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Sex determination of three raptor species using morphology and molecular techniques

Sarah Pitzer,[1,5] Joshua Hull,[1] Holly B. Ernest,[1,2] and Angus C. Hull[3,4]

[1] *Wildlife and Ecology Unit, Veterinary Genetics Laboratory, University of California, Davis, California 95616, USA*
[2] *Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California 95616, USA*
[3] *Golden Gate Raptor Observatory, Building 201, Fort Mason, San Francisco, California 94123, USA*

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ABSTRACT. Accurate identification of sex is important for many raptor studies, but may be difficult to determine in the field for some species. Because of size differences between males and females, morphological measurements have often been used to sex raptors. However, few investigators have examined the accuracy of using measurements of individuals made at one location to sex individuals of the same species at another location. Our objective was to develop more accurate region-specific methods for determining the sex of Red-tailed Hawks (*Buteo jamaicensis*), Red-shouldered Hawks (*B. lineatus*), and Cooper's Hawks (*Accipiter cooperii*) migrating through and wintering in California. We determined sex using a polymerase chain reaction (PCR)-based genetic test and grouped individuals based on sex, age, and geographic area. We did not combine groups due to differences in measurements between age classes and geographic areas. We then compared a suite of morphological measurements between males and females of each combination, and developed both a discriminant function and a flowchart to determine the sex of Red-tailed and Red-shouldered hawks in the field. The flowcharts were more accurate than the functions for both species. We also confirmed the accuracy of the current flowchart used to determine the sex of Cooper's Hawks migrating along the California coast. These region-specific methods for Red-tailed and Cooper's hawks were generally more accurate than published methods, possibly indicating different populations of these species and highlighting the importance of validating sexing methods when using them in different locations.

SINOPSIS. **Determinación del sexo en tres especies de rapaces utilizando morfometría y técnicas moleculares**

La identificación correcta del sexo es importante en muchos estudios con rapaces y puede ser difícil de determinar en el campo para algunas especies. Dada la diferencia en tamaño entre machos y hembras de estas aves, comunmente se ha utilizado la morfometría para sexarlos. Sin embargo, muy pocos investigadores han examinado la exactitud de la morfometría en el sexado de individuos de localidades diferentes. Nuestro objetivo fue el desarrollar un método más exacto y específico a regiones particulares, para determinar el sexo del Halcón de Cola Roja (*Buteo jamaicensis*), el Halcón de Hombro Rojo (*B. lineatus*) y el Halcón de Cooper (*Accipiter cooperi*), los cuales migran y pasan el invierno en California. Determinamos el sexo con técnicas moleculares utilizando una reacción de polimerasa (PCR) y agrupamos los individuos basándonos en el sexo, edad y área geográfica. No combinamos los grupos dadas las diferencias en medidas entre individuos de diferentes edades y areas geográficas. Comparamos un grupo de medidas morfométricas entre hembras y machos de cada combinación, y desarrollamos una función de discriminación y un diagrama de flujo para determinar el sexo en los halcones de Cola Roja y el de Hombro Rojo. El diagrama de flujo resultó mas exacto que la función discriminativa para ambas especies. También confirmamos la exactitud del diagrama de flujo para determinar el sexo de los Halcones de Cooper que migraron a lo largo de la costa de California. Estos métodos específicos a la región, tanto para el Halcón de Cola Roja como el de Cooper, resultaron en gran medida más exactos que el sexado que aparece en publicaciones utilizando otros métodos. Eso tiende a indicar diferencias poblacionales en estas especies y señala la importancia de validar los métodos de sexado, cuando estos se usan en diferentes regiones.

Key words: Cooper's Hawk, discriminant function analysis, polymerase chain reaction, Red-shouldered Hawk, Red-tailed Hawk

Sex determination is important for many studies of raptor biology because behavior, habitat use, and migration can all be affected by sex. However, many species have monomorphic plumage, making discrimination of males and females difficult in the field. Sexing methods include laparoscopy and observation of

[4]Corresponding author. Email: bhull@parkscon servancy.org

[5]Current address: University of California, Davis, Tahoe Environmental Research Center, 291 Country Club Drive, Incline Village, Nevada 89451, USA

sex-specific behaviors, but these techniques can be time-consuming and invasive. Polymerase chain reaction (PCR)-based genetic tests for sexing birds are now available (Griffiths et al. 1996, Kahn et al. 1998, Fridolfsson and Ellegren 1999, Itoh et al. 2001), but have not been tested on all species and are not always practical for field researchers. For field applications, a method for sexing birds of prey using characteristics easily observed or measured in the field would be invaluable. Because many raptor species exhibit sexual size dimorphism, measurement of morphological characteristics may provide a method to determine sex. Several investigators have generated discriminant functions that identify males and females raptors with 90–100% accuracy for a variety of species (Bortolotti 1984a,b, Ferrer and de le Court 1992, Balbontín et al. 2001, Sarasola and Negro 2004). However, for species with widespread geographic distributions, few investigators have determined the accuracy of the discriminant functions for other populations or geographic areas.

Red-tailed Hawks (*Buteo jamaicensis*) and Cooper's Hawks (*Accipiter cooperii*) are widespread species that have been sexed using morphology (Smith et al. 1990, Donohue and Dufty 2006) and exhibit geographic variation in size (Hoffman et al. 1990, Fitzpatrick and Dunk 1999, Pearlstine and Thompson 2004). Because of regional size differences, testing the accuracy of developed sexing methods in additional populations and geographical areas is important. Red-shouldered Hawks (*B. lineatus*) are widely distributed in eastern North America and west of the Sierra Nevada from Baja California to southwestern Oregon (Crocoll 1994), but no method to determine the sex of these hawks using morphological features has been published. Our objectives were to (1) apply a PCR-based genetic sexing test to Red-tailed Hawks, Red-shouldered Hawks, and Cooper's Hawks captured during migration through the Marin Headlands and during winter in the Central Valley of California, (2) determine the accuracy of existing sexing methods, and (3) develop region-specific methods to achieve greater accuracy for these groups of raptors. For Red-shouldered Hawks, we also developed a novel method for in-hand sexing.

METHODS

Sampling. In collaboration with personnel of the Golden Gate Raptor Observatory (GGRO), blood samples were obtained from 267 Red-tailed Hawks (*B. j. calurus*), 46 Red-shouldered Hawks (*B. l. elegans*), and 153 Cooper's Hawks in the Marin Headlands north of San Francisco, California (37° 49′ 52″ N, 122° 29′ 56″ W), from August to December 2003, 2004, and 2005. Blood samples were also collected from 204 Red-tailed Hawks and 45 Red-shouldered Hawks in California's Central Valley (38° 5′ to 39° 57′ N, 121° 22′ to 122° 17′ W) from November to March 2003–2006. Blood (0.5 ml) was taken from the medial metatarsal vein and preserved in Longmire's solution.

The following morphological characters were measured (GGRO Banding Manual 1998): wing chord (±1 mm), flattened wing length (±1 mm), tarsus depth (±0.1 mm), exposed culmen length (±0.1 mm), hallux claw (±0.1 mm), tail length (±0.1 mm), mass (±1.0 g), and for Cooper's Hawks, tail delta (±0.1 mm; the difference in length between the 1 and 6 rectrices on the same side of the tail). Not all measurements were taken for all birds. Individuals were also aged (GGRO Banding Manual 1998).

Molecular methods. Genomic DNA was isolated using a DNeasy 96 Tissue extraction kit (Qiagen, Inc., Valencia, CA, USA) with the following protocol modifications. A total of 20–30 μl of the Longmire's solution was diluted to 200 μl with PBS buffer before adding the Qiagen Proteinase K/ATL buffer solution. Samples were digested at 57°C overnight. After digestion, DNA was washed with Qiagen AW1 and AW2 buffers twice. Both buffers were left on the Qiagen column for 5 min before being centrifuged. A total of 100 μl of Qiagen AE buffer was used to elute the DNA, and was also left on the Qiagen column for 5 min before centrifuging.

PCR for Red-tailed and Red-shouldered hawk samples were performed in a 14 μl mixture containing 50 mM KCl, 0.2 mM dNTPs, 1.75 mM $MgCl_2$, 0.061 μM AWS03 (5′-ACAGTTTGTCTGTCTCCGGGGAA-3′), 0.239 μM USP3 (5′-AGCTGGAYTTCA-GWSCATCTTCT-3′), 0.025 μM CPE15F (5′-AAGCATAGAA-ACAATGTGGGAC-3′), 0.096 μM CPE15R (5′-AACTCTGTCT-

GGAAGGACTT-3′; Itoh et al. 2001), 3.25 μl of 5 M Betaine, and 0.95 units Taq. PCR for Cooper's Hawk samples were performed in the same mixture, except the concentrations of CPE15F and CPE15R were 0.054 μM and 0.214 μM, respectively. The forward primers were M13 labeled with a FAM fluorescent marker (Schuelke 2000). An initial denaturing step of 95°C for 180 s was followed by 27 cycles of 95°C for 80 s, 59°C for 90 s, and 72°C for 60 s. This was followed by eight cycles of 95°C for 80 s, 53°C for 90 s, 72°C for 60 s, and a final annealing step of 72°C for 10 min. PCR products were visualized on an ABI 3730 gene sequencer (Applied Biosystems, Inc., Foster City, CA, USA). PCR was performed at least twice on each sample to confirm the results. Primers were validated using samples from 36 Red-tailed Hawks, 10 Red-shouldered Hawks, and 10 Cooper's Hawks of known-sex.

Morphometric analysis. We determined the sex of 323 Red-tailed Hawks and 65 Red-shouldered Hawks during 2003–2004 and 2004–2005 based on genetic data. Hawks were further categorized as either adults or juveniles based on plumage and into groups from either the Marin Headlands (Marin) or the Central Valley. Individuals with missing data were excluded from analysis, as were eight birds with outlying measurements likely due to measurement or recording errors. The morphological measurements of each combination of sex, age, and geographic area were tested for normality using a one-sample Kolmogorov-Smirnov test. Homogeneity of variance between locations was tested using a two-sample variance test in juvenile Red-tailed Hawks only because of insufficient sample size in other groups. A multivariate analysis of variance (MANOVA) was used to test the morphological measurements of juvenile birds for variation between areas of capture. Testing for variation between age classes was possible only for Red-tailed Hawks from Central Valley because of an insufficient number of adult Red-shouldered Hawks and adult Red-tailed Hawks from Marin. The flattened wing measurement was excluded because it was correlated with wing chord.

Backward step-wise discriminant function analysis (DFA) was performed separately for juvenile Red-tailed Hawks from Marin and the Central Valley, adult Red-tailed Hawks from the Central Valley, and juvenile Red-shouldered Hawks from Marin to determine the morphological measurements most useful in determining sex. A combined Marin/Central Valley function was developed for juvenile Red-tailed Hawks only. Classification tree analysis was also performed and a flowchart for determining sex was derived from the resulting tree. All statistical tests were performed using SYSTAT 11.0 (SYSTAT 2004). All morphological sexing methods were validated using hawks captured during the 2005–2006 field season.

To sex Cooper's Hawks in the field, those with wing chords less than 231 mm were classified as males and greater than 235 mm as females (GGRO 1998). For Cooper's Hawks with wing chords between 231 and 235, those weighing less than 350 g with an empty crop were classified as males and those weighing more than 350 g as females. PCR results for 124 juvenile Cooper's Hawks captured at Marin during the 2003–2006 field seasons were compared to the sex recorded in the field to determine the error rate for the sexing method described above.

RESULTS

Molecular methods. Of 36 blood samples of known-sex Red-tailed Hawks used to validate the primers, DNA was successfully amplified in 30 individuals using the sexing primers. Eight of 10 samples from Red-shouldered Hawks and six of 10 samples from Cooper's Hawks amplified DNA. Genetic sex matched the recorded sex in all cases.

Morphometric analysis. No violations of normality or unequal variances were detected. Backward stepwise discriminant function analysis indicated that tarsus depth, hallux, and wing chord were most important for determining the sex of juvenile Red-tailed Hawks from both Marin and the Central Valley. Tarsus depth, culmen, and wing chord were most important for adult Red-tailed Hawks. For Red-shouldered Hawks, the most important characters were wing chord, mass, and tail (Table 1).

Results from classification tree analysis generally agreed with those from discriminant function analysis. Tarsus and hallux remained the most important characters for determining the sex of juvenile Red-tailed Hawks, whereas wing chord was most important for Red-shouldered Hawks. Tree analysis resulted in the misclassification of four of 30 juvenile Red-tailed Hawks from Central Valley (13.3%), six of 40 juvenile

Table 1. Discriminant functions and validation results for Red-tailed Hawks and Red-shouldered Hawks.

Age	Species	Location	Function[a]	% Incorrectly classified[b]
Juvenile	Red-tailed Hawk	Marin	D = –30.613 + 0.984(tarsus) + 0.287(hallux)+0.028(wing)	10% (4/40)
		Central Valley	D = –40.555 + 0.863(tarsus) + 0.44(hallux)+0.048(wing)	20.7% (6/29)
		Marin and Central Valley	D = –32.569 + 0.948(tarsus) + 0.322(hallux)+0.032(wing)	8.7% (6/69)
	Red-shouldered Hawk	Marin	D = –31.516 + 0.011(mass) + 0.053(wing)+0.056(tail)	0% (0/13)
Adult	Red-tailed Hawk	Central Valley	D = –41.505 + 0.844(tarsus) + 0.385(culmen)+0.056(wing)	0% (0/6)

[a]Hawks with values of D > 0 are females and values of D < 0 are males.
[b]Numbers in parentheses are number of validation birds incorrectly classified/total number of validation birds. Validation birds came from the same location as the birds used to create the function being tested.

Red-tailed Hawks from Marin (15.0%), and one of 13 Red-shouldered Hawks (7.7%). Based on the functions and the classification trees, flowcharts were developed (Figs. 1 and 2). Using these flowcharts, the sex of two of 30 juvenile Red-tailed Hawks (6.7%) from the Central Valley, two of 40 juvenile Red-tailed Hawks (5.0%) from Marin, and no Red-shouldered Hawks ($N = 13$) were misclassified.

The sex of all juvenile Cooper's Hawks from Marin ($N = 124$, Table 2) was determined correctly using both genetic analysis and morphological measurements. For juvenile Red-tailed Hawks from the Central Valley and Marin, males differed in wing chord (MANOVA; $F_{6,144} = 3.7$, $P = 0.002$) and females in mass (MANOVA; $F_{6,101} = 5.2$, $P < 0.0005$; Table 3). For adult and juvenile Red-tailed Hawks from the Central Valley, males differed in wing chord, tail length, and mass (MANOVA; $F_{6,81} = 23.1$, $P < 0.0005$) and females in wing chord and tail length (MANOVA; $F_{6,40} = 13.5$, $P < 0.0005$; Table 4). Several volunteers with varying degrees of experience measured the Marin Headland birds, whereas a few experienced individuals measured the Central Valley birds. To determine if this influenced the results, the wing chords

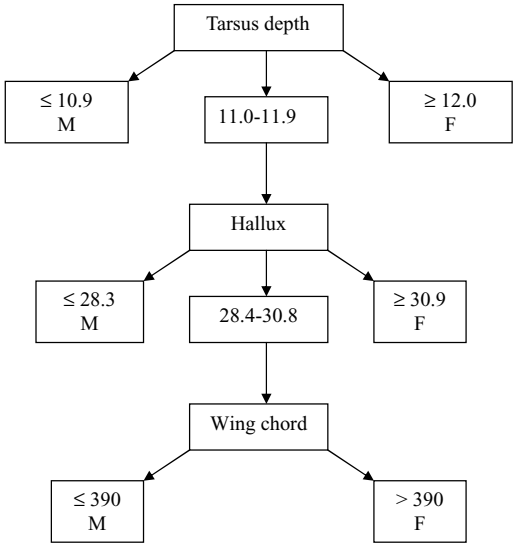

Fig. 1. Juvenile Red-tailed Hawk sex-determination flowchart.

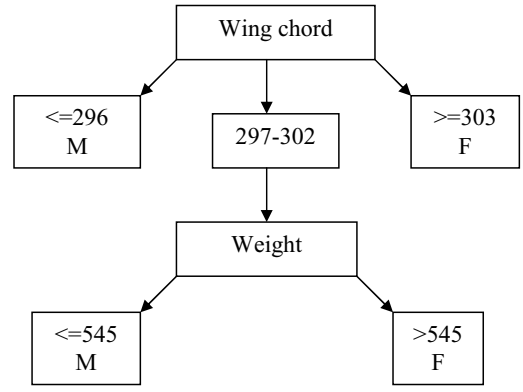

Fig. 2. Juvenile Red-shouldered Hawk sex-determination flowchart.

Table 2. Mean morphological measurements[a] of juvenile Cooper's Hawks captured at Marin, California.

Sex	N	Wing	Flat wing	Tail	Mass	Culmen	Tarsus	Hallux
Male	45	215 ± 0.9	221 ± 0.9	190 ± 0.9	282 ± 3.3	14.7 ± 0.10	5.9 ± 0.05	19.4 ± 0.11
		(205–227)	(210–231)	(169–202)	(249–345)	(13.5–16.8)	(5.3–6.5)	(17.9–21.1)
Female	91	246 ± 0.6	252 ± 0.6	215 ± 0.7	422 ± 3.7	17.6 ± 0.07	7.2 ± 0.04	22.9 ± 0.10
		(235–267)	(240–271)	(193–229)	(329–521)	(15.7–19.3)	(6.3–8.0)	(20.9–24.9)

[a]Values are presented as means ± 1 SE (plus range in parentheses). All measurements are in mm, except mass (g).

of Red-tailed Hawks from Marin handled by experienced banders were compared to those from Central Valley and the difference was still significant (ANOVA; $F_{1,71} = 7.5$, $P = 0.008$). Juvenile male Red-shouldered Hawks from the Central Valley and Marin differed in wing chord and mass (MANOVA; $F_{6,20} = 8.5$, $P < 0.0005$; Table 5), but sample sizes from the Central Valley were small ($N = 6$ males and 9 females).

DISCUSSION

Morphological sexing methods. Despite differences in morphological measurements between age classes and geographic areas among Red-tailed and Red-shouldered hawks, we were able to develop accurate methods of sexing individuals. Two of the three characters that best discriminated between male and female Red-tailed Hawks, tarsus depth and hallux length, did not differ with either age or location. Therefore, the first two steps of our flowchart could be used to determine the sex of any Red-tailed Hawk in either Marin or the Central Valley. Adult Red-tailed Hawks have longer wing chords, however, leading to the possibility of greater overlap in this measurement between adult males and juvenile females.

The combined-region discriminant function for juvenile Red-tailed Hawks sexed both Marin and Central Valley birds more accurately than the individual functions, despite differences in wing chord measurements between the locations. This may be a reflection of the greater sample size when both data sets are combined, but further sampling would be needed to test this hypothesis.

Our functions for determining the sex of Red-tailed Hawks included the variables wing chord, mass, hallux, and culmen. In contrast, Donohue and Dufty (2006) included wing chord, mass, hallux, and culmen in two functions (juvenile and adult) to determine the sex of Red-tailed Hawks from the intermountain west (Goshute Mountains, NV, Manzano Ridge, NM, Chelan Ridge, WA, and Bonney Butte, OR). The function generated by Donohue and Dufty (2006) was more accurate than the function we generated for Central Valley Red-tailed Hawks, incorrectly sexing only two of 27 (7.4%) juvenile birds from that region. However, their function incorrectly sexed nine of 39 (23.1%) juvenile Red-tailed Hawks from Marin. Using the function provided by Donohue and Dufty (2006) for adults, one of six (16.7%) Red-tailed Hawks from the Central Valley was sexed incorrectly. This is a small sample size, however, and further testing is needed. Differences in the error rates between the two locations may indicate that the morphological measurements of juvenile Red-tailed Hawks from the Central Valley are more similar to those of the juvenile, migrant Red-tailed Hawks of the intermountain west studied by Donohue and Dufty (2006) than to those of juvenile Red-tailed Hawks from Marin.

Our results, along with other reports of subspecific (Preston and Beane 1993) and regional (Fitzpatrick and Dunk 1999) morphological variation, suggest that region- or population-specific sex determination functions may be necessary for many species of raptors. The morphological characters we used to determine the sex of Red-shouldered Hawks, wing chord and mass, varied between geographic areas of California. Because subspecies of Red-shouldered Hawks in eastern North America (*B. l. lineatus*, *B. l. alleni*, and *B. l. texanus*), except the Florida subspecies (*B. l. extimus*), are larger than the single subspecies in western North America (*B. l. elegans*; Crocoll 1994), the methods described here should only be used on juvenile birds in the Marin Headlands. When developing sexing methods for widespread and morphologically variable taxa, multiple analyses and discriminant

Table 3. Mean morphological measurements[a] of juvenile Red-tailed Hawks captured at Marin and the Central Valley, California.

Sex	Location	N	Wing chord	Flat wing	Tail	Mass	Culmen	Tarsus	Hallux
Male	Marin	97	379 ± 1.4 (341–406)	387 ± 1.4 (349–418)	226 ± 1.0 (200–254)	890 ± 10.4 (633–1168)	24.9 ± 0.12 (22.2–27.2)	10.7 ± 0.06 (9.3–12.3)	28.2 ± 0.13 (25.5–31.8)
	Central Valley	54	373 ± 1.5 (348–396)	382 ± 1.5 (360–407)	226 ± 1.1 (207–241)	916 ± 11.0 (715–1055)	24.9 ± 0.14 (22.5–26.8)	10.6 ± 0.07 (9.6–11.8)	27.9 ± 0.14 (25.9–30.1)
Female	Marin	82	405 ± 1.5 (367–432)	413 ± 1.5 (379–442)	240 ± 1.1 (220–262)	1104 ± 12.2 (786–1396)	27.0 ± 0.16 (24.0–31.2)	12.1 ± 0.06 (10.8–13.6)	30.8 ± 0.16 (26.6–33.5)
	Central Valley	26	400 ± 2.2 (380–426)	410 ± 2.3 (392–437)	237 ± 1.8 (220–251)	1200 ± 24.0 (975–1405)	26.9 ± 0.25 (24.3–29.2)	12.1 ± 0.14 (10.9–13.4)	30.6 ± 0.26 (28.1–33.8)

[a]Values are presented as means ± 1 SE (plus range in parentheses). All measurements are in mm, except mass (g).

Table 4. Mean morphological measurements[a] of adult Red-tailed Hawks captured at Marin and the Central Valley, California.

Sex	Location	*N*	Wing chord	Flat wing	Tail	Mass	Culmen	Tarsus	Hallux
Male	Marin	6	385 ± 4.2 (370–397)	392 ± 4.0 (378–403)	211 ± 2.4 (205–221)	881 ± 34.1 (732–1001)	25.2 ± 0.52 (23.3–26.7)	10.8 ± 0.17 (10.5–11.4)	28.5 ± 0.26 (27.9–29.4)
	Central Valley	34	380 ± 1.7 (356–398)	388 ± 1.7 (365–403)	212 ± 1.1 (202–227)	972 ± 16.9 (790–1215)	25.0 ± 0.16 (23.3–27.4)	10.8 ± 0.10 (9.4–11.8)	27.8 ± 0.20 (26.1–30.6)
Female	Marin	4	417 ± 4.6 (410–430)	425 ± 5.0 (415–438)	223 ± 3.0 (215–228)	1167 ± 74.5 (1068–1389)	27.6 ± 0.52 (26.6–29.0)	12.3 ± 0.16 (12.0–12.7)	31.4 ± 0.80 (29.7–33.1)
	Central Valley	21	409 ± 1.9 (390–424)	419 ± 1.9 (402–434)	227 ± 1.2 (218–239)	1291 ± 31.4 (1015–1555)	27.6 ± 0.25 (25.4–29.7)	12.5 ± 0.18 (11.4–14.5)	30.9 ± 0.59 (26.9–39.6)

[a]Values are presented as means ± 1 SE (plus range in parentheses). All measurements are in mm, except mass (g).

Table 5. Mean morphological measurements[a] of juvenile Red-shouldered Hawks captured at Marin and the Central Valley, California.

Sex	Location	*N*	Wing chord	Flat wing	Tail	Mass	Culmen	Tarsus	Hallux
Male	Marin	21	291 ± 1.4 (281–310)	299 ± 1.2 (290–313)	195 ± 1.1 (186–204)	467 ± 7.8 (409–536)	20.2 ± 0.25 (17.4–21.8)	7.8 ± 0.10 (7.1–8.5)	21.0 ± 0.22 (18.9–22.4)
	Central Valley	6	282 ± 2.6 (271–289)	289 ± 2.2 (282–298)	195 ± 2.2 (188–203)	542 ± 25.4 (455–625)	19.9 ± 0.25 (18.9–20.4)	7.6 ± 0.31 (6.6–8.5)	20.3 ± 0.27 (19.4–21.2)
Female	Marin	17	314 ± 2.7 (284–327)	320 ± 2.7 (292–334)	210 ± 2.3 (189–225)	582 ± 13.7 (486–695)	21.6 ± 0.28 (19.4–23.5)	8.5 ± 0.18 (7.2–9.8)	22.1 ± 0.28 (20.2–25.4)
	Central Valley	9	310 ± 1.7 (304–317)	316 ± 1.7 (309–324)	209 ± 3.0 (194–225)	656 ± 17.6 (585–740)	21.6 ± 0.40 (19.8–23.3)	8.8 ± 0.14 (8.4–9.6)	21.6 ± 0.22 (20.4–22.4)

[a]Values are presented as means ± 1 SE (plus range in parentheses). All measurements are in mm, except mass (g).

functions may be required to achieve consistently high accuracy.

Our morphometric method for sexing Cooper's Hawks was developed specifically for hawks migrating along the Pacific coast of California and correctly determined the sex of all individuals. This would not have been the case if we had relied upon methodology developed for Cooper's Hawks from other regions because the size of Cooper's Hawks declines from east to west (Smith et al. 1990). Guidelines in the North American Bird Banding Manual (Environment Canada and United States Fish and Wildlife Service 1977) suggest using a wing chord of 251 mm to differentiate the sexes, and Hoffman et al. (1990) suggested using a wing chord of 238 mm to sex all birds west of the Rocky Mountains. Using 251 mm, we incorrectly sexed 79 of 124 Cooper's Hawks (64%) from Marin and, using 238 mm, we incorrectly sexed six (4.8%). In all cases, we incorrectly classified females as males. However, all females incorrectly sexed by wing chord were correctly sexed using the mass suggested by Hoffman et al. (1990), indicating that, in contrast to wing chord, mass may not decline from east to west (Smith et al. 1990). These results provide another example of the importance of region-specific sexing methodology.

Morphological differences between ages and sexes. We found morphological differences between age classes of Red-tailed Hawks. With some exceptions, adult raptors in the genus *Buteo*, including Red-tailed Hawks, tend to have shorter tails, longer secondary flight feathers, and greater masses than juveniles (Ferguson-Lees and Christie 2006). The difference in mass between the Marin Headlands and the Central Valley has also been previously documented (Hull et al. 2006). One possible explanation for this difference is that Red-tailed Hawks at Marin were sampled during migration (August–December), whereas most individuals in the Central Valley were sampled on postmigration (November–March) wintering grounds. Lower mass among migrants might be expected as a consequence of the higher energy demands and stress of migration (Smith 1980, Piersma 2002, Bildstein 2006, Dietz et al. 2007).

We also found differences in the wing chord measurements of male Red-tailed and Red-shouldered hawks captured at Marin and the Central Valley. A comparison of measurements made by experienced and inexperienced

personnel revealed no differences, suggesting that the difference was not due to measurement errors. Mueller et al. (1981) found that wing chords of Cooper's Hawks captured during spring migration were smaller than those captured during the preceding fall migration, and suggested that the difference was due to feather wear. However, many Central Valley birds in our study were captured and measured later (November–March) than those at Marin (August–December) and no apparent differences in feather wear were observed. Although the differences observed in the nonmigratory Red-shouldered Hawks may be a consequence of small sample sizes ($N = 6$ at Marin and 34 at the Central Valley), the difference in wing chords between Red-tailed Hawks at Marin and the Central Valley may by due to differences in point of origin. Band recoveries indicate that the smaller Red-tailed Hawks wintering in the Central Valley originate from outside of central California, particularly the areas from northern California to British Columbia and from southern California (Bloom 1985) to the Great Basin (HawkWatch International 2007). In contrast, band recoveries suggest that Red-tailed Hawks at Marin include a high proportion of individuals resident in central California. This distinction is supported by the observation that Red-tailed Hawks in southern California disperse broadly from California to Wyoming (HawkWatch International 2007), but do not appear to migrate through the Marin Headlands (AH, unpubl. data). This hypothesis is consistent with morphological data, indicating that breeding and migratory Red-tailed Hawks are smaller in the Great Basin than in coastal California (Fitzpatrick and Dunk 1999, Hull et al. in press). Although our study does not address the underlying mechanisms behind regional variation in size, Fitzpatrick and Dunk (1999) suggested that the observed differences between regions may be the result differences in diet, competition, or water stress. Further research is necessary to distinguish among these potential causes.

Validation of genetic sexing. Validation of DNA sexing techniques using birds of known sex is important when using the technique with a new species because mutations in either the Z or W chromosome could confound results (Dawson et al. 2001). Although museum specimens are a reliable source of known-sex birds, errors in determining and recording sex do occur (Lee and Griffiths 2003). Validating morphological sexing methods is also important. Validating a model using an independent data set eliminates bias and tests how well the model generalizes to a new set of cases (Tabachnick and Fidell 2007). When this is not possible, techniques such as jackknife and bootstrap resampling can reduce bias and yield a realistic estimate of model accuracy (Verbyla and Litvaitis 1989).

Additional study is needed of populations of Red-shouldered Hawks in eastern North American and populations of Red-tailed Hawks in the northern part of their range to better characterize relationships among morphology, age, geographic areas, and population structure. In particular, analysis of the Red-tailed Hawk subspecies *B. j. borealis* (eastern North America), *B. j. fuertesi* (southern Texas and northern Mexico), and *B. j. harlani* (central Alaska), and all subspecies of Red-shouldered Hawks in eastern North American is needed. Our findings provide data for comparison and underscore the importance of testing both new and existing sexing models on individuals of the same species in different geographic areas.

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