Sexing Adult Black-legged Kittiwakes by DNA, Behavior, and Morphology

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Abstract.—We sexed adult Black-legged Kittiwakes (*Rissa tridactyla*) using DNA-based genetic techniques, behavior and morphology and compared results from these techniques. Genetic and morphology data were collected on 605 breeding kittiwakes and sex-specific behaviors were recorded for a sub-sample of 285 of these individuals. We compared sex classification based on both genetic and behavioral techniques for this sub-sample to assess the accuracy of the genetic technique. DNA-based techniques correctly sexed 97.2% and sex-specific behaviors, 96.5% of this sub-sample. We used the corrected genetic classifications from this sub-sample and the genetic classifications for the remaining birds, under the assumption they were correct, to develop predictive morphometric discriminant function models for all 605 birds. These models accurately predicted the sex of 73-96% of individuals examined, depending on the sample of birds used and the characters included. The most accurate single measurement for determining sex was length of head plus bill, which correctly classified 88% of individuals tested. When both members of a pair were measured, classification levels improved and approached the accuracy of both behavioral observations and genetic analyses. Morphometric techniques were only slightly less accurate than genetic techniques but were easier to implement in the field and less costly. Behavioral observations, while highly accurate, required that birds be easily observable during the breeding season and that birds be identifiable. As such, sex-specific behaviors may best be applied as a confirmation of sex for previously marked birds. All three techniques thus have the potential to be highly accurate, and the selection of one or more will depend on the circumstances of any particular field study. *Received 2 February 2000, accepted 1 April 2000.*

Key words.—Black-legged Kittiwake, *Rissa tridactyla,* behavior, morphology, DNA, genetics

Waterbirds 23(3): 405-415, 2000

Interpretation of ecological and behavioral data is often improved when the sexes of individual study animals are known. For many avian species, sex can be determined without the need for internal examination, either by observing plumage or sex-specific structural characteristics (such as colored soft-tissue), measuring morphological characteristics, or observing sex-specific behaviors. Recently, DNA-based techniques have also been employed to determine sex in a variety of avian species (Longmire *et al.* 1993; Ellegren 1996; Ellegren and Sheldon 1997; Griffiths *et al.* 1998). It is unlikely, however, that each sexing method would provide the same degree of accuracy, or even be possible, for any given species. Therefore, it becomes necessary to evaluate the applicability and accuracy of each potential technique for the species in question.

Our goal was to assess the accuracy and applicability of using genetic, behavioral, and morphometric techniques to sex Black-legged Kittiwakes (*Rissa tridactyla*), a species increasingly used as an indicator of the health of marine systems throughout Alaska and the circumpolar Arctic (Murphy *et al.* 1991; Gill 1999). Although morphometrics and behavior have been used successfully to sex individuals of this species in the past (Coulson *et al.* 1983; Hatch *et al.* 1993), there has yet to be (1) a morphometric study done on kittiwakes in Alaska, (2) a DNA-based study done in any population, or (3) a comprehensive comparison of the applicability or accuracy of these three techniques for this species in general. Therefore, our objectives were to (1) use DNA-based genetic techniques to determine the sex of adult kittiwakes, (2) use behavior-based techniques to

assess the accuracy of the genetic techniques, and (3) subsequently use sex assignments based on genetic and behavior techniques to develop and evaluate a suite of discriminant function models based on morphological measurements. We also review the limitations and benefits of each technique as it pertains to this species and birds in general.

METHODS

We captured nesting adult Black-legged Kittiwakes (n = 605) during the breeding seasons of 1997 and 1998 on Middleton Island, Alaska (58°28'N, 146°19'W). The primary capture location (n = 585 birds) was an abandoned Air Force radar tower where the effects of food availability on behavior and reproduction of kittiwakes were being examined (Gill 1999). The radar tower was equipped with artificial nesting platforms and one-way glass sliding windows that allowed birds to be easily captured and observed from inside of the tower. The second capture location (n = 20 birds) was a nearby ship grounded on the island in 1944. All captured individuals were banded with unique color combinations, measured, and bled. We assumed that all sampled birds were >3 years old since we knew of only five banded chicks that returned to the island at an age of ≤3 years, and none of these was known to nest on the tower.

Genetic Sex Determination

We used a 27 gauge, 0.5 cc syringe to draw approximately 50-250 µl of blood from the brachial vein of each adult kittiwake shortly after capture. The blood was centrifuged to remove the plasma, and the remainder was stored in 100% ethanol at sub-freezing conditions. DNA was then extracted from red blood cells using a Puregene DNA extraction kit. Samples were analyzed independently at the Alaska Biological Science Center (ABSC) in Anchorage, Alaska, and Queen's University in Kingston, Ontario. We optimized protocols outlined in Griffiths *et al.* (1998) to amplify the CHD-W and CHD-Z genes located on the avian sex chromosomes using P2 and P8 primers (exact protocols are available from R. Lanctot). Gel electrophoresis of the PCR products was then used to reveal the presence of a single or double band representing a male or female, respectively. Eight Black-legged Kittiwakes (four males, four females) that had been sacrificed and sexed in the laboratory were used to confirm this banding pattern. All remaining samples were run without knowledge of the suspected sex of the birds, based on their behavior or morphology.

Behavioral Observations and Analyses

We used sex-specific behaviors to confirm classification as determined by genetic techniques on a sub-sample (n = 285) of the above genetically-sexed birds. We recorded four behavioral interactions to determine sex (Paludan 1955; Baird 1994). These interactions were (1) begging, in which the female bobs her head and pecks and bites at the male's bill, (2) courtship feeding, in which the male regurgitates food to the female, (3) standing, in which the male perches on the back of the female in preparation for copulation, and (4) copulation, in which the male inseminates the female. We used focal group observations to observe pairs at the tower during the courtship and chick-rearing periods from May through August in 1996-1998. Data from 1996 were included in these analyses to increase sample size and investigate the effects of multiple-year behavioral observations on sex determination. We also collected additional observations opportunistically while conducting other tasks at the tower.

We assessed the agreement of behavioral and genetic classification for the sub-sample of 285 banded birds by comparing each type of behavioral interaction independently, and then in combination, with the individual's predicted sex based on genetic analyses. When genetic and behavioral sex determinations disagreed, we attempted to discern the correct classification of the individual by re-examining the behavior data of the focal individual, determining the sex of an individual's former, current, and subsequent mates (via behavioral and genetic techniques), reviewing gel electrophoresis pictures to determine if transcription errors occurred, and, when necessary, re-running genetic samples.

Morphometric Statistical Analyses

Morphometric measurements from the complete sample of 605 captured kittiwakes included exposed culmen length (measured from the tip of the bill to the forehead end of the frontal process of the premaxilla), diagonal tarsus length, and natural wing length (carpel joint to tip of the longest primary). We also measured length of tarsus, head plus bill (hereafter headbill, measured from the tip of the bill to the posterior ridge formed by the parietal-supraoccipital junction) and length of flattened wing in a subset of the birds captured on the tower during a study of daily energy expenditure (hereafter DEE birds, n = 73). We measured lengths of culmen, headbill, and tarsus with dial calipers to ±0.1 mm and wing lengths with a ruler to ±1 mm. All morphometric measures from DEE birds were made by one of the authors (DDR). Most of the measures from the complete sample of birds were made by two of the authors (RBL and VAG), while trained and tested observers made a small proportion of the measurements. (these were checked periodically by RBL and VAG). We calculated the percent measurement error (ME; Yezerinac *et al.* 1992) for culmen, tarsus, and natural wing from birds that were measured in both 1997 and 1998 (n = 33).

We used discriminant function analyses to evaluate sexual size dimorphism in three groups of kittiwakes; the complete group (n = 605), the DEE group (n = 73; a subsample of the complete group), and a mated pair group consisting of all individuals for which both members of a pair were measured (n = 376; a subsample of the complete group). We randomly separated each of the three kittiwake groups into two samples, one used for building the discriminant functions (analysis samples) and one used for verifying the discriminant functions (predictive samples; Hair *et al.* 1995). The number of birds in each analysis sample was as follows: complete-group n = 303 (145 females, 158 males), DEE group n = 47 (24 females, 23 males), and mated-pair group n = 188 (94 females, 94 males). The number of birds in each predictive sample was as follows: complete group n = 302 (144 females, 158 males), DEE group n = 26 (13 females, 13 males), and mated-pair group n = 188 (94 females, 94 males). We divided the DEE group unevenly

so that a larger proportion of the birds were allocated to the analysis sample (Hair *et al.* 1995).

We evaluated 12 discriminant functions, four from each of the three groups. Each discriminant function included subsets of the morphometric variables measured on that group, with the models from the mated pairs being unique in that they also included terms for the difference in measurements of culmen, tarsus, and natural wing between the two individuals of the pair.

For the purposes of these analyses, we assumed the sex of a bird based on genetics was correct (except for 8 cases where behavioral data indicated otherwise, see below). For each of the 12 models tested, we produced a linear discriminant function for each sex. To improve the applicability of the models in field situations, however, we reduced the two original sex-specific discriminant functions to a single discriminant function (we present only the latter). The single function is derived simply by subtracting the constant and each coefficient of the 'male' function from the constant and corresponding coefficients of the 'female' function. We evaluated performance of the models derived from the analysis samples with Wilks' lambda statistic, which decreases as discriminatory power increases, and with the squared canonical correlation value, which indicates the proportion of variance in the dependent variable explained by the independent variables in the model. We evaluated the impact of removing each variable from a model with the removed F-statistic which is based on a Wilks' lambda statistic. We also report allocation rates for each model, which are simply the percentage of individuals correctly identified by that model for each sex and for all birds combined. We also calculated cutting scores for each analysis model so as to later assign sex to individuals in the predictive groups. Cutting scores are the weighted averages of the mean value of discriminant scores (i.e., the value calculated by summing the constant and all coefficients once the data are entered) for each sex (Hair *et al.* 1995).

Each single function derived from the analysis sample was applied to each individual within the predictive sample to verify the discriminant functions. Sex of birds in each predictive group was assigned by entering morphometric variables into the models produced from the analysis samples and determining whether discriminant scores (i.e., values produced by carrying out the equations with the measurement data included) were greater or less than the cutting scores previously determined for each sex. This is the same process that would be used to determine the sex of individuals in the field. We also present allocation rates from predictive models to compare accuracy of the models for assigning sex to individuals in the predictive and analysis samples.

We reviewed morphometric data prior to analyses to insure that assumptions of discriminant function analyses were met. Data did not exhibit multicollinearity ($r < 0.46$ for all pairwise correlations) and there were no significant differences in the variance-covariance matrices of each variable for each sex (Bartlett-Box homogeneity tests, $P > 0.17$).

RESULTS

Genetic and Behavioral Sex Determination

We sexed 270 and 335 adult kittiwakes genetically in 1997 and 1998, respectively. We assessed the reliability of the genetic sexing technique in several ways. First, 71 birds captured in both 1997 and 1998 were sexed independently by technicians in the ABSC and Queen's University molecular ecology laboratories. In all but one case the two technicians agreed on the sex of the birds; this one bird was incorrectly classified as a male by one technician. Second, 98 birds were analyzed twice at the Queen's University molecular ecology laboratory and in only two individuals did the sex designation differ. Two additional analyses of each of these contradictory samples resulted in the same sex designation in three of the four trials. Overall, replicate analyses of blood samples indicated genetic techniques consistently provided the same sex determination.

We collected behavioral observations from 1996-1998 on 285 of the 605 birds whose sex was determined genetically. The median and mean number of behavioral observations per bird was 2.0 (SD = 1.23), and most birds (n = 210) were observed in only one year. Sixty-four birds had behavioral interactions recorded in two years and 11 were recorded in three years. Sex determinations based on all four behavioral interaction categories generally agreed with the genetic determinations for individuals where both behavior and genetics data were available (Table 1). Determining the sex of an adult kittiwake was least reliable when observations of only begging behavior were used. Observations of copulation, standing, and courtship feeding were more reliable, with mistakes typically occurring when observers recorded the male and female backwards (something easily done if male dismountings are not well documented). Each of the behaviors concurred with results from genetic analysis >94% of the time. When behaviors were combined across categories and years, 96.5% of classifications concurred with results from genetic analysis.

Discrepancies between genetic and behavioral sex determinations occurred 18 times out of the 285 occasions in which both genetic and behavior data were available. We reviewed individuals' behavior, reproductive success, and the sex of their former, current,

Table 1. Number of male and female Black-legged Kittiwakes based on genetics and behavioral observations at Middleton Island, Alaska, 1996-1998.

	Sex based on genetic results	
Behavior/sex	Male	Female
Beg		
Male	82	1
Female	5	89
Both[1]	4	1
% Disagree	9.99	3.26
Courtship Feed		
Male	50	0
Female	2	49
Both[1]	1	1
% Disagree	5.66	2.00
Stand		
Male	25	0
Female	2	21
Both[1]	0	0
% Disagree	7.41	0
Copulation		
Male	86	1
Female	4	76
Both[1]	1	1
% Disagree	5.49	2.56
All behaviors combined and mate's behavior and genetic results[2]		
Male	142	0
Female	8	135
% Disagree	5.33	0

[1]Bird was identified as a male and a female during different behavioral observations.

[2]An evaluation of all the behavior and genetic results indicated that for ten individuals sex was incorrectly assigned based on sex and in eight individuals sex was incorrectly assigned based on genetics.

and subsequent mates to determine which of the techniques provided the correct classification. On ten occasions the available evidence supported the genetic results over the behavior results, and on eight occasions the behavior results were supported over the genetic results. Errors in behavior occurred most frequently when different observers within or among years classified the sex of the same bird differently. Errors in the genetic results always occurred when females were incorrectly classified as males. In these cases, the top band that denotes a female was either very light or not present, suggesting that the P2 and P8 primers were competing with one another and thereby reducing the PCR product of one reaction (see discussion in Griffiths *et al.* 1998). Therefore, DNA-based genetic tests identified the correct sex of an individual in 97.2% of cases (277 of 285) where behavioral data were also available. We assumed that for the remainder of the 605 individuals whose sex was determined by genetic analyses alone (n = 320), our sex designation was accurate for 97.2% (n = 311) of these individuals.

Morphometric Statistical Analyses

There were no significant differences in morphometric measurements between years ($-1.49 < t_{604} < 0.39$; $0.14 < P < 0.83$), so data were pooled across years for all analyses. Each morphometric measurement differed between sexes for both the complete sample of birds (Table 2; $-16.41 < t_{604} < -11.79$, $P < 0.0001$) and the DEE birds (Table 2; $-10.16 < t_{71} < -4.15$, $P < 0.0001$). Males were typically larger than females for each characteristic we measured, although the magnitude of difference between sexes varied among measured characteristics (Fig. 1). Coefficients of variation (CVs) within each characteristic were small, ranging only from 2.2 to 4.4%. When morphometric measurements were compared between mates, however, males were almost always larger than females. For example, considering measurements recorded from DEE birds (n = 33 pairs), flattened wing length of males was greater than females in 94% of cases and headbill length of males was greater than females in 97% of cases. Measurement error was low for culmen (0.8%) but higher for tarsus and natural wing length (19.1 and 23.9%, respectively).

Each of the discriminant functions we tested from the complete sample and the DEE sample (hereafter referred to as individual-based models) was significant and explained 32-67% of the variability in the data (see Squared Canonical Correlation values, Table 3). Wilks' Lambda statistics indicated models with measures of headbill and flattened wing were the most powerful for discriminating between sexes, whereas models

Table 2. Body measurements (mean ± 1 SE) of Black-legged Kittiwakes sexed at Middleton Island, Alaska, May-August 1997 and 1998.

	Complete sample		DEE birds[1]	
	Females (n = 289)	Males (n =316)	Females (n = 37)	Males (n = 36)
Wing (mm)[2]	307.81 ± 0.40	316.66 ± 0.41	317.45 ± 1.08	327.89 ± 0.94
Tarsus (mm)	35.08 ± 0.08	36.32 ± 0.07	35.35 ± 0.14	36.95 ± 0.18
Head + bill (mm)	na	na	94.09 ± 0.37	99.62 ± 0.40
Culmen (mm)	37.96 ± 0.09	40.12 ± 0.10	37.76 ± 0.21	39.83 ± 0.34

[1]Bird used in an experiment measuring daily energy expenditure.

[2]Natural wing length was measured for the complete sample of birds; flattened wing length was measured for DEE birds only.

with culmen measures alone were the least powerful (Table 3).

The individual-based discriminant function models we tested correctly classified an individual's sex (i.e., concurred with genetic results) in 73-95% of cases (Table 4). Models including flattened wing measurements were always more accurate than models including natural wing measurements (Table 4). Similarly, models including headbill measurements were always more accurate than models including culmen measurements (Table 4). The highest accuracy achieved with these predictive models (#6; flattened

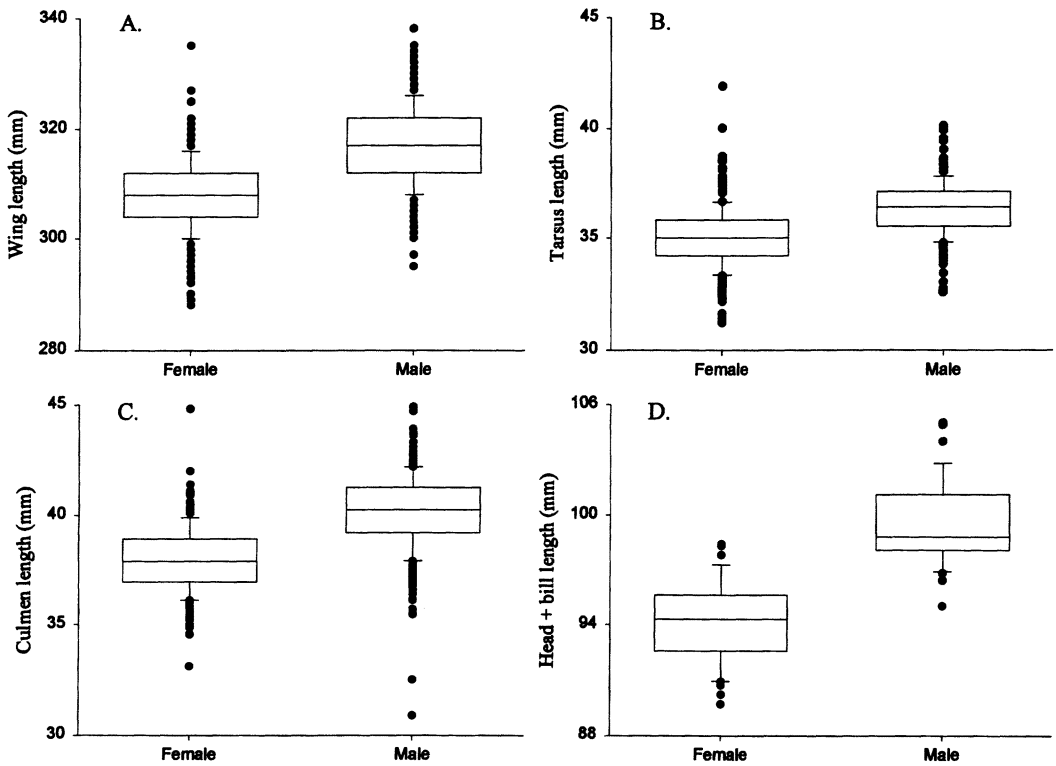

Figure 1. Variation in measurements of (A) flattened wing, (B) tarsus, (C) culmen, and (D) head bill length in male and female Black-legged Kittiwakes from Middleton Island, Alaska, May-August 1997 and 1998. Flattened wing and head bill length were measured from the DEE sample of birds (n = 73); tarsus and culmen length were measured from the complete sample of birds (n = 605). Boxes show median (midline), quartiles (top and bottom of box), 5th and 95th percentiles (bars) and all values (points) beyond the 5th and 95th percentiles.

Table 3. Single discriminant functions[1] based on lengths (mm) of four morphometric variables[2] of Black-legged Kittiwakes captured at Middleton Island, Alaska, May-August 1997 and 1998. Italicized coefficients do not contribute to the explanatory power of the model (i.e., $P < 0.10$, Wilks's Lambda F).

	Discriminant function[3]	Squared canonical correlation	F	DF	P	Wilks' lambda	Cutting score4
Model 1	97.359 - 0.627*cu-0.163*nw-0.620*ta	0.496	97.9	3,299	<0.001	0.504	D < -0.191 = male
Model 2	81.108 - 0.672*cu - 0.173*nw	0.455	125.3	2,300	<0.001	0.545	D < -0.176 = male
Model 3	62.938 - 0.202*nw	0.338	153.7	1,301	<0.001	0.662	D < -0.149 = male
Model 4	31.144 - 0.798*cu	0.321	142.4	1,301	<0.001	0.679	D < -0.033 = male
Model 5	-160.966 + 0.g55*hb + 0.221*fw - *0.073*ta*	0.676	30.0	3,43	<0.001	0.324	D > 0.112 = male
Model 6	161.765 - 0.941*hb-0.218*fw	0.676	46.0	2,46	<0.001	0.324	D < 0.381 = male
Model 7	96.907 - 1.00l*hb	0.611	70.8	1,45	<0.001	0.389	D < 0.102 = male
Model 8	82.623 - 0.256*fw	0.416	32.1	1,45	<0.001	0.583	D < -0.027 = male

[1]Single function derived by subtracting male discriminant function from female discriminant function (see Methods).
[2]CU = culmen, nw = natural wing, fw = flattened wing, ta = tarsus, and hb = head plus bill.
[3]Models 1-4 are for the complete sample; models 5-8 are for the DEE sample.
[4]Cutting score calculated as weighted averages of the group centroids (i.e., the mean value of discriminant scores for each sex; Hair *et al.* 1995).

Table 4. Allocation rates (i.e., proportion of birds correctly sexed) for discriminant functions of analysis and predictive groups of Black-legged Kittiwakes measured on Middleton Island, Alaska, May-August 1997 and 1998.[1]

	% female sexed correctly	% males sexed correctly	% of males and females sexed correctly
Analysis - complete sample			
Wing + Tarsus + Culmen	88.27	87.34	87.79
Wing + Culmen	86.21	82.28	84.16
Wing	80.69	69.62	74.92
Culmen	81.38	79.11	80.20
Prediction- complete sample			
Wing + Tarsus + Culmen	82.64	85.58	84.16
Wing + Culmen	79.86	81.13	80.53
Wing	68.06	77.36	72.94
Culmen	78.47	74.84	76.57
Analysis - DEE birds			
Wing + Tarsus + Head bill	91.67	100.00	95.47
Wing + Head bill	91.67	95.65	93.62
Head bill	91.67	91.30	91.48
Wing	75.00	86.96	80.85
Prediction - DEE birds			
Wing + Tarsus + Head bill	92.31	84.61	88.46
Wing + Head bill	100.00	84.61	92.31
Head bill	84.61	92.31	88.46
Wing	92.31	76.92	84.61

[1]Natural wing length was measured for the complete sample of birds; flattened wing length was measured for DEE birds only.

wing and headbill) was similar to that obtained using behavioral techniques (Tables 1 and 4). Headbill alone correctly classified individuals in 88.5% of cases and was the best single factor model we tested (model 7; Tables 3 and 4). When headbill measurements from the DEE birds were plugged into model 7 and the cutting scores sorted, individuals were classified as females when the headbill measurement was <96.8 mm. The predictive accuracy of the single element headbill model was improved by 3.8% when flattened wing was added as the second element to the model. Adding tarsus measurement as a third element did not improve the accuracy of the two element headbill and flattened wing model, however (Table 3 Model 5 and Table 4).

The four discriminant function models derived from mated pairs (hereafter referred to as mate-based models; Table 5) were each more accurate at assigning sex than the individual-based models using the same variables. The full mate-based model (#9) that contained all three measurements and their difference terms predicted the sex of individuals more accurately (96.2% of cases) than any other model we tested (see below and Table 4) and approached classification levels of both genetic analysis and behavioral observations. This model also had the highest squared canonical correlation and lowest Wilks' lambda of any model tested (Tables 4 and 5). A slightly reduced model (#8) which excluded tarsus measurements was only slightly less accurate then the mate-based full model, predicting sex correctly in 92.0% of cases.Simpler models that only included terms for either culmen or natural wing measurements and their differences between mates correctly classified sex in 88.3 and 79.2% of cases, respectively. For each mate-based model, the coefficients for the difference terms did contribute to the explanatory power of the models while those for the measurements of the individual did not contribute to the explanatory power of the models (Table 5). It is

Table 5. Single discriminant functions[1] from the differences in lengths (mm) of four morphometric variables[2] between males and females from 188 mated pairs of Black-legged Kittiwakes at Middleton Island, Alaska, May-August 1997 and 1998. Italicized coefficients do not contribute to the explanatory power of the model (i.e., $P < 0.10$, Wilks's Lambda *F*).

	Discriminant function	Squared Canonical correlation	F	DF	P	Wilks' lambda	Cutting score[3]
Model 9	-13.544 + *0.3674*cu* - 1.1643*dcu - *0.0115 *nw* - 0.1398*dnw + *0.0811 *ta*-1.0367*dta	0.714	74.9	6,180	<0.001	0.2B6	D < -0.0684 = male
Model 10	-7.845 + *0.2535*cu* - 1.1011*dcu - *0.0063*nw* - 0.1531*dnw	0.650	84.4	4,182	<0.001	0.350	D < -0.0452 = male
Model 11	4.916 - *0.01576*nw* - 0.19014*dnw	0.475	83.8	2,185	<0.001	0.525	D < -0.0005 = male
Model 12	-10.6348 + *0.2753*cu* - 1.2540*dcu	0.566	120.1	2,184	<0.001	0.434	D < -0.0475 = male

[1]Single function derived by subtracting male discriminant function from female discriminant function (see Methods).
[2]CU = culmen, nw = natural wing, ta = tarsus; a "d" preceding these abbreviations is the difference in size between paired males and females.
[3]Cutting score calculated as weighted averages of the group centroids (i.e., the mean value of discriminant scores for each sex; Hair *et al.* 1995).

therefore not surprising that culmen and wing mate-based models were only slightly more accurate than assigning sex based simply on the assumption that male culmen and wing were always longer than female culmen and wing (86.6 and 77.5% correct, respectively).

DISCUSSION

Black-legged Kittiwakes at Middleton Island, Alaska, can be reliably sexed using DNA-based genetic techniques, morphology, or behavior. Among the three techniques we evaluated, DNA-based genetic techniques and behavioral observations were the most accurate, with morphology a close third. Each technique has its benefits and limitations and the appropriate method for a study would be dictated by the field conditions and finances available. Below we discuss each technique in turn.

Behavioral observations are best used to sex kittiwakes during the breeding season when sex-specific behavior is most common and when birds from each sex are present simultaneously. Our data suggest that previously identified sex-specific behaviors in kittiwakes (Paludan 1955) can be used as reliable indicators of a bird's sex. Sexing of kittiwakes via behavior was most accurate when multiple behaviors were observed for each individual and least accurate when only begging was observed. Error rates in sexing were 3-10% when only begging was observed. Such errors are more pronounced when low intensity begging is observed (J. Coulson, pers. comm.). Other behaviors that have been documented in kittiwakes and which confound sexing accuracy include reverse mounting and female-female pairing (J. Coulson, pers. comm.). We found no evidence of these two activities in our study and instead attribute most of the behavior-based sexing errors that occurred to recording observations incorrectly.

Behavior is best suited for sexing kittiwakes when each individual under consideration is marked in a way that allows for long-term identification (e.g., color-banded, radio-tagged, or dyed) and the identity and be-

havior of individual birds can be observed with ease. Without such a marking scheme sex needs to be re-established each time the individual is encountered, with no means to ensure consistent classification. The observation windows and the long-term banding study present on the abandoned radar tower on Middleton Island also allowed birds to be captured with relative ease and observers to view birds at a distance of <3 m. This is unlikely to be the case at many other kittiwake colonies where birds typically nest on cliff faces surrounded by open ocean.

The genetic procedure used in this study was the most accurate of those we assessed, although still not 100% accurate. Errors within the genetic procedure may occur even though sexes are always distinct and analytical protocols have been optimized. For example, samples must be collected and labeled correctly in the field. Sample analysis and interpretation also must be conducted without error in the laboratory. This may be problematic given the many steps each sample undergoes during DNA extraction, PCR amplification and gel electrophoresis. Ideally all samples should be analyzed twice to detect inconsistency in results. This approach, however, will not remove errors occurring in the field and can be logistically and financially costly when large numbers of samples are being processed. Our approach of analyzing a proportion of the samples twice may be more realistic for assessing the accuracy of a genetic sexing protocol. Furthermore, the ability to employ genetic procedures may be limited by the need to capture individuals to obtain blood or tissue samples. DNA-based techniques also are not able to provide immediate sex classification in the field, a benefit of both morphometrics and behavior once the appropriate relationships are established. Nonetheless, the genetic procedures we used were relatively simple and expedient, resulting in highly accurate sex determination for this species. Genetic techniques may prove most useful in situations where morphometric analyses or behavioral observations cannot separate males from females. For example, we used this technique to determine the sex of newly hatched kittiwake young (Lanctot, Gill, and Hatch unpublished data). Our results indicate, however, that genetic sex identification should not be considered error-free.

The morphometric analyses we conducted provided reasonably high allocation rates for each group of kittiwakes we tested. We suspect that the predictive abilities of our models would have been higher had we definitive sex classifications for all individuals (i.e., we were only able to verify sex classifications with both behavior and genetics for 285 of the 605 birds). Consequently, it was likely that some birds (about 3%) in the analysis and prediction sub-samples were misclassified. Our comparison of behavior and genetic sex determinations (Table 1) suggested that any errors probably resulted in females being misclassified as males. Lower allocation rates for males than females in the complete sample support this possibility (Table 4). The DEE sample did not show this pattern, however, perhaps because these birds had all been sexed previously from behavior observations (i.e., having a known number of males and females was important for the daily energy expenditure study), making misclassifications unlikely.

The allocation rates from discriminant models of the DEE sample were always greater than those from their counterpart models of the complete sample. We therefore recommend using headbill and flattened wing versus culmen and natural wing when sexing kittiwakes. It is worthwhile, however, to investigate the cause of this disparity. For example, the difference in allocation rates may be a result of having one person measuring all kittiwakes from the DEE sample versus a few people measuring kittiwakes from the complete sample. Alternatively, there may be a difference in the natural variability of the length of the characters used in each data set (which may contribute to reduced sexual dimorphism for that character), or there may be differences in the overall precision and accuracy with which the characters in the different data sets can be measured. For example, the lower allocation rates from the culmen versus the headbill model do not appear to be a result of measurement error.

The ME for culmen was low (<1%) despite having numerous people measuring birds during two different years. Although we could not calculate a ME for headbill, we suspect it also would be low (Yezerinac *et al.* 1992). The CV associated with culmen length (4.2% when averaged for both sexes) was, however, almost twice that of headbill length (2.4%). Given the low ME for culmen it appears that the higher CV for culmen was due to natural variability. In fact, there appears to be greater overlap in culmen length between the sexes than in headbill length between the sexes (Fig. 1).

It is more difficult to determine why allocation rates were lower for the natural versus the flattened wing models. Despite having similar CV values for the two measures, models using flattened wing allocated an additional 10% of the individuals correctly. Caution should be applied, however, when using any measure of wing length as the measured value may be affected by wing-tip wear or moult.

Allocation rates obtained from discriminant models in this study were similar to those obtained for other larids. For example, either headbill length alone or in combination with other bill measures provided greater than 90% accuracy in sexing a variety of gulls occupying habitats from arctic to subtropical regions (Ingolfsson 1969; Fox *et al.* 1981; Coulson *et al.* 1983; Hanners and Paton 1985; Bosch 1996; Mawhinney and Diamond 1999). Headbill length was the best single variable available for predicting sex of kittiwakes in our study and its accuracy rate of 88% indicates it can serve as a simple but effective tool for quickly sexing kittiwakes in the field. Headbill measures have been successfully used to discriminate between sexes of Black-legged Kittiwakes in other geographic areas as well. For example, kittiwakes were sexed with an accuracy rate of at least 83% in England (Coulson *et al.* 1983), Norway (Barrett *et al.* 1985), and Russia (Tatarincova and Shklyarevich 1978), using headbill measures. It is critical to assess, however, whether a need exists for population-specific analyses of morphometrics. For example, mean headbill lengths of female and male kittiwakes were smaller in England (85.8 mm and 91.4 mm, respectively; Coulson *et al.* 1983), Norway (89.2 and 94.5 mm, respectively; Barrett *et al.* 1985), and Russia (90.3 mm and 95.0 mm, respectively; Tatarincova and Shklyarevich 1978) relative to the birds in our study area (94.1 and 99.6 mm, respectively, see Table 2). Had we attempted to sex our birds using data or discriminant functions from any of these studies, our misallocation rates would have been substantially higher. This highlights one of the shortcomings of using discriminant functions; the range and values of the measurements in question must be similar to those used to construct and test the models.

The ability to measure both birds from mated pairs improved the accuracy of our morphometric models considerably and resulted in classification levels nearly as high as those obtained from both genetics and behavior. We suspect that we would have achieved even higher levels of classification in our mate-based discriminant function models if we could have used headbill and flattened wing measures instead of culmen and natural wing measures; unfortunately, small sample sizes prohibited such an analysis.

This study suggests Black-legged Kittiwakes may be sexed accurately using either behavior, morphology, or DNA-based genetic techniques. Assuming birds are readily observable, behavior may be best suited to confirm results from the other two techniques once birds are captured and marked. The method of choice for sexing individuals, therefore, will be dictated by the situation at hand.

ACKNOWLEDGMENTS

We are grateful for office and field assistance from N. Bargmann, C. Bishop, S. Blanchard, E. Claus, B. Kempenaers, L. LeBourg, K. MacDougall, L. Pajot, A. Patirana, S. Schulte, R. Wilhite, and especially T. Mills, B. Toner, and K. Turco. Genetic analyses were conducted by G. Ibarguchi and I. Williams. T. Van Pelt provided blood samples from known-sex kittiwakes and J. Pearce helped test genetic primers. S. Talbot and V. Friesen kindly allowed us use of their molecular ecology laboratories. This research was supported by an Angus Gavin Memorial Bird Research Grant, the U.S. Geological Survey's Alaska Biological Science Center, the Alaska Natural Heritage Program, the Alaska Predator Ecosystem Experiment (APEX) and the *Exxon Valdez* Oil Spill Trustee Council (project no. 98163 G; the findings and conclusions are those of the authors and do not neces-

sarily reflect views or positions of the Trustee Council), the Konrad Lorenz Institute for Comparative Ethology, and the Oregon Cooperative Fish and Wildlife Research Unit. Support during the final stages of data analysis and writing was provided to RBL by the Belgium Fund for Scientific Research. John C. Coulson, David D. Duffy and an anonymous reviewer provided valuable comments on the manuscript.

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