Determining the Sex of Reed Parrotbills *Paradoxornis heudei* from Biometrics and Variations in the Estimated Sex Ratio, Chongming Dongtan Nature Reserve, China

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Anthony Boulord, Xiao-Ting Yang, Tian-Hou Wang, Xiao-Ming Wang, and Frédéric Jiguet (2011) Determining the sex of Reed Parrotbills *Paradoxornis heudei* from biometrics and variations in the estimated sex ratio, Chongming Dongtan Nature Reserve, China. *Zoological Studies* **50**(5): 560-565. Differences in gender morphology of the near-threatened Reed Parrotbill *Paradoxornis heudei* were analyzed to obtain equations discriminating individuals according to sex. Results were used to define sex ratios during the breeding period in both harvested and unharvested reedbeds. We caught 281 individuals and sexed them from biological criteria and by subsequent observations of display behavior. A discriminant analysis was performed on biometrics from 100 adults for which the sex was confirmed by a genetic analysis, and then the predictive accuracy was tested with the remaining individuals. The sex ratio of birds captured and its changes during the nesting period were investigated in both harvested and unharvested reedbeds. All tested parameters significantly differed between sexes in adult Reed Parrotbills. Bill measurements displayed the greatest sexual dimorphism. A discriminant function using bill height and culmen length was derived which was found to correctly identify the gender of 100% of tested individuals. This function will be useful for rapidly sexing individuals in the field. The species evidenced a balanced sex ratio in both harvested and unharvested areas. This study provides supplementary evidence that the Reed Parrotbill shares the same social system as the Vinous-throated Parrotbill *P. webbianus* and shows that harvesting activities do not seem to influence the sex ratio during the nesting period.
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The Reed Parrotbill *Paradoxornis heudei* inhabits reedbeds of lower regions of the Yangtze River, the Yellow Sea coast, the northeastern part of China, extreme eastern Mongolia, and extreme southeastern Russia (Zheng 2002). Its populations are presumably declining because of habitat loss and degradation, and the species is classified as near threatened by extinction by the International Union for the Conservation of Nature (BirdLife International 2008). Although the species forms winter flocks, it is considered seasonally monogamous (Ma 1988, Xiong and Lu 2008) with biparental care (unpubl. data) and weak territoriality (unpubl. data); biological and ecological basics are however still poorly documented. Among parrotbill species, the ecology of the Vinous-throated Parrotbill *P. webbianus* is better known. That species has a balanced sex ratio which plays a role in determining its social behavior (Lee et al. 2009a). We believe that the Reed Parrotbill shares the same social behavior and thus expect to find the existence of the same balanced sex ratio in adults in our studies.

The Reed Parrotbill exhibits no evident sexual

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dimorphism in ornaments or plumage chromatism, and no formal method exists to determine the sex of in-hand individuals based on the phenotype. In species with no evident plumage dimorphism, discriminant analyses based on biometric features are widely used to detect sexual dimorphism in size and thus determine the sex of individuals (Genovart et al. 2003, Svagelj and Quintana 2007, Alarcos et al. 2007).

The aims of this paper were 1) to search for the existence of a consistent pattern of sexual size dimorphism in the Reed Parrotbill and test the obtained discriminant function's ability to easily and correctly determine the sex of in-hand individuals based on gender-related morphological features, 2) to estimate the adult sex ratio during the breeding season, and 3) to check for an effect of harvesting activities on the breeding sex ratios, as Boulord et al. (2010) showed that nest and individual densities are significantly lower in harvested reedbeds than unharvested reedbeds and that the sex ratio can be correlated to habitat quality (Fretwell and Calver 1970, Zanette 2001, Johnson et al. 2006).

MATERIALS AND METHODS

Fieldwork

Fieldwork was carried out in reedbeds of the Chongming Dongtan National Nature Reserve (121°50'-122°05'E, 31°25'-31°38'N), a complex of coastal wetland ecosystems listed as a Ramsar site of international importance and situated on Chongming I., Shanghai Municipality, China.

Reed Parrotbills were captured with mist nets during the breeding season, from late Apr. to early Sept. 2009 (21 wk) in unharvested reedbeds (121 adults, 232 capture-recaptures) and from early May to late Aug. 2010 (16 wk) in both harvested (61 adults, 101 captures-recaptures) and unharvested (99 adults, 192 capture-recaptures) reedbeds. Individuals were banded with a numbered metal ring provided by the National Bird Banding Center of China and also 3 colored rings to allow distant individual recognition. Five body measurements were obtained from each captured bird: wing length (flattened wing chord), culmen length (from the bill tip to the feathers), bill height (maximum height), tarsus length (from the intertarsal joint to the distal end of the tarso-metatarsus), and body mass. For bill and tarsus lengths, we used a digital vernier caliper (to the nearest 0.1 mm). Wing length was measured with a ruler (to the nearest 0.5 mm), and

birds were weighed with a 0.5 g precision Pesola scale (©Pesola AG, Switwerland). Given that body mass can vary throughout the year and during the day (Brothers 1985, Croxall 1995), we excluded this latter measurement from the analysis, but report the average values we found.

Sex determination

The sex was first determined in the field by checking for the presence of a cloacal protuberance and further confirmed by intensive observations of breeding behavior (detecting color-ringed singing males). Both sexes develop brood patches, and we thus excluded this characteristic from the sex determination. Sex was also confirmed by a molecular analysis of the 1st 100 individuals caught in 2010. Blood samples (10 μl) were obtained by brachial venipuncture and stored at room temperature in a solution of 30% TE-buffer/70% absolute ethanol. DNA was extracted using the commercial DNeasy® Blood and Tissue Kit from QIAGEN (QIAGEN China (*Shanghai*) Co Ltd CA, USA). We amplified DNA sequences corresponding to the chromo-helicase-DNA-binding protein (CHD) by a polymerase chain reaction (PCR) using the CHD1F/CHD1R primer pair (see Lee et al. 2010 for a description). PCR fragments were separated by gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide and viewed under ultraviolet light. The CHD gene is carried by avian sex-determining chromosomes. Females carry both a W and Z chromosome, whereas males carry 2 copies of the Z chromosome (Griffiths et al. 1998). Thus, 2 stained bands on a gel sample indicated blood from a female, while 1 band indicated that from a male.

Sex discrimination analysis

Significant differences between males and females were tested for each variable using a two-sample Student's *t*-test. The sexual size dimorphism (SSD) index was calculated for each variable as: SSD = ((male mean/female mean) - 1) × 100 (from Weidinger and Franeker 1998). For all variables, we also calculated the coefficient of within-sex variation (CV = standard deviation/mean) to estimate the degree of variability in each measurement (Fletcher and Hamer 2003).

We used a multivariate analysis of variance (MANOVA) with Pillai's trace to test for the discriminatory power of multivariate combinations

of biometrics of the 100 genetically sexed individuals to discriminate sex. Pillai's trace is preferred as it is the most robust statistical test used in MANOVA (Olson 1976). We then ran a linear discriminant analysis (LDA) based on all biometrics used together or combined in twos on the 100 genetically sexed individuals as Pillai's traces were significant at $p < 0.001$ for all combinations. The effectiveness of the LDA was first assessed in terms of the proportion of correctly classified individuals (as male or female) after performing leave-one-out cross-validation. We finally applied the function to the 181 remaining individuals to check the proportion of individuals correctly classified in that sample.

Sex ratio

We tested for deviance in the sex ratio (male number/adult number) from a 1: 1 ratio using a binomial test for the 3 datasets (unharvested reeds 2009, unharvested reeds 2010, and harvested reeds 2010). Weekly sex ratios based on capture-recapture data were tested with a Chi-squared test to detect variations in the sex ratio during the nesting period. Weekly capture numbers which did not reach 5% of the total number of captures were grouped together, as that is a prerequisite to perform this test (Cochran 1952).

All statistical analyses were performed with R software vers. 2.10.1 (R Development Core Team 2009).

RESULTS

Sex discrimination

Though ranges of all biometrics, except bill height, slightly overlapped between the sexes, all measurements of males were significantly larger than those of females (Table 1). Culmen length and body weight exhibited the highest sexual size dimorphism and within-sex variations, whereas wing and tarsus lengths were the least dimorphic features for both inter-sex and within-sex classes. Based on these tests, bill height (which exhibited high sexual size dimorphism and low within-sex variation) was the most discriminant measurement (Table 1).

The LDA was applied to the 4 biometric measurements. Only the univariate function modeled with bill height successfully classified all individuals using both cross-validations and the test dataset (Table 2). All bivariate functions that included bill height with a 2nd biometric measurement combined in twos also provided results with 100% successful classification of gender. Due to the absence of overlapping, univariate function is simpler than the bivariate one and provided the same accuracy in our results. However, we provide a bivariate function which is more appropriate for bill height overlapping situations. The equation of the function using culmen length and bill height combined in twos is:

$$D = 3.525 \times \text{Bill height} - 0.015 \times \text{Culmen length} - 39.212.$$

Table 1. Male and female biometric measurements (mean ± standard deviation (range)), coefficients of sexual size dimorphism (SSD), and coefficients of variation (CVs) of captured Reed Parrotbills. All Student's *t*-tests were significant at $p < 0.001$

Biometric	Males (n = 124)	Females (n = 157)	Student's *t*-test	SSD (%)	CV (%)
Wing length (mm)	64.8 ± 1.7 (61.0 - 69.0)	60.5 ± 1.6 (57.0 - 67.5)	t_{279} = - 22.01	7	2.6
Culmen length (mm)	16.0 ± 0.7 (14.3 - 18.3)	13.3 ± 0.6 (10.9 - 15.2)	t_{279} = -32.20	20	4.4
Bill height (mm)	12.5 ± 0.4 (11.4 - 13.4)	10.2 ± 0.3 (9.3 - 11.1)	t_{278} = -56.12	23	3.1
Tarsus length (mm)	25.6 ± 0.7 (23.2 - 27.3)	24.0 ± 0.8 (22.3 - 25.6)	t_{277} = -17.02	7	3.0
Weight (g)	24.5 ± 1.4 (20.0 - 28.0)	19.5 ± 1.5 (17.0 - 26.0)	t_{267} = -27.33	26	6.7

Using the discriminant function, D, to class individuals, a negative value indicates a female and a positive value indicates a male (Fig. 1).
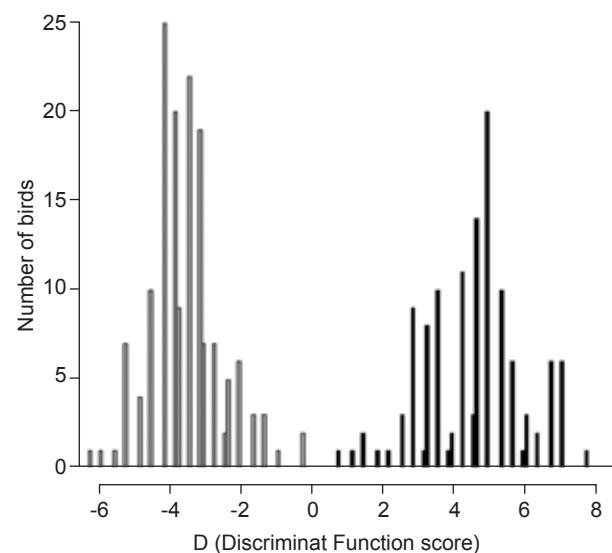
Sex ratio

After removing recaptured individuals from the capture data, we captured fewer males than females during the 2 yr and in the 2 habitat types studied, but the sex ratio did not significantly deviate from 1: 1 (binomial test; 2009: 0.44, p = 0.20; 2010 harvested reeds: 0.48, p = 0.80; 2010 unharvested reeds: 0.40, p = 0.06). No significant variation in the sex ratio was observed during the nesting period during the 2 yr or across the 2 habitat types (2009: χ^2_9 = 4.94, p = 0.84; 2010 harvested reedbeds χ^2_5 = 0.60, p = 0.99; 2010 unharvested reedbeds: χ^2_6 = 3.47, p = 0.75).

DISCUSSION

Sex determination

In many passerines, males are larger than females (Nilsson 1992, Borras et al. 1998, Lezana et al. 2000, Gutiérrez-Corchero et al. 2002). This pattern is also found in the Reed Parrotbill as demonstrated by significant sex differences in bill height, and wing, culmen, and tarsus lengths that we recorded in our study. Bill height proved the most useful measure to determine the sex of in-hand individuals in the field, as no overlap between sexes was observed in our dataset. However, the difference between the highest female bill and lowest male bill was only 0.3 mm, and in a larger dataset, we would expect some bill height overlap to occur. We thus proposed using the discriminant function, D, presented above which incorporates culmen length, the 2nd most accurate characteristic, as a complementary measure. With 100% of individuals correctly classified using

Fig. 1. Projection of individual scores obtained using the discriminant function, D, defined by the combination of bill height and culmen length in twos. Females (gray color) have negative score values, whereas males (black color) have positive ones.

Table 2. Accuracy of the discriminant functions tested (percentage of correctly classified individuals). Functions were created using 100 genetically sexed individuals and tested with a different dataset of 181 individuals

Model	Leave-one-out cross-validation		Validation dataset	
	Male (n = 47)	Female (n = 53)	Male (n = 77)	Female (n = 104)
WL	94	94	65	99
CL	98	91	99	99
BH	100	100	100	100
TL	94	74	92	67
WL, CL	98	98	94	99
WL, BH	100	100	100	100
WL, TL	94	94	78	98
CL, BH	100	100	100	100
CL, TL	100	94	99	98
BH, TL	100	100	100	100
WL, CL, BH, TL	100	100	100	100

WL, wing length; CL, culmen length; BH, bill height; TL, tarsus length.

this methodology, our results showed that the use of biometric features to sex Reed Parrotbills, especially during the winter when males have no cloacal protuberance, is an excellent substitute to molecular sexing, is cheaper and faster, and evidenced a high degree of accuracy. In the literature, we found no proposed method to sex the 19 *Paradoxornis* species listed by the IUCN (BirdLife International 2008). Based upon ecological similarities between Reed Parrotbills and the better-known Vinous-throated Parrotbill, it is quite likely that other species of parrotbills display a similar sexual dimorphism in size, especially in bill size. We thus strongly promote this discriminant analysis be applied to biometric features in these species.

Sex ratio

The Vinous-throated Parrotbill has unusual social behavior in which individuals live in flocks composed of relatives during the winter. Pair formation occurs within winter flocks (Kim et al. 1992, Kim 1998). All individuals breed independently in monogamous pairs, and broods are raised by biparental efforts with no helpers. However, individuals maintain kin associations during the nesting period as relatives breed in close proximity and exhibit only weak territoriality (Severinghaus 1987 in Lee et al. 2009a, Kim 1998, Lee et al. 2009b). Before the nesting period, flocks exhibit a balanced sex ratio which should reduce individual movements to stabilize social structure. Thus, group members have little chance of losing breeding opportunities due to a lack of potential mates (Lee et al. 2009a). Our results revealed that a similar balanced sex ratio during the entire nesting period also occurs in the Reed Parrotbill. This constitutes a circumstantial indication that the species exhibits a similar social structure to that of the Vinous-throated Parrotbill. Therefore, the well-known species, the Vinous-throated Parrotbill, provides an excellent example and basis for comparative behavioral research on the ecology and social structure of the threatened Reed Parrotbill.

For conservation purposes, a crucial point is to define if reed harvesting activities, which were recognized as unsuitable for the species (Xiong et al. 2007, Boulord et al. 2010), lead to adverse modifications of the social structure or reproductive behavior/success of Reed Parrotbills. Indeed, genetic relatedness within groups in the nesting environment can influence the reproductive success of individuals and/or groups (Double et al. 2005, Fowler 2005, Lee et al. 2009b). Our results give the beginning of an answer by showing that a balanced sex ratio, which possibly plays an important role in the maintenance of the Reed Parrotbill social structure, was not affected by reed harvesting during the breeding period.

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