adult breeding plumage specimens is correct. To reduce observer bias, only one researcher (WMI) collected morphometric measurements. Because the condition of each specimen varied, a complete set of measurements could not always be obtained. All efforts were made by the researcher to make the same morphometric measurement at the same anatomical location on each study specimen used. When such measures could not be replicated on a given study specimen, or a complete set of measures could not be obtained, the morphometric measurements from that specimen were eliminated from our statistical analyses.

After eliminating winter (basic) plumaged birds, specimens whose museum labels indicated "juvenile" or "sex unknown," and specimens with an incomplete set of measurements, we had a total sample of 190 known-sex plover specimens (males, $n = 112$; females, $n = 78$) in alternate plumage for inclusion in our analysis. The measurements we collected were the lengths (to nearest 0.1 mm) of unflattened wing chord; outer or 10th primary; central rectrix; outer rectrix; total head (from the posterior end of the occipital crest of the skull to the anterior end of the upper mandible); exposed culmen (from the base of the upper mandible at the beginning of the feather tracts to the anterior end of the upper mandible); culmen (from the anterior end of the nares to the anterior end of the upper mandible); bill depth (measured at the anterior end of the gonys); bill width (measured at the anterior end of the gonys); and tarsus (from the intertarsal joint to the distal end of the last leg scale before the toe emerges). We chose these external measures because of their common use among bird banders and because differences between the sexes would most likely be evident in these features (Prater et al. 1977, Hayman et al. 1986, Pyle 1997). Body mass recorded on museum labels was not used because too few specimens had this information (17 of 190 specimens).

To develop a simple mathematical equation to predict the sex of Mountain Plovers, we first tested for sex differences using a univariate approach with a logistic regression model using the logit link function in the PROC GENMOD procedure in SAS (SAS Institute 1990). Sex was the dependent variable in our regression models and followed a binomial distribution. The importance of each measurement for explaining sex was tested using a chi-square test with 1 degree of freedom (df). For morphometric variables showing significant differences between sexes, we used the intercept and regression coefficient to obtain a logistic regression equation to predict the sex of our museum specimen data set. For these analyses we used $\alpha = 0.05$ as the level of statistical significance. We also used a multivariate approach with a stepwise discriminant function analysis to predict sex in Mountain Plovers in accordance with other morphometric studies (Brennan et al. 1984, Johnstone and Niven 1989). Using the same 10 variables listed above, we attempted to classify plovers as male or female using the Fisher discriminant function analysis in PROC DISCRIM in SAS (SAS Institute 1990). A stepwise discriminant function analysis was used to identify which subset of the 10 variables was most useful for discriminating between male and female plovers. We then used the Fisher discriminant functions for all 10 variables from DISCRIM

TABLE 1. Univariate measurements ($\bar{x} \pm s$) between female ($n = 78$) and male ($n = 112$) adult Mountain Plover museum specimens in breeding plumage.

Variable	Females (mm)	Male (mm)	χ^2	$P > \chi^2$
Unflattened wing chord	147.2 ± 4.1	147.5 ± 4.4	0.1	0.69
10th primary length	103.3 ± 4.6	102.4 ± 8.2	0.66	0.42
Central rectrix length	61.2 ± 3.5	61.6 ± 4.1	0.41	0.52
Outer rectrix length	59.5 ± 4.1	59.6 ± 3.9	0.05	0.82
Total head length	53.9 ± 1.7	53.3 ± 3.7	1.41	0.24
Exposed culmen length	21.4 ± 1.1	21.1 ± 1.1	2.58	0.11
Culmen length	13.9 ± 0.9	13.6 ± 0.9	4.70	0.03
Bill depth	4.4 ± 0.3	4.4 ± 0.3	0.72	0.40
Bill width	4.3 ± 0.3	4.3 ± 0.4	0.02	0.88
Tarsus length	39.8 ± 1.4	39.6 ± 1.5	1.09	0.30

to generate predictive equations of group (male or female) affiliation. For these analyses we used $\alpha = 0.15$ as the probability of entry into, and of remaining in, the model in PROC DISCRIM and $\alpha = 0.05$ as the level of statistical significance for all other tests.

RESULTS

We first regressed each of the 10 variables separately with sex as the response variable. Of the 10 measures we analyzed, only culmen length ($\chi^2 = 4.70$, df = 1, $P = 0.03$) showed a significant difference between the sexes (Table 1). None of the remaining measurements differed by sex in Mountain Plovers. We further tested our ability to predict the sex of all birds in our sample using the regression equation with only culmen length. Using the intercept and regression coefficient obtained when we regressed culmen on sex, we calculated a predicted sex for each of the 190 birds (Table 2). We predicted sex using the regression equation:

$$\text{Xbeta} = 5.4353 - 0.3691 * \text{Culmen}$$

Xbeta values were then mapped into the logit-link function of:

$$\text{Sex} = \frac{1}{1 + e^{-Xbeta}}$$

where if Sex > 0.5, the bird was classified as a male, and if Sex < 0.5, the bird was classified as a female. Although we found significant differences in culmen length between sexes, this measure alone was a poor predictor of the sex of our Mountain Plover specimens (Table 2). Using this procedure, we were able to correctly classify 62% of our birds ($n = 117$), with most correctly classified specimens being male (91%). Most of the misclassifications were of females that were classified as males (81%).

The discriminant function analysis produced similar results using Fisher discriminant functions for predicting sex and a model using all 10 variables (Table 3). Of the 10 morphometric variables, the Fisher discriminant function analysis found only culmen length ($F_{1,188}$, $P = 0.03$) as a significant predictor of sex. Similarly, in the STEPDISC discriminant function analysis, only culmen met the criteria for retention in the model. We used these equations to classify each plover as male or female, as above (Table 2). Discriminant function analysis was able to correctly classify only 63% of our birds ($n = 119$), with a pattern of misclassification similar to that found with the logistic regression results (74% misclassification for females and 12% misclassification for males).

DISCUSSION

The results of our study indicate that for 9 of 10 morphometric measurements we collected, Mountain Plover body sizes were similar between the sexes, suggesting that they are a monomorphic species. Culmen length, as in other studies (Skeel 1982, Brennan et al. 1984, Jehl and Murray 1986, Sandercock 1998), was the most useful of the 10 measures, but only successfully identified the sex of 62%–63% of our museum specimens. Mean values for culmen length between male and female specimens, although significantly different, were slight, potentially negating the usefulness of

TABLE 2. Comparison of known to predicted sex of Mountain Plover specimens in breeding plumage based on culmen length (n = 190). The 1st value is percentage estimate from the logistic regression (LR) model and the 2nd value is the percentage estimate from the discriminant function (DF) analysis.

	Predicted sex				
	Female		Male		Total
Known sex	LR (n)	DF (n)	LR (n)	DF (n)	n
Female	19% (15)	36% (20)	81% (63)	74% (58)	78
Male	9% (10)	12% (13)	91% (102)	88% (99)	112
Total	12% (25)	17% (33)	87% (165)	83% (157)	190[a]

[a]Overall number of correct classifications (logistic regression: 117 of 190 or 62%; discriminant function analysis: 119 of 190 or 63%).

TABLE 3. Fisher discriminant function analysis for predicting sex of Mountain Plover specimens in breeding plumage based on a 10-variable model (n = 190). The model includes measures of unflattened wing chord, 10th primary, central rectrix, outer rectrix, total head length, exposed culmen, culmen, bill depth, bill width, and tarsus length.

Variable	Female	Male
Constant	–958.52	–953.34
Unflattened wing chord	7.57	7.63
10th primary length	–1.18	–1.22
Central rectrix length	–0.26	–0.22
Outer rectrix length	0.16	0.15
Total head length	2.26	2.23
Exposed culmen length	7.43	7.37
Culmen length	3.60	3.32
Bill depth	15.16	14.64
Bill width	11.10	11.28
Tarsus length	12.16	12.10

this single measure alone in predicting the sex of Mountain Plovers. The results of our logistic regression analysis suggest that the value of culmen length as a predictor of sex in Mountain Plovers is questionable. Our discriminant function analysis yielded results similar to those found using logistic regression, further strengthening claims that the Mountain Plover is a monomorphic species.

The use of museum specimens in sex discrimination can be helpful, especially when studying avian species that are in decline or are restricted in their handling or collection. However, the use of linear morphometric measurements solely from museum specimens for field application warrants some caution. Linear morphometric measurements from museum specimens can vary due to feather wear, museum preparation and storage, and potential shrinkage (Johnston 1990, Winker 1993). However, incorporation of other field measures from live-caught Mountain Plovers, such as body mass, may lead to a more useful sex determination criterion. Johnstone and Niven (1989) demonstrated in their field study on Grey-faced Petrels (*Pterodroma macroptera gouldi*) that a discriminant function classification formula based on bill depth alone yielded a predictability rate of 63% and 65% for males and females, respectively; however, by incorporating body mass into this formula, accuracy rose to 93% and 91% for males and females, respectively. Unfortunately, few of the museum specimens used in our analyses had reliable body mass data recorded on museum labels (only 17 of the 190 specimens). Also, body mass measures from live birds should be used with caution as mass can fluctuate due to several variables, including reproductive status (such as presence of eggs in the oviducts of females), migratory condition (such as fat deposition), and general physiological condition of the bird. However, the incorporation of body mass measures in this study will have to depend on future field studies involving live-capture and measurement of Mountain Plovers.

Other sex determination criteria, such as field plumage characteristics, should also be considered for sexing Mountain Plovers. Field studies on this species have indicated that plovers suspected of being males tend to have a brighter alternate plumage, including a more distinct head pattern and brighter rufous or orange color on the neck and mantle (Graul 1973b, Jehl and Murray 1986, SJD personal observation). If such observational data of dichromatism in this species could develop standardized measurements of plumage coloration, a potential field methodology for sexing Mountain Plovers could be established. Other sexing criteria, such as genetic markers (Kahn

et al. 1998, Dinsmore et al. 2002) obtained from blood or feather samples, may prove even more effective as these techniques are improved and become more widely available.

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