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Sexual size dimorphism, assortative mating and sex identification in the Whiskered Tern *Chlidonias hybrida*



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I examined sexual size dimorphism (SSD) and assortative mating and estimated a discriminant function for predicting sex in the Whiskered Tern *Chlidonias hybrida*, a medium-sized marsh tern. I measured and molecularly sexed 241 breeding adults captured in southern Poland from 2005 to 2010. Males were significantly larger than females in all six measurements: total head length, bill length, bill depth at gonys, tarsus length, wing length and body mass. The largest dimorphism index (DI) was found in head and bill measurements and body mass. Within pairs, the total head length, bill length and bill depth were positively correlated. Two discriminant functions, one based on the length and depth of the bill and the second including only total head length, showed the same high sex classification success of 95%. DI in Whiskered Terns was greater than in other terns probably due to stronger sex specialization in foraging niche.

Key words: assortative mating, *Chlidonias*, discriminant function, DNA analysis, sex identification, sexual size dimorphism, Whiskered Tern

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In birds, one of the most abundant differences between the sexes is body size (Owens & Hartley 1998). The range and variation of sexual size dimorphism (SSD) are linked to ecological characteristics as mating systems, frequency of extra-pair paternity, sex differences in parental investment and sex-specific niche utilization (Andersson 1994, Dunn et al. 2001, Lindenfors et al. 2003, Blanckenhorn 2005, Székely et al. 2007). SSD may be due to sexual selection, where larger males are usually favoured in competition for females (Andersson 1994). SSD may also be a result of natural selection, with male and female adaptations to different ecological factors (Selander 1966). For example, body size differences may arise when males and females occupy different foraging niches (González-Solis et al. 2000, Temeles et al. 2000, Phillips et al. 2004). As a first step in understanding why the sexes differ in size, information on SSD and assortative mating is needed (Dunn et al. 2001, Blanckenhorn 2005, Székely et al. 2007).

Terns are monogamous, long-lived, colonial birds, with no obvious sexual dimorphism in plumage, but

slight sexual size dimorphism is common (Gochfeld & Burger 1996). Discrimination between sexes based on external measurements is usually not very precise due to overlap in body dimensions. The Whiskered Tern Chlidonias hybrida is a medium-sized, socially monogamous marsh tern, which exhibits no differences in plumage between sexes, but shows some degree of SSD (Gochfeld & Burger 1996). Whiskered Terns breed across large areas of the Palearctic, Africa and Australia. Three to six subspecies have been described (Mees 1977, Gochfeld & Burger 1996). Intersexual differences in measurements have been reported in non-breeding birds from Australia (Dostine & Morton 1989), and in the nominate subspecies from Europe and North Africa (Cramp & Simons 1985). Sample sizes in these studies were small including no more than 20 individuals, lacking some common body measurements, and measurements were done on skins. These studies did not report on within-pair size dimorphism, assortative mating or sex determination by discriminant analysis. Here, I present data from a large sample describing sexual size dimorphism in the nominate subspecies of the



Figure 1. Frequency distributions of morphometric measurements and body mass of male and female Whiskered Terns nesting in southern Poland in 2005–2010.

Table 1. Mean, range and sample size of morphometrics of molecularly sexed Whiskered Terns caught during the breeding seasons of 2005–2010 in southern Poland. *F*-scores and *P*-values refer to results of ANOVA's testing male–female differences. The dimorphism index (DI) was calculated as log(mean male/mean female).

	Male			Female			Differ	Difference male-female		
Measurement	Mean ± SD	Range	n	Mean \pm SD	Range	n	F	Р	DI	
Head length (mm)	67.8 ± 1.3	64.2–71.4	123	63.0 ± 1.5	58.8–65.9	118	687.84	< 0.001	0.032	
Bill length (mm)	30.7 ± 1.0	28.4–33.3	123	27.6 ± 1.0	25.4–30.0	118	557.13	< 0.001	0.046	
Bill depth (mm)	7.0 ± 0.3	6.2–7.9	119	6.5 ± 0.3	5.7-7.2	109	141.87	< 0.001	0.032	
Tarsus length (mm)	23.5 ± 0.9	21.0-26.6	107	22.8 ± 0.9	18.9–24.6	103	32.58	< 0.001	0.013	
Wing length (mm)	237.6 ± 5.2	226-253	86	230.4 ± 5.3	217-243	64	69.86	< 0.001	0.013	
Body mass (g)	90.3 ± 5.5	78 –110	119	84.1 ± 5.3	72–99	106	75.01	< 0.001	0.031	

Whiskered Tern and make comparisons with other terns. I show the occurrence of assortative pairing and estimated a discriminant function for predicting sex using morphological measurements.

METHODS

Study site and field procedures

Whiskered Terns were caught in breeding colonies on carp ponds in the Upper Vistula Valley River in southern Poland near the town of Zator (49°59'N, 19°26'E) from 2005 to 2010. In total, 241 adult breeding birds, including 61 pairs, were trapped on nests from about the tenth day after clutch completion until the downy chick period. Birds were caught with a roof trap or a loop trap. Individuals were ringed, measured, weighed and in some cases colour marked using a dye. Six morphological measurements were taken: (1) total head length: from the tip of the bill to the back of the skull; (2) culmen: from the bill tip to the posterior edge of ramphoteca; (3) bill depth: vertical height of the closed bill from the ventral tip of the gonys to the analogous dorsal surface of the upper mandible; (4) tarsus length: tarsometatarsus bone; (5) wing length: of maximum flattened wrist-wing tip length; (6) body mass. All linear measurements were recorded to the nearest 0.1 mm with callipers, except wing length which was measured with a ruler to the nearest 1 mm. Wing length was measured only if the tip of the wing was not damaged. Body mass was measured to the nearest 1 g with a Pesola spring balance. All measurements were taken by the author.

Molecular sex determination

The sex of Whiskered Tern was determined by the CHD gene on sex specific chromosomes (Griffiths et al. 1998). A blood sample of c. 0.2 ml was taken from the tarsus vein and was stored in 98% ethanol. Blood samples were frozen at -20°C until processing. Prior to extraction, the blood clot was dried at 50°C and 200 μ l Phosphate Buffered Saline was added. DNA was extracted using the Qiamp DNA Mini Kit (Qiagen). Cycling parameters for the PCR were as follows: 95°C for 4 min, then 40 cycles of 30 s at 95°C, 1 min at 53°C, 2 min at 72°C, ending with 10 min at 72°C. Each 10 μ l PCR sample contained: DNA, 4 mM MgCl₂, 800 μ M of each dNTP, 0.5 U Taq polymerase, 1x buffer and primers: F2550 (5'-GTTACTGATTCGTCTACGAGA-3'), R2718 (5'-ATTGAAATGATCCAGTGCTTG-3'). Products were separated by electrophoresis in 1.5% agarose gel. Negative controls were employed during extraction and

PCR to check for contamination of reagents.

A male was indicated by one band (650 bp), while female DNA produced two bands (650 bp and 450 bp). However, 16 females (14%) produced only one band (450 bp). Duplicate PCR analyses were conducted for all samples. Samples of females that produced only one band (450 bp) were run four times, and all analyses gave equal outcomes. The accuracy of this technique was tested using four dead adult birds of known sex. Additionally, the sex of 22 birds was confirmed by observations on courtship feedings and copulations.

Statistical analysis

ANOVA was used to test differences in measurements between sexes. Because no significant differences were found between years, individuals from all years were pooled. Dimorphism index (DI) was calculated for each morphometric character following Greenwood (2003) as log (mean male/mean female). Data of measurements of other terns were taken from the original papers.

A discriminant function was estimated by stepwise discriminant analysis using SPSS 17.0. Parameters were not linear combinations of each other and correlations between variables in the discriminant analysis were below 0.75 (Sikora & Dubiec 2007). At each step the variable that minimized the overall Wilk's Lambda ratio was added into the model. The default minimum partial F for entering was 3.84 and maximum partial F for removal was 2.71. The discriminant equation is presented with unstandardized canonical discriminant function coefficients. Canonical standardized discriminant function coefficients are presented to show the contribution of one variable in the presence of the other variables in the model.

The cut-off point to assign individuals to the male or female sex was estimated as the weighted average of male and female values (Garson 2010). A jackknife procedure was used to estimate classification success rate by classifying each case using a discriminant function based on all cases except the given case.

RESULTS

Genetic sexing identified 123 males and 118 females. Males were significantly larger than females for all morphological measures (Table 1). The largest DI was found in head and bill measurements and body mass, the smallest in tarsus and wing length. For all measurements, there was overlap between the sexes (Fig. 1). Head length and bill length showed the smallest overlap. Three measurements were used in a step-wise discriminant analysis: bill length, bill depth and tarsus length. Body mass was not used because of the large variation during the season (Wendeln & Becker 1996, 1999). Also wing length was excluded because the tip of wing was often damaged and did not allow for reliable measurements. Head length was not used because of the strong correlation with bill length. The discriminant analysis selected bill length and bill depth (Wilks' Lambda = 0.275, $\chi_2^2 = 257.193$, P < 0.0001) resulting in the following unstandardized equation:

 $D = -31.434 + 0.902 \times Bill \text{ length} + 0.773 \times Bill \text{ depth.}$

The cut-off point was D = 0.0977. This function correctly classified 95.2% of the individuals: 97.5% of males (n = 119) and 92.7% of females (n = 109; Fig. 2A). A jackknife cross-validation showed a similar classification. The canonical standardized discriminant function coefficients for bill length and bill depth were 0.902 and 0.773, respectively, showing that bill length had the highest discriminatory power. The probability (*P*) of classification for any D (Fig. 2B) was:

 $P = 1 / (1 + e^{-3.236 \times (D + 0.048)}).$

When bill length was replaced by head length, the stepwise discriminant analysis selected only head length (Wilks' Lambda = 0.257, χ_2^2 = 271.33, *P* < 0.0001) producing the following unstandardized equation:

 $D = -45.528 + 0.697 \times Head length$

(cut-off point: 0.0522),

with $P = 1 / (1 + e^{-3.388 \times (D + 0.050)})$.

This function correctly sexed 95.9% of males (n = 123) and 93.2% of females (n = 118), which gave an overall correct classification of 94.6% of all individuals. A jack-knife cross-validation showed a similar classification success rate.

Head length, bill length and bill depth were positively correlated between male and female partners

Table 2. Within-pair differences and Pearson correlation coefficients between morphometric measurements of Whiskered Terns nesting in southern Poland (2005–2010).

Measurement	Female ≥ male	Female < male	Paired <i>t</i> -test	r
Head length (mm)	2	59	22.2*	0.32*
Bill length (mm)	1	60	20.8*	0.36*
Bill depth (mm)	5	51	11.40*	0.30*
Tarsus length (mm)	7	34	4.66*	0.28
Wing length (mm)	3	19	5.81*	0.32
Body mass (g)	12	43	5.52*	-0.03

* *P* < 0.05.



Figure 2. (A) Frequency distribution of discriminant scores for male and female Whiskered Terns. Scores based on estimates from the equation based on bill length and bill depth. The arrow indicates the cut-off point. (B) The probability of being male in relation to the discriminant scores.

(Table 2). Other linear measurements and body mass of partners were not significantly correlated. Within pairs all measurements were larger in males.

DISCUSSION

In this study 14% of females produced only the W-chromosome-specific band whereas other females produced two bands – from the Z and W chromosomes. This result is not consistent with previous studies on Black Tern *Chlidonias niger* and Arctic Tern *Sterna paradisaea* (Devlin *et al.* 2004, Shealer & Cleary 2007), where two bands were produced in all females. However, it is consistent with a previous study on Greater Flamingo *Phoenicopterus roseus* reporting 50% of females producing only one band (Balkiz *et al.* 2007). All of these studies used the same set of primers described by Fridolfsson & Ellegren (1999), who suggested that preferential amplification of the shorter CHDW intron might lead to a non-detectable CHDZ product in females of some species. However, in this study a lack of one of the bands occurred in only a low percentage of the females. Absence of a band might be also due to variation in the quality of DNA at the start of amplification (Balkiz *et al.* 2007). In this study, no errors were found when comparing molecular data with behavioural observations (22 birds) and with samples from 4 dead birds. Additionally, in all 61 pairs studied, molecular analysis confirmed there were no same-sex pairs and duplicate PCR analyses produced equal results. In sum, the results confirm the appropriateness of the molecular method for correctly sexing Whiskered Terns.

I showed that male Whiskered Terns are larger than females in all characters, particularly in head and bill measurements and body mass. These results are consistent with previous studies on Whiskered Terns (Table 3) and other tern species (Table 4). However, differences between sexes in body mass were not found in five other tern species (Table 4). In only two species sexes differed significantly in body mass – in Brown Noddy *Anous stolidus* males were heavier than females (Chardine & Morris 1989) and in Common Terns *Sterna hirundo* females were heavier (Wendeln 1997, Nisbet *et al.* 2007). As in my data, non-breeding Australian Whiskered Tern males were significantly heavier than females (Dostine & Morton 1989; Table 3, 4). Body mass of both sexes of terns and gulls changes during

Table 3. Morphometric measurements	(means ± SD) of male and female	e Whiskered Terns f	rom different po	opulations
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Population		Bill length (mm)		Tarsus length (mm)		Wing length (mm)		Body mass (g)	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Mean n	30.1 ± 1.7 19	28.1 ± 1.1 18	26.4 ± 0.9 16	$\begin{array}{c} 25.2\pm0.8\\ 18\end{array}$	217.9 ± 7.1 19	$\begin{array}{c} 212\pm8.5\\20\end{array}$	82.4 ± 7.1 18	73.2 ± 7.9 20
2	Mean Range n	31.6 ± 1.1 30–33 8	28.5 ± 1.5 26–30 11	23.3 ± 0.6 23–24 9	22.6 ± 1.4 21–24 11	242 ± 7.1 231–250 8	232 ± 3.5 228–238 12	- -	
3	Mean Range n	30.7 ± 1.0 28.4–33.3 123	27.6 ± 1.0 25.4-30.0 118	23.5 ± 0.9 21.0-26.6 107	22.8 ± 0.9 18.9–24.6 103	237.6 ± 5.2 226-253 86	230.4 ± 5.3 217–243 64	90.3 ± 5.5 78–110 119	84.1 ± 5.3 72–99 106

1 = Australia, non-breeding, shot birds, body mass minus weight of stomach contents, no data for range (Dostine & Morton 1989), 2 = Europe and North Africa, May-August, non-breeding and breeding, skins (Cramp & Simmons 1985), 3 = Southern Poland, this paper. - = no data.

Table 4. Sexual size dimorphism index in various tern species calculated from literature data. The dimorphism index was calculated as log(mean male/mean females).

Source ^a	Head length	Bill length	Bill depth	Tarsus length	Wing length	Body mass
1	0.016*	0.023*	0.021*	0.007*	0.002	-0.019*
2	0.014*	_	_	0.004*	0.005*	-0.014
2	0.015*	_	_	0.005*	0.006*	-0.004
3	0.018*	0.023*	0.024*	0.005	0.006*	-0.004
4	0.019*	0.022*	0.026*	0.007*	0.004	-0.003
5	0.010*	0.007	0.039*	0.011	0.002	-0.005
6	0.006*	0.013*	0.017*	0.012	0.003	-0.008
7	0.013*	-	0.031*	-	0.008*	0.030*
8	0.011*	0.010	0.023*	0.012*	0.005*	_
9	0.015*	0.020*	0.016*	_	0.006*	0.001
10	0.019*	0.031*	_	_	_	_
11	-	0.041*	_	0.020*	0.012*	0.051*
12	-	0.045*	-	0.013	0.018*	-
13	0.032*	0.046*	0.032*	0.013*	0.013*	0.031*
	Source ^a 1 2 3 4 5 6 7 8 9 10 11 12 13	Source ^a Head length 1 0.016* 2 0.014* 2 0.015* 3 0.018* 4 0.019* 5 0.010* 6 0.006* 7 0.013* 8 0.011* 9 0.015* 10 0.019* 11 - 12 - 13 0.032*	Source ^a Head length Bill length 1 0.016* 0.023* 2 0.014* - 2 0.015* - 3 0.018* 0.023* 4 0.019* 0.023* 5 0.010* 0.023* 6 0.019* 0.023* 7 0.013* - 8 0.011* 0.010 9 0.015* 0.020* 10 0.019* 0.031* 11 - 0.041* 12 - 0.045* 13 0.032* 0.046*	Source ^a Head length Bill length Bill depth 1 0.016* 0.023* 0.021* 2 0.014* - - 2 0.015* - - 3 0.018* 0.023* 0.024* 4 0.019* 0.023* 0.024* 4 0.019* 0.022* 0.026* 5 0.010* 0.007 0.039* 6 0.006* 0.013* 0.017* 7 0.013* - 0.031* 8 0.011* 0.010 0.023* 9 0.015* 0.020* 0.016* 10 0.019* 0.031* - 11 - 0.041* - 12 - 0.045* - 13 0.032* 0.046* 0.032*	Source ^a Head length Bill length Bill depth Tarsus length 1 0.016* 0.023* 0.021* 0.007* 2 0.014* - - 0.004* 2 0.015* - - 0.005* 3 0.018* 0.023* 0.024* 0.005* 4 0.019* 0.022* 0.026* 0.007* 5 0.010* 0.007 0.039* 0.011 6 0.006* 0.013* 0.017* 0.012 7 0.013* - 0.031* - 8 0.011* 0.010 0.023* 0.012* 9 0.015* 0.020* 0.016* - 10 0.019* 0.031* - - 11 - 0.041* - 0.020* 12 - 0.045* - 0.013 13 0.032* 0.046* 0.032* 0.013*	Source ^a Head length Bill length Bill depth Tarsus length Wing length 1 0.016* 0.023* 0.021* 0.007* 0.002 2 0.014* - - 0.004* 0.005* 2 0.015* - - 0.005* 0.006* 3 0.018* 0.023* 0.024* 0.005 0.006* 4 0.019* 0.022* 0.026* 0.007* 0.004 5 0.010* 0.007 0.039* 0.011 0.002 6 0.006* 0.013* 0.017* 0.012 0.008* 7 0.013* - 0.031* - 0.008* 8 0.011* 0.010 0.023* 0.012* 0.005* 9 0.015* 0.020* 0.016* - 0.006* 10 0.019* 0.031* - - - 11 - 0.041* - 0.013* 0.012* 12

^aSource: 1 = Nisbet *et al.* 2007, 2 = Fletcher & Hamer 2003, 3 = Devlin *et al.* 2004, 4 = Bluso *et al.* 2006, 5 = Ackerman *et al.* 2008, 6 = Quinn 1990, 7 = Chardine & Morris 1989, 8 = Reynolds *et al.* 2008, 9 = Shealer & Cleary 2007, 10 = Stern & Jarvis 1991, 11 = Dostine & Morton 1989 (shot birds, body mass minus weight of stomach contents), 12 = Cramp & Simmons 1985 (skins), 13 = this study. * P < 0.05, - = no data.

the breeding season (Wendeln & Becker 1996, 1999, Moe *et al.* 2002). Data on body mass in this study were collected from the tenth day after clutch completion until the downy chick period, which is similar to that in the other tern studies. Therefore differences in male and female body mass in Whiskered Tern and other tern species does not result from birds being measured at different stages of the breeding season.

Whiskered Tern populations display greater SSD in almost all measurements compared to other terns (Table 4). The DI's of the majority of measurements in Whiskered Terns were at least twice as high as in other terns: the largest differences were found in bill length. head length and body mass. The degree of SSD in Whiskered Terns in comparison with other terns may result from their stronger sex-specific foraging niche utilization than in other terns (Lewis et al. 2002). Male and female Whiskered Terns differ significantly in type and size of prey. In non-breeding Australian Whiskered Tern males consumed more vertebrates while females took more small invertebrates (Dostine & Morton 1989). On carp ponds in southern Poland males were bringing over 60% of all vertebrates (mainly fish, frogs, tadpole), whereas females delivered over 90% of all invertebrates (mainly small dragonflies; Betleja 2003, Ledwoń 2010). Some studies reported tern males capturing larger vertebrates than females (Wagner & Safina 1989, Uttley 1992, Fasola & Saino 1995). Even a small degree of sexual dimorphism in body size leads to differential flight performance (Andersson & Norberg 1981, Norberg 1995). Natural selection may lead to sex-related foraging niche partitioning because SSD decreases intrapair food competition and enables exploitation of a wider range of prey items (Gosler 1987, Przybylo 1995).

I showed positive assortative mating based on head length, bill length and bill depth. Assortative pairing – mostly positive – in terms of morphological measurements, body condition, age and moult patterns has been described in terns (Stern & Jarvis 1991, Wendeln 1997, González-Solis *et al.* 1999, Bridge & Nisbet 2004, Ludwig & Becker 2008), though other studies reported a lack of assortative mating (Chardine & Morris 1989, Devlin *et al.* 2004, Nisbet *et al.* 2007, Ludwig & Becker 2008).

Assortative mating may positively affect reproductive success (Black & Owen 1995, Fasola *et al.* 2001, Tryjanowski & Šlimek 2005). When the abundance of food is high, like on carp ponds where this study was conducted (Ledwoń 2010), selection on pairing with high quality mates should be strong (Tryjanowski & Šlimek 2005). Since the effectiveness of delivering high-quality prey to chicks depends on body size (bill and head size contributes to the snapping power of the bill; Ashmole 1968), pairs consisting of large mates are expected to be more successful in breeding than pairs of smaller individuals.

Head length was the best single variable for predicting sex, accurately predicting 94.6% of cases. Similar success was achieved when bill length and bill depth were used. Accuracy of sex determination in terns as collected from the literature varied from 70% to 90% (Table 4), which is the result of relatively weaker sexual size dimorphism in terns other than Whiskered Terns. The comparison of head and bill length within pairs allowed correct sexing of 97% and 98% of birds, respectively (Table 2). Other studies on terns reported also better classification rates in within-pair comparison that in discriminate function analysis of all measured individuals (Fletcher & Hamer 2003, Devlin *et al.* 2004, Shealer & Cleary 2007).

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SAMENVATTING

Grootteverschillen tussen seksen ('sexual size dimorphism', of SSD) kan het resultaat zijn van zowel natuurlijke als seksuele selectie. De factoren en mechanismen die hieraan ten grondslag liggen, zijn echter moeilijk vast te stellen. Het meten van de SSD en een niet-random partnerkeuze alsmede het bestuderen van de ecologie van een soort zijn cruciaal voor het toetsen van oorzaken en effecten van SSD. In het onderhavige onderzoek is de SSD en niet-random partnerkeuze gemeten bij Witwangsterns Chlidonias hybrida, een moerasstern van gemiddelde grootte. Vervolgens is met behulp van een discriminantanalyse een vergelijking opgesteld die de sekse voorspelt. De auteur heeft in 2005-2010 in het zuiden van Polen 241 volwassen broedvogels gevangen waaraan een aantal maten zijn genomen en waarvan de sekse via moleculaire technieken is bepaald. Mannetjes waren significant groter dan vrouwtjes voor alle zes genomen maten: totale koplengte (inclusief snavel), snavellengte, snavelhoogte, tarsuslengte, vleugellengte en lichaamsgewicht. De grootste dimorfie-index werd vastgesteld bij kop- en snavelmaten en bij het lichaamsgewicht. Koplengte, snavellengte en snavelhoogte waren positief gecorreleerd binnen paren. Twee discriminantfuncties, één gebaseerd op snavellengte en snavelhoogte en één gebaseerd op koplengte, voorspelden beide 95% van de seksen correct. Grootteverschil tussen de seksen is bij Witwangsterns sterker dan bij andere sternensoorten, waarschijnlijk door een sterkere sekseafhankelijke voedselspecialisatie. (PW)

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