Differentiation of subspecies and sexes of Beringian Dunlins using morphometric measures

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ABSTRACT. Five subspecies of Dunlins (*Calidris alpina*) that breed in Beringia are potentially sympatric during the non-breeding season. Studying their ecology during this period requires techniques to distinguish individuals by subspecies. Our objectives were to determine (1) if five morphometric measures (body mass, culmen, head, tarsus, and wing chord) differed between sexes and among subspecies (*C. a. actites, arcticola, kistchinski, pacifica,* and *sakhalina*), and (2) if these differences were sufficient to allow for correct classification of individuals using equations derived from discriminant function analyses. We conducted analyses using morphometric data from 10 Dunlin populations breeding in northern Russia and Alaska, USA. Univariate tests revealed significant differences between sexes in most morphometric traits of all subspecies, and discriminant function equations predicted the sex of individuals with an accuracy of 83–100% for each subspecies. We provide equations to determine sex and subspecies of individuals in mixed subspecies groups, including the (1) Western Alaska group of *arcticola* and *pacifica* (known to stage together in western Alaska) and (2) East Asia group of *arcticola*, *actites*, *kistchinski,* and *sakhalina* (known to winter together in East Asia). Equations that predict the sex of individuals in mixed groups had classification accuracies between 75% and 87%, yielding reliable classification equations. We also provide equations that predict the subspecies of individuals with an accuracy of 22–96% for different mixed subspecies groups. When the sex of individuals can be predetermined, the accuracy of these equations is increased substantially. Investigators are cautioned to consider limitations due to age and feather wear when using these equations during the non-breeding season. These equations will allow determination of sexual and subspecies segregation in non-breeding areas, allowing implementation of taxonomic-specific conservation actions.

RESUMEN. **Diferenciación entre subespecies y sexos de *Calidris alpina* usando medidas morfométricas**
Cinco subespecies de *Calidris alpina* que se reproducen en Beringia son potencialmente simpátricas durante la temporada no reproductiva. El estudio de la ecología durante este periodo requiere la clasificación de los individuos por subespecies. Nuestros objetivos en este estudio son determinar (1) si cinco medidas morfométricas (masa corporal, culmen, cabeza, tarso y cuerda alar) son diferentes entre los sexos y las subespecies (*C. a. actites, arcticola, kistchinski, pacifica,* y *sakhalina*) y (2) si estas diferencias son suficientes para permitir la correcta clasificación de los individuos usando ecuaciones derivadas de un análisis de función discriminante. Realizamos los análisis usando datos de morfología provenientes de 10 poblaciones de *Calidris alpina* que se reproducen en el norte de Rusia y en Alaska. Pruebas univariadas revelaron diferencias significativas entre los sexos en casi todos los caracteres morfométricos y todas las subespecies. Además, las ecuaciones de las funciones discriminantes predijeron el sexo de los individuos con una precisión del 83–100% para cada subespecie. Proveemos las ecuaciones para determinar el sexo y las subespecies de individuos que conforman grupos mixtos de subespecies, incluyendo los grupos de (1) Alaska Occidental compuesto por *arcticola* y *pacifica* (conocidos por agruparse en el oeste de Alaska) y (2) Asia Oriental compuesto por *arcticola, actites, kistchinski* y *sakhalina* (conocidos por pasar el invierno juntos en el este de Asia). Las ecuaciones que predicen el sexo de los individuos en grupos mixtos tuvieron una precisión en la clasificación de 75–87% resultando en ecuaciones de clasificación confiables. También proveemos ecuaciones que predicen la subespecie de los individuos con una precisión de 22–96% para diferentes grupos mixtos de subespecies. Cuando el sexo de los individuos puede ser previamente determinado, la precisión de estas ecuaciones

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incrementa sustancialmente. Sugerimos que los investigadores tomen las precauciones necesarias considerando las limitaciones de las ecuaciones debido a la edad y el desgaste del plumaje cuando se usen en individuos durante la temporada no reproductiva. Estas ecuaciones permitirán la determinación sexual y la segregación de las subespecies en áreas no reproductivas permitiendo la implementación de acciones de conservación que sean específicas para cada taxón.

Migratory birds travel long distances between breeding and non-breeding areas, frequently using multiple stopover or staging sites along the way. Segregation arising from different migration patterns has been documented across subspecies, populations, sexes, ages, and morphological types (Nebel et al. 2002, O'Hara et al. 2006, Nebel 2007). Moreover, segregation has been observed over large geographic distances (Nebel et al. 2002) and at local levels (Fernández and Lank 2006, Choi et al. 2011). The extent to which individuals from the same breeding area segregate spatially or temporally at non-breeding areas can differentially affect mortality and reproduction, and consequently affect population size (Webster et al. 2002). Consequently, determining how populations and particular groups of individuals are distributed away from breeding areas is an important challenge for conservation.

Dunlins (*Calidris alpina*) breed in the circumpolar Arctic and sub-Arctic and migrate to more southerly portions of the Northern Hemisphere to spend the non-breeding season. Of 10 described subspecies (Engelmoer and Roselaar 1998), five breed in distinct areas of Beringia, i.e., the geographic region comprising the Bering Strait and adjacent lands of the Russian Far East and Alaska (Fig. 1). All five of these subspecies have migratory or wintering ranges that partially overlap those of at least one other subspecies (Warnock and Gill 1996, Message and Taylor 2005, Marthinsen et al. 2007). Dunlins breeding in western (*pacifica* subspecies) and northern Alaska (*arcticola* subspecies) co-occur on the Yukon-Kuskokwim Delta in western Alaska during the fall staging period (Warnock and Gill 1996), although the extent of the temporal and spatial overlap is poorly known (Gill et al. 2013, Warnock et al. 2013). The *pacifica* subspecies then migrates along the Pacific Flyway to winter along the west coast of North America, whereas the *arcticola* subspecies continues its migration along the East Asian-Australasian Flyway to winter in Japan, Taiwan, North and South Korea, and China (Warnock and Gill 1996, Lanctot et al. 2009). On the non-breeding grounds, the *arcticola* subspecies co-occur with three other subspecies (*actites*, *kistchinski,* and *sakhalina*) that migrate to East Asia from breeding areas in northern Russia (Bamford et al. 2008, Cao et al. 2009, Lanctot et al. 2009). As with *pacifica* and *arcticola* in western Alaska, little is known about how these subspecies, which differ greatly in population size (estimates range from 2000 to 1 million birds; Blokhin et al. 2004, Bamford et al. 2008, Andres et al. 2012), distribute spatially and temporally in this vast area. Sexual segregation has been documented for the *pacifica* subspecies along the west coast of North America and for Dunlins (no subspecies identified) in Taiwan, but additional data are needed to confirm these patterns (Shepherd et al. 2001, Yang et al. 2012).

Our objective was to develop reliable and easily implemented equations to determine the subspecies and sex of individual Dunlins captured in non-breeding areas in East Asia and the Pacific coast of North America. Because Dunlins sampled at these locations and times are generally not in breeding plumage, we used body measurements (e.g., culmen, head, tarsus, and wing chord) and DNA analysis to determine sex. We focused our analysis on groups of subspecies known to overlap at migratory stopover and wintering sites. Because female Dunlins are generally larger than males (e.g., Hayman et al. 1991), we also developed statistical models to predict sex. We then incorporated this information into our subspecies models to determine if determination of sex improved model performance. We used morphological characters that exhibit little variation throughout the annual cycle and that can be quickly measured to determine subspecies and sex in the field. Similar techniques have been used to identify subspecies (Merendino et al. 1994, Saitoh et al. 2008, Yeung et al. 2009) and
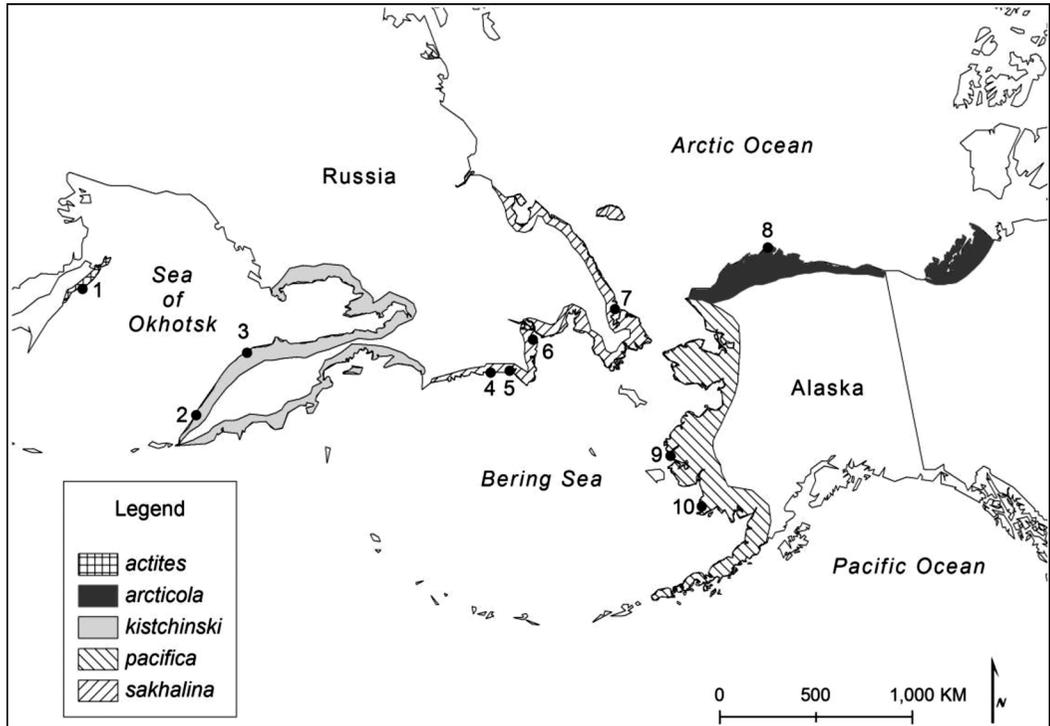
Fig. 1. Breeding distribution and sampling sites for the five Beringian Dunlin subspecies included in this study. Subspecies breeding ranges from original taxonomic descriptions and field studies (Browning 1991, Engelmoer and Roselaar 1998, Lappo et al. 2012). Numbers in figure refer to sampling sites described in Table 1.

sexes (Shealer and Cleary 2007, Meissner and Pilacka 2008, Dechaume-Moncharmont et al. 2011) in a variety of avian taxa.

METHODS

Field methods.   We captured or collected adult Dunlins during the breeding season (mid-June to mid-July) in 10 separate breeding populations representing five subspecies (Table 1, Fig. 1). Most of *kistchinski* and *sakhalina* individuals were collected and measurements were taken on fresh specimens within 24 h. All *actites*, *pacifica*, and *arcticola* individuals were captured on nests, measured within 30 min, and then released. We relied on data collected from Dunlins sampled within the breeding ranges to develop our models because birds sampled in wintering areas cannot be accurately assigned to a subspecies. Dial calipers were used to measure lengths (±0.1 mm) of the culmen (from bill tip to the base of the feathering near the nares),

head (from the back of the skull to the tip of the bill), and tarsus (from the tibio-tarsal joint diagonally to the end of the tarsal bone; Gratto-Trevor 2004). A stop-end ruler was used to measure the length of the wing chord (±1 mm; from the wrist to the tip of the longest primary, measured flattened and straightened; Gratto-Trevor 2004). To reduce between-observer measurement biases, we limited the number of people contributing data to those well trained in standardized shorebird measurements at each site. The wings of birds molting their outer primaries were not measured. Because wings of *actites* were flattened, but not straightened, we used linear regression to relate flattened and straightened wing-chord measurements to flattened wing-chord measurements for a sample of *sakhalina* birds (flattened and straightened wing chord = 28.115 + 0.808 * flattened wing, $r^2 = 0.75$, $N = 88$; P. Tomkovich, unpubl. data). We used this relationship to adjust the *actites* wing-chord measurements, but suspect this resulted

Table 1. Sampling sites, years of sampling, and sample sizes for Dunlins used in building discriminate function models. Site numbers match those in Fig. 1.

Subspecies	General location	Site	Latitude and longitude	Year(s)	*N*
actites	Sakhalin Island, Russia				
	Chaivo Bay	1	52°31′N, 143°17′E	2007, 2009	25
kistchinski	Kamchatka, Russia				
	Bolshaya River	2	52°48′N, 156° 25′E	2009	30
	Fchun River	3	56°30′N, 155°59′E	1989	13
sakhalina	Chukotka, Russia				
	Khatyrka River	4	62°7′ N, 175° 26′E	2005	2
	Meinypilgyno	5	62°31′N, 177°1′E	2009	8
	Vtoraya River	6	64°22′N, 177°25′E	2000	23
	Belayaka Spit	7	67°3′N, 174° 37′W	1986 – 2002	29
arcticola	Northern Alaska, USA				
	Barrow	8	71°14′N, 156° 33′W	2003 – 2009	341
pacifica	Western Alaska, USA				
	Manokinak River and	9	61° 11′N, 165° 5′W	2009	38
	Kanaryarmiut Field Station				
	Platinum Spit	10	59° 1′N, 161°49′W	2009	8

in a small amount of error given *sakhalina* wing chords are longer than *actites* wing chords in general. Body mass was determined using a 100-g Pesola® scale (±1 g).

As a source of DNA from captured individuals, we collected either blood (10–50 μl) from the brachial vein or the following feathers: 1–2 primaries, 1–2 secondaries, or 10–15 breast feathers. Blood samples were typically stored in buffer (Longmire et al. 1988) or, less frequently, in 90% ethanol. Feathers were stored in dry paper envelopes until processed in the laboratory.

Sexing. We determined the sex of 542 individuals using DNA analysis (Jae-Ik et al. 2009) and 86 individuals by examination of gonads during specimen preparation. Of 491 individuals used to generate discriminant function models, 82% were sexed using DNA and 18% by examination of gonads. We extracted DNA from blood or feathers and purified it using a modified version of the salting-out protocol described by Medrano et al. (1990). We amplified portions of the CHD-W/CHD-Z genes via the polymerase chain reaction (PCR) using the P2 and P8 primers identified in Griffiths et al. (1998). Primer and PCR procedures are available from the senior author. We electrophoresed fluorescently labeled PCR products through an 18-cm, 6% polyacrylamide gel on a LI-COR 4200L automated sequencer (LI-COR, Lincoln, NE, USA). We scored images using GeneImageIR™

4.05 software (Scanalytics, Inc., Fairfax, VA, USA).

For confirmation of DNA diagnosis of sex, we analyzed DNA from three males and five females whose sex had been confirmed by dissection. Repeat tests yielded identical results from 58 separate PCRs of the same blood-derived DNA samples and 68 separate PCRs of the same feather-derived DNA samples. Separate samples collected independently from the same individual in different years gave identical PCR results in 79 of 80 cases. The one discrepancy was an individual where a blood sample and a feather sample each amplified as male, and a second feather sample collected in another year amplified repeatedly as female. We could not distinguish between misidentification, mislabeling, contamination, or mutation as the cause of this discrepancy, and consequently did not use this individual in our analyses. The apparent error rate for sexing (1.25%) was low.

Data analysis. We tested each morphometric character for sexual dimorphism and differences between subspecies using a two-way ANOVA with subspecies and sex as factors, and used Tukey-Kramer honestly significant difference (HSD) tests for multiple comparisons (Zar 1999). We tested the morphometric characters of each subspecies for sexual dimorphism using a one-way ANOVA with sex as a factor. For subspecies with multiple sampling locations, we examined possible differences in morphometric

values across locations using a one-way ANOVA with location as a factor. We then used Tukey-Kramer HSD tests for multiple comparisons.

We used multivariate methods that depend upon complete sets of measurements for each individual. We used a restricted maximum likelihood method to generate "imputed" values for individuals missing head measurements using the variance–covariance matrix generated from individuals with complete sets of measurements (Krzanowski 1988). Imputed values were generated separately for each subspecies. This method has been shown to generate reliable estimates for missing measurements in morphometric analyses (Strauss et al. 2003). In total, we imputed head length for 41 individuals (8% of $N = 491$), including three male and one female *actites* (16%, $N = 25$), nine male and four female *kistchinski* (25%, $N = 53$), and 17 male and seven female *sakhalina* (67%, $N = 46$).

We developed three different groups of models and used discriminant function analysis to classify individuals to sex (Hair et al. 2010). In the first group, we separately developed discriminant functions for each subspecies that incorporated one or more of the morphometric measurements. This allows researchers studying a particular subspecies on the breeding grounds to differentiate the sex of individuals. In the second group, we considered all individuals from the two subspecies that co-occur in Alaska (*arcticola* and *pacifica*); in the third, all individuals from the four subspecies that co-occur in East Asia (*arcticola*, *actites*, *kistchinski*, and *sakhalina*). These second and third groups assess whether sex ratios in mixed-subspecies assemblages can be estimated without knowing the subspecies of each individual. We excluded body mass from the predictor variables in the second and third groups because shorebird mass can be highly variable away from the breeding grounds (Warnock et al. 2013). In all models, "prior probabilities" for each sex were set as equal and we used the resubstitution method to estimate the proportion of individuals classified correctly (Hair et al. 2010). We present results for the best discriminant function model with and without mass or, in some cases, the two best models when their classification rates and squared canonical correlation (SCC) values were similar. To classify an individual as male or female, its measurements are substituted into the discriminant function equation to calculate a discriminant function score. Individuals with discriminant function scores above the cutting score are predicted to be female; those below the cutting score are predicted to be male. Discriminant function scores further from the cutting score imply a higher probability that an individual is correctly assigned.

We developed two different groups of models to classify individuals to subspecies. We considered the subset of subspecies that co-occur in Alaska (*arcticola* and *pacifica*) in the first group, and the subspecies that co-occur in Asia (*arcticola*, *actites*, *kistchinski*, and *sakhalina*) in the second group. For each group, we developed models under two scenarios: where both sex and subspecies were unknown, and where an individual's sex was known, but not its subspecies. The second scenario assesses whether estimation of subspecies ratios in mixed-subspecies assemblages can be improved if sex is assessed independently, such as from DNA. Prior probabilities for each subspecies were set as equal (i.e., each individual had an equal chance of being in any subgroup). To classify subspecies using the first group of models, an individual's measurements were substituted into the discriminant function to calculate a discriminant function score. Individuals with discriminant function scores above the cutting score were predicted to be *pacifica*, and those with scores below the cutting score were predicted to be *arcticola*. To classify subspecies using the second group of models, an individual's measurements were used to calculate a discriminant function score for each of the four subspecies-specific models. The subspecies model that yielded the highest discriminant function score was the most probable subspecies (Hair et al. 2010). Body mass was excluded as a variable in these analyses. We used JPM (8.0.2, 2008–2010) software for all statistical analyses.

RESULTS

Morphometric comparisons between subspecies and sex. Body measures differed significantly among subspecies and between sexes (culmen: $F_{5,485} = 164.3$, head: $F_{5,485} = 170.6$, tarsus: $F_{5,485} = 31.7$, wing chord: $F_{5,485} = 74.4$, and mass: $F_{5,485} = 156.8$, all $P < 0.001$, Fig. 2), and most morphological measures differed between subspecies in pairwise comparisons (Fig. 2). Females were significantly larger than males for all morphometric measurements
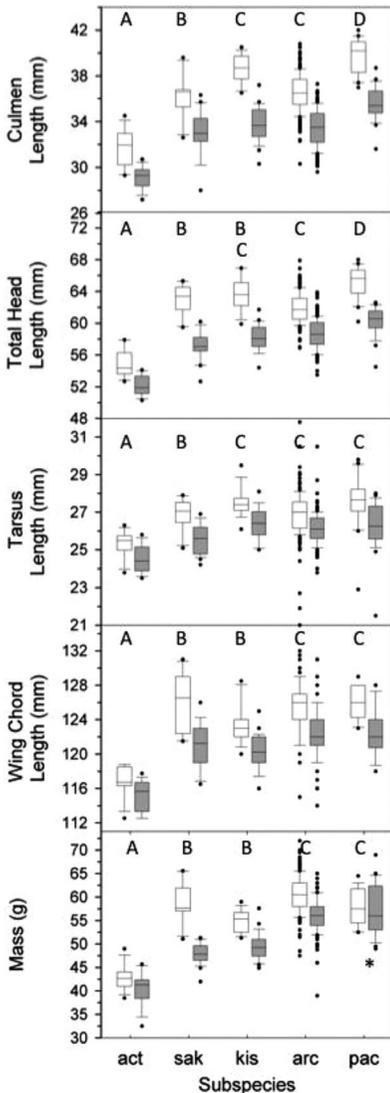
Fig. 2. Distribution of morphometric measurements of adult male (dark gray) and female (white) Dunlins belonging to five subspecies. Box plots include the median (horizontal bar within box), quartile range (box), 10th and 90th percentiles (whiskers), and outliers (filled circles). Combined male and female measurements from subspecies identified with the same letter (noted along the top of each figure) were not statistically different. All measurements differed significantly between the sexes of each subspecies except one noted with a "*" ($P > 0.05$). Subspecies along the x-axis are organized geographically from east to west, south to north: *C. alpina actites* (act, $N = 12$ females, 13 males), *sakhalina* (sak, $N = 10$ females, 26 males), *kistchinski* (kis, $N = 17$ females, 26 males), *arcticola* (arc, $N = 181$ females, 160 males), and *pacifica* (pac, $N = 20$ females, 26 males).

in all subspecies (all $P < 0.05$) except body mass of *pacifica* females was the same as that of males ($F_{1,44} = 0.2$, $P = 0.66$). The magnitude of these differences varied with subspecies and morphological character (Fig. 2).

Morphometric comparisons between subspecies measured at more than one location. Certain morphological features differed significantly between sampling locations for *pacifica*, *kistchinski*, and *sakhalina*. For *pacifica*, tarsus ($F_{1,24} = 13.6$, $P = 0.001$) and mass ($F_{1,24} = 5.6$, $P = 0.027$) differed between locations for males, and wing chord ($F_{1,18} = 5.8$, $P = 0.027$) differed for females. For *sakhalina*, mass differed among females ($F_{1,8} = 5.5$, $P = 0.047$). For *kistchinski*, the head ($F_{1,24} = 5.7$, $P = 0.025$), tarsus ($F_{1,24} = 12.2$, $P = 0.002$), and wing chord ($F_{1,24} = 5.6$, $P = 0.027$) differed between locations for males, and head ($F_{1,15} = 6.9$, $P = 0.019$) differed for females. The *actites* and *arcticola* subspecies were sampled at only one location.

Discriminant function models to determine subspecies and sex. Discriminant function models predicted the sexes of individuals of each subspecies with an accuracy of 83–100% (Table 2). Models to predict the sexes of individuals in mixed groups of *arcticola* and *pacifica* or mixed groups of *actites*, *arcticola*, *kistchinski*, and *sakhalina* had classification accuracies of 75–87% (Table 3). The best discriminant function model for distinguishing *arcticola* and *pacifica* when sexes were unknown correctly classified 78% and 69% of *pacifica* and *arcticola*, respectively (Table 4). Subspecies classification was more accurate when sex was known; the best model involving only males correctly classified 89% and 82% of *pacifica* and *arcticola* individuals, respectively, and the best model involving only females correctly classified 75% and 81% of *pacifica* and *arcticola* individuals, respectively (Table 4). When sex was unknown, a discriminant function model for distinguishing among *actites*, *kistchinski*, *arcticola*, and *sakhalina* subspecies correctly classified 96% of *actites* individuals, 72% of *kistchinski* individuals, and <45% of *arcticola* and *sakhalina* individuals (Table 5). Models for known-sex birds had higher classification accuracies (Tables 5 and 6). The best model involving only males correctly classified 100% of *actites*; all other subspecies had correct classification rates below 60%. The best model involving only females correctly classified 100% of *actites*

Table 2. Discriminant function models for predicting the sex of five subspecies of adult Dunlins that occur in Beringia. Models were developed using five morphometric measures: culmen (C), head (H), tarsus (T), wing chord (W), and body mass (BM). Two models are presented for each subspecies; the second model does not include body mass. Sample sizes in italics.

Discriminant function models	SCC[a]	Wilks' Lambda	F-value	df	Cutting score >= ♀	Sample size and % correct classification		
						♀	♂	Total
Sex of *actites*						*11*	*14*	*25*
0.6641 * (C) + 0.1472 * (H) + 0.8976 * (T) − 0.0125 *(W) − 0.1271 * (BM)	0.790	0.4	6.3	19	43.74	92	100	96
0.6747 * (C) + 0.7659 * (T)	0.771	0.4	16.1	22	39.68	83	100	92
Sex of *kistchinski*						*17*	*26*	*43*
0.5955 * (C) + 0.1684 * (BM)	0.888	0.2	74.6	40	30.50	100	96	98
0.5549 * (C) + 0.0384 * (H) + 0.2869 * (T) + 0.1395 * (W)	0.881	0.2	32.8	38	47.18	100	96	98
Sex of *sakhalina*						*10*	*26*	*36*
0.4531 * (C) + 0.6021 * (H) + 0.5504 * (T) + 0.1094 * (W) + 0.1474 * (BM)	0.915	0.2	30.9	30	56.14	90	100	97
0.3951 * (C) + 0.2703 * (W)	0.774	0.4	24.7	33	47.31	100	96	97
Sex of *arcticola*						*181*	*160*	*341*
0.4513 * (C) + 0.0615 * (T) + 0.1244 * (W) + 0.0762 (BM)	0.734	0.5	98.2	336	37.31	88	86	87
0.5049 * (C) + 0.1034 * (T) + 0.1408 * (W)	0.721	0.5	121.5	337	37.87	87	85	86
Sex of *pacifica*						*20*	*26*	*46*
0.2743 * (C) + 0.2794 * (H) + 0.2227 * (T) + 0.1468 * (W) + 0.0102 * (BM)	0.861	0.3	23.0	40	52.70	95	96	96
0.2855 * (C) + 0.2693 * (H) + 0.2215 * (T) + 0.1489 * (W)	0.861	0.3	29.4	41	52.12	95	96	96

[a]Squared canonical correlation.

(100%) and 94% of *kistchinski*, but <60% of *arcticola* and *sakhalina* individuals.

DISCUSSION

The discriminant function equations we developed correctly identified between 86% and 98% of males and females for each subspecies of Dunlin (sexes combined, Table 2). Relative to other studies of Dunlins, our models for predicting sex performed well. For example, our 96% classification success rate for *pacifica* Dunlins was more reliable than reported by Brennan et al. (1984; 91.5%), Shepherd et al. (2001; 88%), and Page (1974; 69%). Comparable subspecies models have not been developed for the other Beringian subspecies.

When subspecies were pooled to mimic mixed-subspecies aggregations that occur away from the breeding grounds, we were able to correctly determine the sex of >75% of individuals (Table 3). Other studies attempting to determine sex of waterbirds using discriminant function analysis report similar ranges (76–96%) of classification accuracy (Jodice et al. 2000, Meissner 2005, Gunnarsson et al. 2006, Shealer and Cleary 2007, Meissner and Pilacka 2008). Studies of survival, site fidelity, habitat use, lifetime productivity, and natural history are greatly enhanced when the sex of individuals is known (Dinsmore et al. 2002, Sandercock et al. 2005). Conversely, analyses without sex-specific data can lead to erroneous interpretation (Hanowski and Niemi 1990, Ellegren and Sheldon 1997).

Table 3. Discriminant function models for determining sex of adult Dunlins that occur in the East-Asian Australasian Flyway (i.e., *actites, kistchinski, sakhalina,* or *arcticola*) and in western Alaska (i.e., *arcticola* and *pacifica*). Models were developed using four morphometric measures: culmen (C), head (H), tarsus (T), and wing chord (W). Sample sizes in italics.

Discriminant function models	SCC[a]	Wilks' Lambda	*F*-value	df	Cutting score $> = ♀$	Sample size and % correct classification		
						♀	♂	Total
Sex of *actites, kistchinski, sakhalina,* and *arcticola*						*60*	*84*	*144*
0.0521 * (C) + 0.3196 * (H) − 0.1036 * (T) − 0.0204 * (W)	0.559	0.7	15.8	139	15.30	75	82	79
Sex of *arcticola* and *pacifica*						*46*	*54*	*100*
0.1324* (C) + 0.2499* (H) + 0.1646 * (W)	0.718	0.5	34.1	96	40.46	83	87	85

[a]Squared canonical correlation.

Table 4. Discriminant function models for determining if adult Dunlins are either *arcticola (arc)* or *pacifica (pac)* subspecies, both when sex is known and not known. Models were developed separately for males and females, and with males and females combined to simulate a situation where the sex of an individual was not known. Models were developed from four morphometric measures: culmen (C), head (H), diagonal tarsus (T), and wing chord (W). Sample sizes in italics.

Discriminant function model	SCC[a]	Wilks' Lambda	*F*-value	df	Cutting score $> = pac$	Sample size and % correct classification		
						arc	pac	Total
Sex is unknown						*54*	*46*	*100*
0.6755 * (C) − 0.2210 * (H) + 0.0152 * (T) − 0.1578 * (W)	0.553	0.7	10.4	95	−8.33	69	78	73
Sex is male						*28*	*26*	*54*
0.7476 * (C) − 0.0833 * (H) + 0.0745 * (T) − 0.0617 * (W)	0.653	0.6	9.1	49	15.14	82	89	85
0.7537 * (C) − 0.0937 * (H) − 0.0663 * (W)	0.652	0.6	12.3	50	12.21	82	89	85
Sex is female						*26*	*20*	*46*
0.6299 * (C) − 0.0203 * (H) + 0.0532 * (T) − 0.0432 *(W)	0.726	0.5	11.4	41	18.73	81	75	78
0.6104 * (C) + 0.0521 *(T) − 0.0440 * (W)	0.726	0.5	15.6	42	19.15	81	75	78

[a]Squared canonical correlation

When investigators need to determine the sex of individuals with greater certainty, molecular techniques outperform morphometric-based approaches, although these techniques are also not 100% accurate (Jodice et al. 2000, Dubiec and Zagalska-Neubauer 2006, Robertson and Gemmell 2006, our study).

We also examined the feasibility of using morphometric measures to differentiate two subspecies that aggregate during fall staging in western Alaska, and four subspecies that potentially aggregate during migration and the non-breeding season in East Asia. In general, these models performed less well than the sex-specific models, with classification rates of 62–73% for the best models (Tables 4 and 5). These models improved when the sex of individuals was included, reaching 78–85% classification accuracy for the *arcticola* and *pacifica* group,

Table 5. Discriminant function models for determining whether adult Dunlins are *actites* (*act*), *arcticola* (*arc*), *kistchinski* (*kis*), or *sakhalina* (*sak*) subspecies when sex is not known. Models were developed separately for males and females and with males and females combined to simulate a situation where the sex of an individual was not known. Models were developed from four morphometric measures: culmen (C), tarsus (T), head (H), and wing chord (W). See Table 6 for model constants, variables and their associated coefficients for models where sex is known, and the Methods section for information concerning how to use models to determine the most likely subspecies of an individual. Sample sizes in italics.

Discriminant function models	SCC[a]	Wilks' Lambda	Est.-F	df	Sample size and % correct classification				
					act	*arc*	*kis*	*sak*	*Total*
Sex is unknown					*25*	*30*	*30*	*30*	*115*
$0.2102*(C)+0.2089*(W)+0.2352*(T)$	0.730	0.3	17.1	9	96.0	43.3	73.3	40.0	61.7
$0.2199*(C)+0.2216*(W)$	0.728	0.4	24.6	6	96.0	36.6	63.3	40.0	57.4
$0.3287*(W)$	0.698	0.5	35.2	3	88.0	46.7	33.3	20.0	45.2
Sex is male					*13*	*19*	*26*	*26*	*84*
See Table 6 for model equations	0.881	0.2	20.3		100	58	39	53	57
Sex is female					*12*	*21*	*17*	*10*	*60*
See Table 6 for model equations	0.867	0.2	17.1		100	57	94	60	77

[a] Squared canonical correlation.

Table 6. Discriminant function model constant and coefficients for classifying adult Dunlins to subspecies (*actites*, *arcticola*, *kistchinski,* or *sakhalina*) when sex is known. Models were developed using measures of exposed culmen, diagonal tarsus, head, and flat wing chord. "n/a" indicates that the character was not included in the model. See Methods section for information concerning how to use models to determine the most likely subspecies of an individual.

	Constant	Culmen	Head	Tarsus	Wing
Male					
actites	−5447.489	−157.970	197.429	n/a	45.032
arcticola	−6265.053	−167.900	211.802	n/a	47.863
sakhalina	−6137.366	−164.803	209.203	n/a	47.199
kistchinski	−6102.507	−164.547	208.142	n/a	47.346
Female					
actites	−2390.473	17.0620	n/a	5.384	35.141
arcticola	−2730.033	19.5194	n/a	4.663	37.429
sakhalina	−2777.058	21.4103	n/a	6.340	36.876
kistchinski	−2821.453	19.8164	n/a	4.965	38.010

and 57–77% for the four subspecies that aggregate in East Asia (Tables 4 and 5). The accuracy of our models was better for identifying *arcticola* during fall staging in western Alaska than in East Asia. Within the East Asia subspecies group, the *actites* subspecies were easiest to identify (100% assignment for both sexes), followed by *kistchinski* females (94%), and the remaining subspecies and sex combinations (39–60%, Table 5). Researchers using similar analytical tools to discriminate subspecies of Canada Geese (*Branta canadensis*) and Arctic Warblers (*Phylloscopus borealis*) reported higher rates of correct classification (76–96%; Merendino et al. 1994, Saitoh et al. 2008), possibly because these subspecies were more morphologically distinct.

Overlap in morphometric measures among subspecies caused certain discriminant functions to have low classification accuracies. This overlap was likely reinforced by differences in morphological traits among populations within a subspecies. We suspect that within-subspecies differences result from local adaptation or clinal variation (Ely et al. 2005, Conklin et al. 2011). Previous researchers noted overlap in morphometric measurements among subspecies

and differentiated them based on differences in alternate plumage color and pattern (Nechaev and Tomkovich 1987, Browning 1991, Choi et al. 2011). However, the alternate plumage of Dunlins is not retained throughout the annual cycle and provides no insight into their likely subspecies during the non-breeding period. Other methods for identifying subspecies during the non-breeding period also have limitations. For example, resighting birds in non-breeding areas that were originally banded on the breeding grounds suffers from low return rates and biases due to differences in the geographic scope of search effort and reporting (Lanctot et al. 2009). Use of genetic and stable isotope markers is expensive and suffers from other limitations. Genetic markers require fixed allelic differences between subspecies, differences that may not be present depending on the evolutionary history and gene flow between populations (e.g., Wennerberg 2001). Stable isotope markers require known geographic isoscapes, knowledge of when animals incorporate isotopes into tissues, and turnover times within tissue types (Hobson and Wassenaar 1997). Given these limitations, both genetic and isotopic analyses have yielded imprecise and inconclusive results in previous studies (Wenink et al. 1996, Wennerberg 2001, Lovette et al. 2004, Kelly et al. 2008). In recent years, light-level geolocation has enhanced our ability to estimate spatial and temporal movements of individuals (Clark et al. 2010), but this approach is expensive, typically results in data from small numbers of individuals, and provides limited geographic resolution. In short, although our approach does not always provide 100% accuracy, our models rely on simple morphological measurements and provide an inexpensive and rapid method for identifying Dunlin subspecies. In certain cases, morphometric data sets already exist for retrospective assessment of temporal and spatial segregation from different parts of western Alaska (Gill et al. 2013), China (Z. Ma, pers. comm.), and Taiwan (C. Chiang, pers. comm.). The equations we present could be used to identify subspecies and calculate sex ratios in non-breeding areas.

Our equations have some limitations for use on the non-breeding areas. First, because we had to collect morphological data from breeding birds to ensure subspecies identification, we could not control for changes in morphological measurements that take place during migration.

For example, feather wear on wings is likely to occur and may differ among subspecies because they migrate different distances. However, wing chord length is not always included in our models and frequently has the lowest weighting factor. Nevertheless, this could decrease the difference in wing chord length between some subspecies, lessening our ability to differentiate between them. It seems less likely that males and females experience different flight feather wear because both sexes migrate similar distances. Feather wear might also vary by age, although two studies on *pacifica* Dunlin revealed no relationship between wear and bird age (Brennan et al. 1984, Shepherd et al. 2001; but see studies of other Dunlin subspecies; Meissner 2005, Yosef and Meissner 2006). Nevertheless, we caution investigators at non-breeding sites to limit use of these equations to after-hatch year birds (the age class our data were predominantly drawn from), and to weigh their results against the likelihood of correctly assigning subspecies and sexes to individuals. For example, analyses indicating the presence of the *actites* subspecies, which had nearly 100% correct assignment rates, should be considered more reliable than analyses indicating the presence of *arcticola* or *sakhalina*, which had much lower correct assignment rates. Further, our discriminant function analysis equations would benefit from independent validation and having data from more individuals, particularly for the Russian-breeding subspecies. Our small sample sizes for these subspecies may have artificially inflated correct classification rates if within-group variability was underestimated (Dechaume-Moncharmont et al. 2011). Also, our discriminant function models were developed under the assumption that all subspecies within each of our groupings had an equal probability of being present. This is not likely true, given the large differences in population sizes among subspecies (Blokhin et al. 2004, Bamford et al. 2008, Andres et al. 2012). However, adjusting the models to account for these differences is not possible until we have more accurate information about population size and knowledge of how these subspecies segregate. Despite these limitations, our models will likely be useful for differentiating sex and subspecies in many situations. We recommend mathematically combining this approach with others (see above) to improve assignment accuracy.

Spatial and temporal segregation of Beringian Dunlins by subspecies or sexes on the non-breeding grounds has important conservation implications. First, subspecies may be differentially affected by habitat degradation along the East Asian-Australasian Flyway. For example, natural intertidal areas in the Yellow Sea region of China and South Korea are being converted for human use at higher rates than in other regions in the flyway (Yang et al. 2011, MacKinnon et al. 2012). Second, certain areas of the Asian non-breeding grounds have had widespread avian disease outbreaks, including the highly pathogenic avian influenza virus H_5N_1, which has proven particularly virulent for Dunlins (Hall et al. 2011). Knowledge of how the four Dunlin subspecies that use this area segregate in time and space is important for evaluating likelihood of exposure to H_5N_1 and other pathogens (Gilbert et al. 2007), which, in turn, informs how birds may transmit disease to other areas of the world (Ip et al. 2008). Third, temporal segregation of Dunlins at non-breeding sites could make certain groups more susceptible to seasonal management practices. For example, seasonal changes in aquaculture practices reduce the amount of habitat available to shorebirds wintering in East Asia during certain times of the year (Yang and Lee 2007, Ma et al. 2009).

Information about the temporal and geographic segregation of Dunlins by subspecies and sex is limited in both the Pacific and East Asian-Australasian flyways. Temporal segregation of Dunlins belonging to different age classes may also occur (Buchanan et al. 1986). For example, the ratio of adults to hatch-year birds shifts throughout the year at Chongming Dongtan estuarine wetland near Shanghai, China (Choi et al. 2011). Within the *pacifica* subspecies, Shepherd et al. (2001) postulated that there were two distinct populations that segregate along the Pacific Coast of the United States, and females in each population winter farther north. Comparable information from the East Asian-Australasian flyway is sparse despite efforts to understand migratory connectivity (Lanctot et al. 2009, Yang et al. 2012). Segregation has been documented in other shorebirds in both flyways, including Western (*C. mauri*) and Least (*C. minutilla*) sandpipers in the Pacific Flyway (Nebel et al. 2002, Nebel 2006), and Curlew Sandpipers (*C. ferruginea*),

Eastern Curlews (*Numenius madagascariensis*), Bar-tailed Godwits (*Limosa lapponica*), and Sanderlings (*C. alba*) in the East Asian-Australasian flyway (Nebel 2007). In these species, individuals were segregated by age, sex, and morphometrics. Spatial segregation of the Dunlin subspecies using the East Asian-Australasian Flyway would be especially important to know for the *actites* subspecies because its small population size makes it especially vulnerable to population perturbation.

Management and conservation of long-distance migratory bird populations requires an understanding of annual distribution and migratory connectivity. At present, we have insufficient information about the distribution of Beringian Dunlins outside the breeding season to draft effective conservation plans. Applying our models to the five non-breeding populations of Dunlin discussed herein, however, will help determine spatial and temporal distribution patterns and enable relevant conservation action. To best use our equations for subspecies delineation, we recommend determining the sex of individuals and then applying equations where sex is known, limiting analyses to after-hatch-year birds, and weighing the results based on the classification accuracy for each subspecies. We further recommend that large sample sizes (i.e., 200–300 individuals) be obtained at non-breeding locations to minimize potential sample biases (Brennan et al. 1991).

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