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Dorsal color variation among subspecies of the Oregon Dark-eyed Junco (*Junco hyemalis* [*oreganus*]) group

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ABSTRACT—Initial descriptions of avian subspecies were based on expert opinions of geographic variation in phenotypes and were inherently subjective. Although best practices for subspecies delimitation continue to be debated, reassessing subspecies limits with current, quantitative methods is important toward refining and improving taxonomic treatments. Plumage coloration is the basis of many subspecies diagnoses, but is potentially problematic because of the historical lack of quantitative methods to measure color. Recently developed methods, such as colorimetry, provide repeatable measurements of color variation that can be used to reassess subspecies limits. In this study, we reassess color variation among subspecies of the Oregon Dark-eyed Junco (*Junco hyemalis* [*oreganus*]) group, in which differences in back and hood color were established as diagnostic characters. We measured back and hood brightness and hue in 206 museum specimens among 5 Oregon Dark-eyed Junco subspecies using a colorimeter. We then compared mean measurements among subspecies and conducted a discriminant function analysis to assess how well dorsal color predicted subspecies. Our data correctly classified only 67.9% of males and 82.5% of females to their presumed subspecies. Furthermore, no adjacent subspecies pairs passed the "75% rule" due to extensive overlap in plumage characters. Thus, back color alone is not as effective in diagnosing Oregon Dark-eyed Junco subspecies as originally described, suggesting the need for a taxonomic revision. Specifically, similarity in phenotypic and genetic data suggests that some combination of *thurberi*, *montanus*, and *shufeldti* may be lumped to recognize broad, clinal variation in dorsal color alongside clinal variation in other phenotypes and extensive gene flow. *Received 3 December 2020. Accepted 10 February 2022.*

Key words: classification, color, Passerellidae, subspecies delimitation, taxonomy.

Variación en el color dorsal entre las subespecies del grupo Oregon del junco *Junco hyemalis oreganus*

RESUMEN (Spanish)—Las descripciones iniciales de las subespecies de aves se basaban en opiniones expertas de variación geográfica de los fenotipos y son inherentemente subjetivas. Si bien las mejores prácticas para la delimitación de subespecies siguen debatiéndose, la redeterminación de los límites entre subespecies con métodos actuales y cuantitativos es importante hacia el refinamiento y mejora de los tratamientos taxonómicos. La coloración del plumaje es la base de la diagnosis de muchas subespecies, si bien es potencialmente problemática por la histórica carencia de métodos cuantitativos para medir el color. Métodos desarrollados recientemente, como la colorimetría, proveen mediciones repetibles de variación de color que pueden ser utilizadas para redeterminar límites entre subespecies. En este estudio, redeterminamos la variación de color entre subespecies del complejo Oregon del junco *Junco hyemalis oreganus*, en el cual las diferencias en el color del dorso y la capucha fueron establecidas como caracteres diagnósticos. Hicimos mediciones del brillo y el tono del dorso y la capucha en 206 especímenes de museo entre 5 subespecies del grupo Oregon usando un colorímetro. A continuación comparamos las mediciones medias entre subespecies y llevamos a cabo un análisis de funciones discriminantes para determinar qué tan bien predecía el color dorsal a las subespecies. Nuestros datos clasificaron correctamente solo al 67.9% de los machos y el 82.5% de las hembras a la subespecie a la que se presumía que pertenecían. Más aún, ningún par de subespecies adyacentes pasó la "regla del 75%" dado el extenso traslape en caracteres del plumaje. Por ello, el color del dorso por sí mismo no es efectivo para diagnosticar a las subespecies del grupo Oregon de este junco tal y como se describieron originalmente, lo que sugiere la necesidad de una revisión taxonómica. Específicamente, la similitud entre los datos fenotípicos y genéticos sugiere que una combinación de *thurberi*, *montanus* y *shufeldti* puede ser agrupada para reconocer una amplia variación en el color dorsal a la par de una variación gradual en otros fenotipos y un extenso flujo génico.

Palabras clave: clasificación, color, delimitación de subespecies, Passerellidae, taxonomía.

Avian subspecies delimitation is a controversial taxonomic practice that has been debated and refined since its initial conceptualization (Mayr 1943, Wilson and Brown 1953, Zink 2004, Patten 2010, Remsen 2010, Winker 2010, Patten 2015). In birds, subspecies are generally thought to represent phenotypically and/or genotypically differentiated populations within a species that occupy a geographic region (Patten and Remsen 2017). However, original subspecies descriptions were based mostly on expert opinions of geographic patterns and infraspecific partitioning in phenotypic variation, resulting in subjective classifications that often fail to meet current best practices (James 2010).

Today, delimiting subspecies is still far from straightforward: taxonomists continue to differ on what subspecies represent and optimal criteria for determining how many subspecies exist within a given species (Fitzpatrick 2010, Gill 2014, Cronin

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et al. 2015, Weckworth et al. 2015, Vinarski 2015). Nonetheless, the development and implementation of quantitative metrics and statistical analyses promote a more consistent and standardized subspecies classification system (Patten 2010). Many subspecies described prior to the development of current practices are equivocal and would benefit from reassessments using modern approaches. Such reassessments are important for current applied and basic research initiatives, many of which use subspecies as units of biodiversity and indices of ecological and evolutionary processes (Phillimore and Owens 2006, Haig and D'Elia 2010).

Phenotypic variation provided the foundation for initial subspecies descriptions and remains important in infraspecific taxonomy, even as genetic data play a growing role in subspecies delimitation (Winker 2009, Patten and Remsen 2017). Coloration in particular has played a prominent role in avian taxonomy for multiple reasons: First, plumage coloration and pattern are influenced by selective pressures, such as natural selection favoring coloration that promotes camouflage or sexual selection favoring bright, showy colors that promote mate choice (Hill and McGraw 2006, Mason and Bowie 2020). Thus, differences among populations may represent evolutionary changes in response to local conditions (Zink and Remsen 1986, Zamudio et al. 2016). Second, differences in coloration are readily observable by the human eye and were easily detected by early taxonomists (Endler 1990).

However, historical assessments of color differences relied on individual taxonomists matching plumage patches to color swatches (Ridgway 1912), which may have introduced qualitative, subjective differences among observers (Zuk and Decruyenaere 1994, Butler et al. 2011). Today, colorimetry, spectrophotometry, and digital photography offer affordable ways to accurately measure color variation in a consistent, quantitative manner (Burns et al. 2017, Mason and Bowie 2020). Recently, colorimetry has been used to quantify color variation and reexamine subspecific taxonomy in various groups, including Willow Flycatchers (*Empidonax traillii*; Paxton et al. 2010), Least Terns (*Sternula antillarum*; Johnson et al. 1998), and Sagebrush/Bell's Sparrows (*Artemisiospiza* spp.; Patten and Unitt 2002). Nonetheless, many subspecies groups are still in need of quantitative reevaluations of color variation and diagnosability among taxa.

The Oregon Dark-eyed Junco (*Junco hyemalis* [*oreganus*]) is one such subspecies group that would benefit from a quantitative reexamination of subspecies limits using modern empirical methods. At a broader, species-level taxonomic scale, Dark-eyed Juncos exhibit pronounced intraspecific plumage variation, with 14 subspecies assigned to 7 subspecies "groups" (i.e., a distinctive, field-identifiable, subspecies or set of subspecies *sensu* Clements et al. 2019) that have recently diversified across North America (Milá et al. 2007, Friis et al. 2016, Clements et al. 2019).

In western North America—from Baja California north to Alaska—7 subspecies comprise the Oregon Dark-eyed Junco group (Clements et al. 2019, Nolan et al. 2020). Following initial assessments of Dark-eyed Junco subspecies by early taxonomists (Ridgway 1901, Dwight 1918, Miller 1941) solidified the current subspecific taxonomy of the Dark-eyed Junco by matching specimens' hoods and backs with graded color samples and examining pigments under a microscope (see Table 1 for subspecies descriptions). Despite long-standing use of Miller's (1941) classification, it is still unknown whether these subspecies represent diagnosable taxa that meet current guidelines and conventions for subspecies delimitation.

In this study, we reevaluated Miller's (1941) classification of the Oregon Dark-eyed Junco complex using colorimetry. Specifically, we compared back and hood color variation between 5 subspecies from the Oregon Dark-eyed Junco complex (*pinosus*, *thurberi*, *shufeldti*, *montanus*, and *oreganus*) and excluded 2 subspecies from Mexico (*pontillis*, *townsendi*) for which we lacked samples. We compared mean values of brightness and hue measurements among sexes, age classes, and subspecies. We also quantified subspecies diagnosability of males and females using a discriminant function analysis. Finally, we tested the "75% rule" (Amadon 1949, Patten and Unitt 2002) to see if quantitative color variation among subspecies passed a widely used "yardstick" of diagnosability. In doing so, we reassessed the validity of back color as a diagnostic character for Oregon Dark-eyed Junco subspecies, and reconsidered subspecies limits within the complex.

Table 1. Descriptions of the 5 subspecies' phenotypes and breeding ranges of the Oregon Dark-eyed Junco complex included in this study following descriptions provided by Miller (1941).

Subspecies	Plumage color description	Breeding range
J. h. oreganus	Dark reddish-brown back Dark brown flanks Black hood in male Gray hood in female	Southeast Alaska to south-central British Columbia
J. h. shufeldti	Grayer, browner, less red than *oreganus* Cinnamon flanks Black hood in male Gray hood in female	Southwest British Columbia through western Washington and Oregon
J. h. montanus	Dark gray-brown back Cinnamon brown flanks Blackish to slate hood in male Gray hood in female	Central interior British Columbia and southwest Alberta through northwest Montana, western Idaho, eastern Washington, eastern Oregon
J. h. thurberi	Back rich coffee brown Sides cinnamon brown Lighter and more pinkish than *J. h. shufeldti* Hood as *J. h. shufeldti*	Southern Oregon to coastal California, interior California
J. h. pinosus	Ruddier back and flanks than *thurberi* Sides and flanks bright cinnamon brown Bright russet back Grayer hood than *thurberi*—blackish to slate in male, gray in female	Resident central coastal California

Methods

We measured plumage reflectance from 206 specimens of Oregon Dark-eyed Junco (Fig. 1; Supplemental Tables S1, S2). Our sampling was drawn from specimens housed in 3 collections: the San Diego Natural History Museum, Natural History Museum of Los Angeles County, Los Angeles, and the University of California, Los Angeles. We measured a minimum of 16 individuals from each of 5 subspecies, including (from north to south) *J. h. oreganus* ($n_{\text{male}} = 13$; $n_{\text{female}} = 3$), *J. h. montanus* ($n_{\text{male}} = 15$; $n_{\text{female}} = 14$), *J. h. shufeldti* ($n_{\text{male}} = 13$; $n_{\text{female}} = 4$), *J. h. thurberi* ($n_{\text{male}} = 51$; $n_{\text{female}} = 74$), and *J. h. pinosus* ($n_{\text{male}} = 12$; $n_{\text{female}} = 7$). Only specimens falling in the subspecies' ranges described by Miller were included. For example, if a specimen was labeled as *montanus* but was collected outside the range of *montanus* as described by Miller, or in a portion of the range overlapping with other subspecies, it was not included in our analysis. We also omitted specimens with significantly worn plumage, faded or missing feathers, juveniles with irregular streaking, diffuse brown dorsal plumage, obvious truncations in tail or greater coverts, or completely unossified skulls. Juvenile birds were identified and omitted via aging criteria, tag labels noting partially ossified skulls, or labels noting the developmental stage of gonads. Birds for which none of this information was obvious or available were considered adults. Thus, each individual included in the study was classified as either juvenile or adult.

We measured the coloration of the center of the hood and the back of each specimen using a Konica Minolta CR-300 Chroma Meter (Ramsey, New Jersey, USA). We quantified color in CIELAB color space (i.e., $L^*a^*b^*$ color space; Kuehni 2003), which includes 3 metrics: (1) L^*, or brightness, in which higher values corresponded to brighter plumage; (2) a^*, or redness, in which lower values corresponded to greener hues and higher values corresponded redder hues; and (3) b^*, or yellowness, in which lower values corresponded to bluer hues and higher values corresponded to yellower hues. We repeated each measurement 3 times and subsequently obtained the mean of those measurements.

We constructed separate linear models (LMs) for each of the 6 dorsal color measurements (hood L, hood a, hood b, back L, back a, and back b) with a Gaussian distribution of error in the R programming environment (R Core Team 2020).

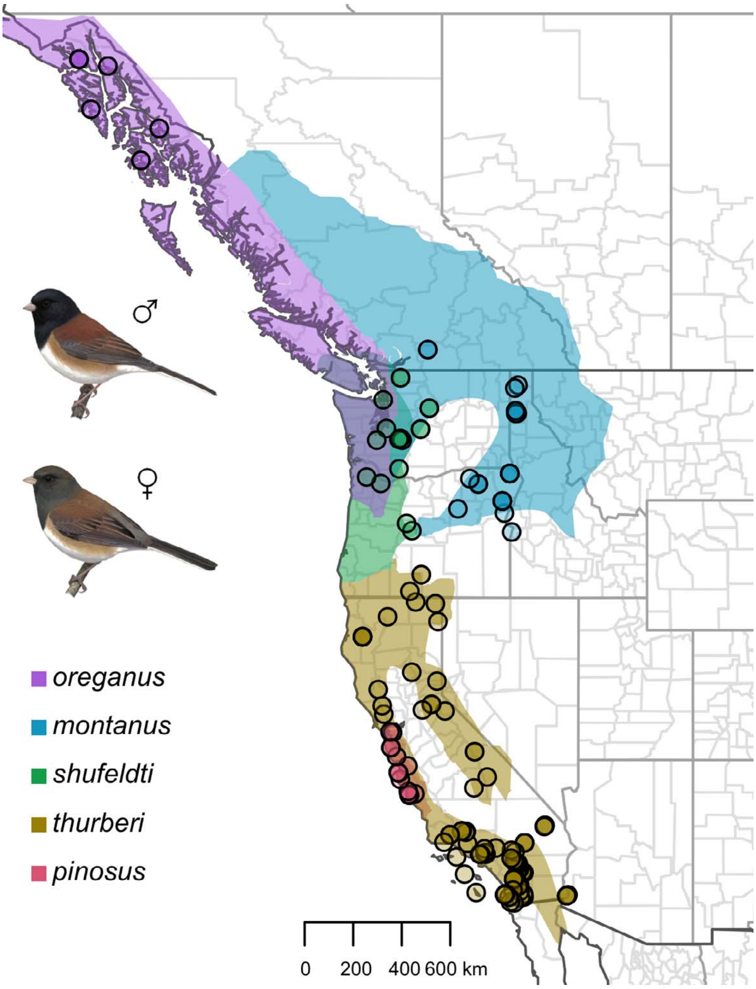

Figure 1. Sampling map of vouchered specimens included in our study of plumage reflectance among subspecies of the Oregon Dark-eyed Junco. Plates of male and female Oregon Dark-eyed Junco are shown on the left. Approximate breeding ranges of each subspecies are shown in different colors and have been modified following the descriptions of Miller (1941). As our study included wintering as well as breeding birds, some circles fall outside of their expected range due to nonbreeding seasonal movements. Sampling localities are indicated with circles with subspecies indicated by the fill color of the circle. Some dots may represent more than 1 individual sampled from the same locality. Illustrations are provided courtesy of Lynx Edicions.

We included subspecies, sex, age class, days since molt, and years since collection as main effects in each model. In our LMs, the base model had male sex, adult age class, and *oreganus* subspecies. We examined the distribution of residuals for each model to ensure that they approximated a normal distribution (Supplemental Fig. S1).

Dark-eyed Juncos undergo 2 body molts per year: an alternate plumage molt from February to April and a basic molt from July to October (Pyle 1997). Definitive basic and alternate plumages are nearly identical in Dark-eyed Juncos (Nolan et al. 2020), but color may change as freshly molted feathers wear and abrade over time (Tökölyi et al. 2008). We therefore incorporated the number of days since molt into our LMs as the difference between the collection date and the most recent molt event of either 15 March or 1 September. Furthermore, because specimens can fade and change color over years since their initial collection (Doucet and Hill 2009), we also incorporated the number of years since collection into our LMs. To account for the effect of specimen age in downstream statistical analyses, we calculated "age-corrected" values for each plumage metric that was associated with specimen age. Specifically, we extracted the coefficient corresponding to specimen age from each linear model, and then multiplied that coefficient times the number of years since collection for each specimen to obtain a corrected value that accounted for any changes in back or hood *L*, *a*, or *b* values due to specimen age. We subsequently performed a series of Tukey's Honestly Significant Difference (HSD) tests to quantify differences in the mean values of each plumage metric among sexes, age classes, and subspecies using the R package *agricolae* v1.3.1 (de Mendiburu 2019).

We also performed a discriminant function analysis (DFA) on adult males ($n = 91$) and females ($n = 83$) separately to determine the diagnosability of Oregon Dark-eyed Junco subspecies based on hood and back coloration using the *MASS* package (Venables and Ripley 2002) in R. We subsequently performed a "leave-one-out" (i.e., jackknifed) cross validation on our DFA to predict the subspecies of each individual specimen and test the diagnosability of each subspecies for both sexes (i.e., quantify the proportion of individuals that were correctly predicted as their identified subspecies based on colorimetry data).

Finally, we implemented a widely used diagnosability test commonly referred to as the "75% rule" (Amadon 1949, Patten and Unitt 2002). In brief, the "75% rule" involves a pairwise test that determines whether 75% of the distribution of a single trait for 1 subspecies falls outside of 99% of another subspecies' distribution for the same trait. The derivation of the test statistic is described in detail in Patten and Unitt (2002) and is briefly described here.

Equation 1:

$$D_{ij} = \left(\underline{x_i} - S_i(t_{0.25,df_i})\right) - \left(\underline{x_j} + S_j(t_{0.99,df_j})\right)$$

In Equation 1, $\underline{x_i}$ is the mean value and S_i is the standard deviation of a given trait for subspecies *i*. In this case, the mean for subspecies *i* is higher than *j*. Thus, the term $\underline{x_i} - S_i(t_{0.25,df_i})$ indicates the 25% percentile for subspecies *i*, while $\underline{x_j} + S_j(t_{0.99,df_i})$ represents the 99th percentile for subspecies *j*. If the D_{ij} statistic is >1, then subspecies *i* is diagnosable from subspecies *j* such that 75% of individuals in subspecies *i* have a value higher than 99% of individuals in subspecies *j*. For the "75% rule" to be met, the converse has to be true (i.e., $D_{ji} > 0$) such that subspecies *j* is also reciprocally diagnosable from subspecies *i*. We have provided R code for performing these diagnosability tests (https://github.com/mason-lab/oregonjuncocolor/blob/master/Scripts/pattenunitt75.R).

In this study, we tested pairwise diagnosability via the "75% rule" for each subspecies pair with overlapping ranges. This resulted in 5 reciprocal diagnosability tests (from northernmost to southernmost): (1) *oreganus* and *montanus*, (2) *oreganus* and *shufeldti*, (3) *montanus* and *shufeldti*, (4) *shufeldti* and *thurberi*, and (5) *thurberi* and *pinosus*. We only included adults and performed a DFA on each sex and subspecies pair separately and then used scores from the first DFA axis to calculate the 2 summary statistics, D_{ij} and D_{ji}, for adult males and females.

Results

We uncovered variation in hood and mantle coloration among sexes, age classes, and 5 subspecies within the Oregon Dark-eyed Junco complex. The number of days since molt did not exhibit any associations with any back or hood measurements. However, the number of years since collection exhibited positive correlations with hood *b*, back *a*, and back *b* values (Table 2). This is consistent with a pattern of "foxing" or increasing red and yellow coloration corresponding with years since collection. Specifically, our linear models estimated that for each year since collection, hood *b* values increased by 0.02 per year, back *a* value increased by 0.008 per year, and back *b* values increased by 0.2 per year. We therefore used corrected values for hood *b*, back *a*, and back *b* color measurements in downstream analyses.

When we compared hood and back color among males and females, we found that females had higher hood *L* (dark-light) and hood *b* (yellow-blue) values, but lower hood *a* (green-red) values (Fig. 2, Table 2). Among back measurements, females had higher back *L*, back *a*, and back *b*

Table 2. Results from linear model (LM) analyses for each of 6 response variables: hood *L (dark-light axis)*, hood *a (green-red axis)*, hood *b (blue-yellow axis)*, back *L*, back *a*, back *b*. Significant model effects ($P < 0.05$) are displayed with a bold font for the *P* value. LMs were generated with a Gaussian distribution of error. Categorical terms are compared to a base model with male sex, adult age class, and *oreganus* subspecies. The terms for days since molt indicate change in each metric per day, while the terms for specimen age displays change in parameter values per year.

Hood *L*	β ± SE	*t* value	*P* value
(Intercept)	27.183 ± 0.617	44.036	>**0.0001**
Sex Female	0.768 ± 0.267	2.875	**0.0045**
Age Class Juvenile	−0.772 ± 0.314	−2.463	**0.0146**
Subspecies *montanus*	4.118 ± 0.589	6.991	>**0.0001**
Subspecies *shufeldti*	3.4 ± 0.641	5.302	>**0.0001**
Subspecies *thurberi*	5.861 ± 0.53	11.05	>**0.0001**
Subspecies *pinosus*	3.826 ± 0.627	6.103	>**0.0001**
Days Since Molt	0.002 ± 0.003	0.798	0.4261
Specimen Age	−0.004 ± 0.004	−1.012	0.3126

Hood *a*	β ± SE	*t* value	*P* value
(Intercept)	5.156 ± 0.259	19.928	>**0.0001**
Sex Female	−0.243 ± 0.112	−2.168	**0.0313**
Age Class Juvenile	0.458 ± 0.131	3.485	**0.0006**
Subspecies *montanus*	−1.401 ± 0.247	−5.676	>**0.0001**
Subspecies *shufeldti*	−0.683 ± 0.269	−2.543	**0.0117**
Subspecies *thurberi*	0.014 ± 0.222	0.065	0.9483
Subspecies *pinosus*	1.555 ± 0.263	5.92	>**0.0001**
Days Since Molt	−0.001 ± 0.001	−0.974	0.3312
Specimen Age	0.002 ± 0.002	1.075	0.2837

Hood *b*	β ± SE	*t* value	*P* value
(Intercept)	13.171 ± 0.436	30.195	>**0.0001**
Sex Female	0.542 ± 0.189	2.869	**0.0046**
Age Class Juvenile	0.194 ± 0.222	0.874	0.383
Subspecies *montanus*	−1.867 ± 0.416	−4.485	>**0.0001**
Subspecies *shufeldti*	−0.333 ± 0.453	−0.734	0.4639
Subspecies *thurberi*	1.123 ± 0.375	2.996	**0.0031**
Subspecies *pinosus*	2.466 ± 0.443	5.566	>**0.0001**
Days Since Molt	−0.002 ± 0.002	−1.047	0.2966
Specimen Age	0.016 ± 0.003	6.128	>**0.0001**

Back *L*	β ± SE	*t* value	*P* value
(Intercept)	15.892 ± 1.102	14.416	>**0.0001**
Sex Female	5.988 ± 0.477	12.555	>**0.0001**
Age Class Juvenile	2.839 ± 0.56	5.068	>**0.0001**
Subspecies *montanus*	2.112 ± 1.052	2.007	**0.0461**
Subspecies *shufeldti*	1.207 ± 1.145	1.054	0.2933
Subspecies *thurberi*	1.242 ± 0.947	1.312	0.1912
Subspecies *pinosus*	1.548 ± 1.12	1.383	0.1682
Days Since Molt	0.006 ± 0.005	1.261	0.2087
Specimen Age	0.007 ± 0.007	1.082	0.2804

Table 2. Continued.

Back *a*	β ± SE	*t* value	*P* value
(Intercept)	1.296 ± 0.203	6.396	>**0.0001**
Sex Female	0.417 ± 0.088	4.757	>**0.0001**
Age Class Juvenile	0.627 ± 0.103	6.089	>**0.0001**
Subspecies *montanus*	−0.187 ± 0.193	−0.967	0.3348
Subspecies *shufeldti*	0.154 ± 0.211	0.732	0.4647
Subspecies *thurberi*	0.158 ± 0.174	0.907	0.3657
Subspecies *pinosus*	0.537 ± 0.206	2.609	**0.0098**
Days Since Molt	−0.001 ± 0.001	−1.651	0.1004
Specimen Age	0.008 ± 0.001	6.251	>**0.0001**

Back *b*	β ± SE	*t* value	*P* value
(Intercept)	4.053 ± 0.582	6.959	>**0.0001**
Sex Female	2.196 ± 0.252	8.713	>**0.0001**
Age Class Immature	2.11 ± 0.296	7.132	>**0.0001**
Subspecies *montanus*	−0.768 ± 0.556	−1.382	0.1687
Subspecies *shufeldti*	0.375 ± 0.605	0.619	0.5364
Subspecies *thurberi*	0.32 ± 0.5	0.64	0.5231
Subspecies *pinosus*	1.29 ± 0.591	2.182	**0.0303**
Days Since Molt	−0.003 ± 0.003	−1.366	0.1736
Specimen Age	0.019 ± 0.003	5.488	>**0.0001**

values compared to males (Fig. 2, Table 2). Generally, females show lighter and buffier (higher red and yellow values) coloration in their hoods and backs compared to males. Although average values differed between males and females, there was still substantial overlap in the range of plumage coloration measurements between sexes.

Age classes also differed in dorsal color. Hood *a* was higher in juveniles compared to adults, while juveniles also had higher back *L*, *a*, and *b* measurements (Fig. 3, Table 2). Together, these results confirm that juvenile birds included in our study tended to have lighter, more "buffy" backs compared to adult juncos.

We also documented differences in coloration among subspecies of the Oregon Dark-eyed Junco group (Fig. 4, Table 2). We observed greater differentiation among subspecies in the mean values of hood measurements than back measurements. Using the Tukey's HSD test, we identified as many as 4 groups of mean hood coloration values among subspecies (Fig. 4A, B, and C). The northernmost subspecies, *J. h. oreganus*, had the lowest average hood reflectance, or *L* values, while *J. h. thurberi* had the highest average *L* values. The resident subspecies, *J. h. pinosus*, had the highest average hood *a* values, or "redness," while *J. h. montanus* had the lowest average hood *a* values. Patterns for hood *b* values, or "yellowness," followed a similar pattern to hood *a* values, but exhibited more overlap among subspecies.

Although mean hood measurements varied substantially among subspecies, differences in

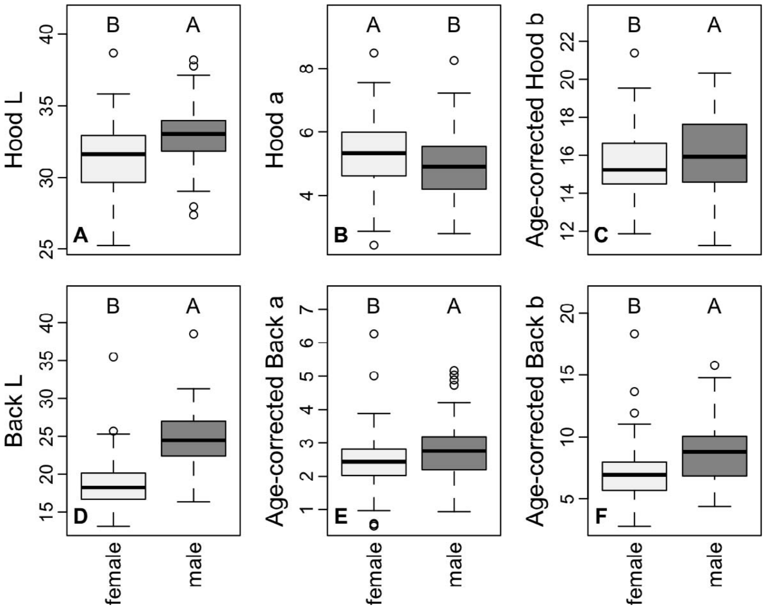

Figure 2. Hood and back coloration measurements among males and females of the Oregon Dark-eyed Junco complex. For the back and hood, "*L*" corresponds to brightness with higher values indicating brighter colors; "*a*" corresponds to redness, in which higher values correspond to more red coloration; and "*b*" corresponds to yellowness, in which higher values correspond to more yellow coloration. Shown above each box is the group classification following a Tukey's HSD test ($\alpha = 0.05$) for pairwise differences in mean values with the alphabetical order of groupings corresponding to descending differences in mean values among groups.

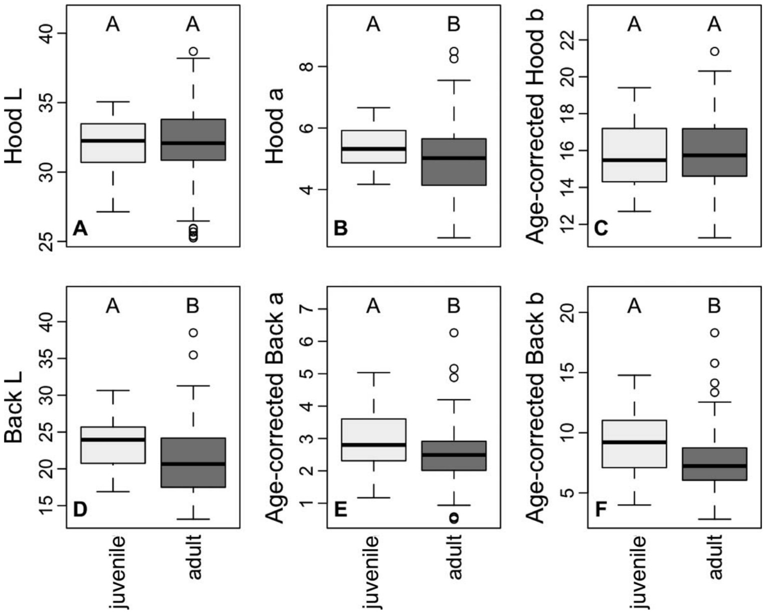

Figure 3. Hood and back coloration measurements among juvenile and adult individuals of the Oregon Dark-eyed Junco complex. For the back and hood, "*L*" corresponds to brightness with higher values indicating brighter colors; "*a*" corresponds to redness, in which higher values correspond to more red coloration; and "*b*" corresponds to yellowness, in which higher values correspond to more yellow coloration. Shown above each box is the group classification following a Tukey's HSD test ($\alpha = 0.05$) for pairwise differences in mean values with the alphabetical order of groupings corresponding to descending differences in mean values among groups.

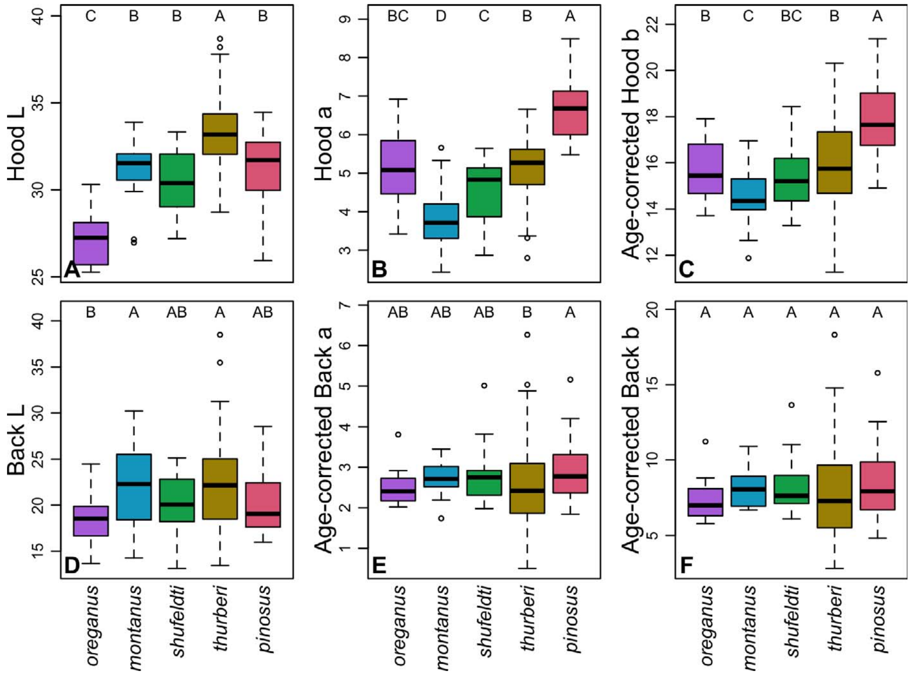

Figure 4. Hood and back coloration among subspecies of the Oregon Dark-eyed Junco complex. For the back and hood, "*L*" corresponds to brightness with higher values indicating brighter colors; "*a*" corresponds to redness, in which higher values correspond to more red coloration; and "*b*" corresponds to yellowness, in which higher values correspond to more yellow coloration. Shown above each box is the group classification following a Tukey's HSD test ($\alpha = 0.05$) for pairwise differences in mean values with the alphabetical order of groupings corresponding to descending differences in mean values among groups.

back coloration were less pronounced (Fig. 4, Table 2). The maximum number of back groupings we recovered via Tukey's HSD was 2 (Fig. 4D, E, and F). Differences among subspecies in mean back *L* values, or reflectance, were similar to those observed among hoods, albeit with greater overlap among subspecies.

The discriminant function analysis using the colorimetry data on adult specimens correctly assigned 55 out of 81 males to their identified subspecies (67.90%; Fig. 5A) and 66 out of 80 females (82.50%; Fig. 5B). Diagnosability varied among subspecies. For males, hood and back color successfully categorized (from north to south) 69.23% of *oreganus* (9/13), 75.00% of *montanus* (9/12), 20.00% of *shufeldti* (2/10), 85.71% of *thurberi* (30/35), and 45.55% of *pinosus* (5/11). For females, hood and back color correctly categorized 50.00% of *oreganus* (1/2), 75.00% of *montanus* (9/12), 0% of *shufeldti* (0/3), 91.07% of *thurberi* (51/56), and 71.43% of *pinosus* (5/7).

Among all pairs of subspecies and sexes for which we tested the "75% rule," 4 comparisons were diagnosable with D_{ij} values $\geq$ 0: male *J. h. oreganus* were diagnosable from male *J. h. montanus* (D_{ij} = 0.08; Table 3), female *J. h. oreganus* were diagnosable from female *J. h. montanus* (D_{ij} = 1.89; Table 3), female *J. h. shufeldti* were diagnosable from female *J. h. montanus* (D_{ij} = 0.04; Table 3), and female *J. h. pinosus* were diagnosable from *J. h. thurberi* (D_{ij} = 0.06; Table 3). In all of these cases, the converse comparisons did not meet the requirements of the "75% rule." All other pairwise comparisons had largely overlapping distributions of discriminant function scores and therefore failed to meet the "75% rule" threshold of diagnosability.

Discussion

Using a colorimeter, we documented variation in the coloration of hoods and backs between

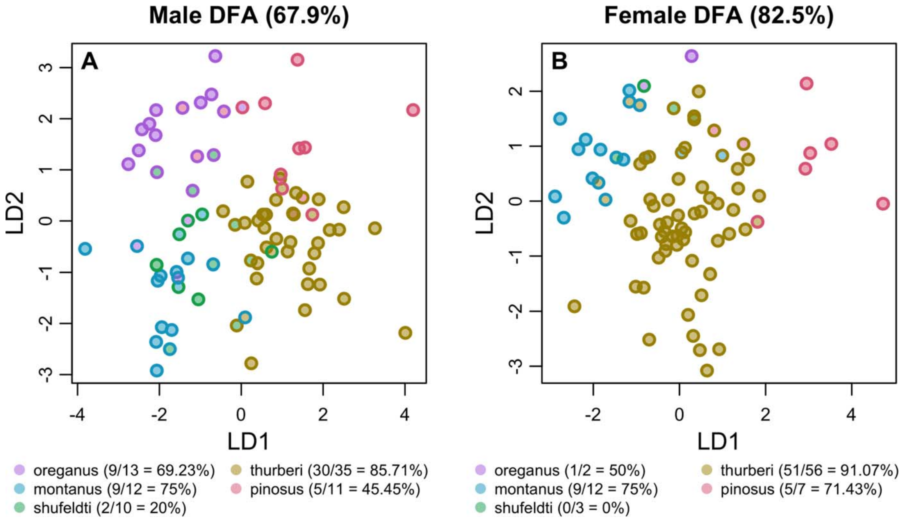

Figure 5. Discriminant function analysis and cross-validation analyses based on hood and back coloration for adult (A) males and (B) females among subspecies within the Oregon Dark-eyed Junco complex. Values for hood yellowness, back redness, and back yellowness have been corrected for specimen age. The percentage of total correct classifications across subspecies is shown at the top of each plot. Within each plot, the center of each point corresponds to the subspecies identity associated with the metadata of each specimen, while the outside ring of each point corresponds to the predicted subspecies of each individual.

subspecies, sexes, and age classes within the Oregon Dark-eyed Junco complex. Diagnosability of Oregon Dark-eyed Junco subspecies on the basis of back and hood coloration was limited for both sexes. Only 4 sex-specific subspecies pairwise comparisons passed the "75% rule" often used to delimit intraspecific taxonomy in birds (Patten and Unitt 2002). In each of these cases, the reciprocal comparison did not pass the "75% rule." Furthermore, cross-validation of our DFA was only able to accurately predict the subspecies grouping of 67.90% of males and 82.50% of females.

In contrast, Miller (1941) claimed that back color allows a 90% "separation rate" between *shufeldti* and *thurberi* in interior ranges and a 75% separation rate along the coast, a 92% separation rate between *oreganus* and *shufeldti,* and a 97% separation between *oreganus* and *montanus*. Similarly high separation rates are reported for other subspecies pairs. When comparing *montanus* and *shufeldti*, Miller (1941) reported a 75–80% "separation rate" but acknowledged that *shufeldti* is variable enough to "include the original *montanus* series."

The methodology Miller (1941) used to generate this output is unclear. Whether the high "separation rates" he found between subspecies represent the extent of overlap between back color

Table 3. Pairwise comparisons of Oregon Dark-eyed Junco complex subspecies. Results from diagnosability tests of the "75% rule" formalized by Patten and Unitt (2002) using scores from the first axis of the discriminant function analysis with all 5 subspecies pairs. When the test statistic (D) is >0, then 75% of the distribution for the first subspecies lies outside of 99% of the other subspecies.

		Male		Female	
Subspecies 1	Subspecies 2	D_{12}	D_{21}	D_{12}	D_{21}
oreganus	*montanus*	**0.08**	−0.54	**1.89**	−63.64
oreganus	*shufeldti*	−1.59	−1.04	−5.54	−20.43
montanus	*shufeldti*	−1.15	−0.79	−6.57	**0.04**
shufeldti	*thurberi*	−0.65	−0.91	−0.92	−1.28
thurberi	*pinosus*	−1.04	−0.75	−0.97	**0.06**

of subspecies, correct identification rate based on back color, or another method of differentiation is unknown. The most direct interpretation is that they represent the percentage of individuals that were able to be identified to subspecies by back color alone. In this case, our diagnosability rates fell short of what Miller (1941) reported, suggesting that subspecies of Oregon Dark-eyed Juncos exhibit weaker differentiation in dorsal coloration than has been heretofore asserted via qualitative assessments.

Importantly, we had low sample sizes for a few subspecies, most notably the nonmigratory *pinosus* ($n_{\text{male}} = 11$, $n_{\text{female}} = 7$), which has a restricted geographic range and correspondingly few specimens in most collections. We also had low sample sizes for *oreganus* ($n_{\text{male}} = 13$, $n_{\text{female}} = 2$), which decreased our statistical power to detect diagnosable differences for these taxa and contributed to the output of the "75% rule" tests involving *oreganus* and *pinosus*. These low sample sizes may have contributed to some of the discrepancies between Miller's study and ours.

Beyond dorsal coloration, many subspecies in the Oregon Dark-eyed Junco complex exhibit broadly overlapping, clinal variation in other phenotypes. For example, many subspecies exhibit substantial overlap in morphological characters, such as tail length, wing length, and the extent of white on rectrices (Ferree 2013). Furthermore, 4 out of 5 of the Oregon Dark-eyed Junco subspecies included in this study (*thurberi*, *shufeldti*, *montanus*, and *oreganus*) exhibit little to no genetic population structure based on genomic analyses involving thousands of loci (Friis et al. 2018). *J. h. pinosus*, however, exhibits pronounced genomic differentiation (Friis et al. 2018), is nonmigratory, and has the shortest wings of all Oregon Dark-eyed Junco subspecies (Ferree 2013), which we argue supports its status as a valid subspecies.

Although *oreganus* exhibits less genomic differentiation than *pinosus*, *oreganus* is also distinct in its darker plumage and partially geographically isolated range, suggesting it too may be a valid subspecies. On the other hand, *montanus*, *shufeldti*, and *thurberi* are less distinct: while *montanus* is duller than either *shufeldti* or *thurberi*, the broadly overlapping ranges between *shufeldti* with both *thurberi* and *montanus* and low genomic differentiation suggest a potential taxonomic revision for the 3 subspecies. Based on observed similarity in phenotype and genotype, one taxonomic solution may be to treat *montanus*, *shufeldti*, *and thurberi* as a single, widely distributed taxon with broad clinal variation across its range. Another solution would be to either lump *shufeldti* and *thurberi* together or to lump *thurberi* and *montanus*. Finally, the most conservative option would be to retain all existing taxonomic classifications and continue to recognize *montanus*, *shufeldti*, and *thurberi* as distinct subspecies.

In summary, our quantitative, colorimetric analysis of dorsal values does not support preexisting assertions of subspecies diagnosability within the Oregon junco complex. Miller's (1941) method for assessing pigmentary characters, which consisted of matching color effect in a given area of plumage with a graded series of color swatches and microscopic examination of pheomelanin and eumelanin, yielded results that are inconsistent with our colorimetry data. Our findings suggest a possible taxonomic revision of the Oregon Dark-eyed Junco group whereby fewer subspecies are recognized in light of broadly overlapping phenotypic and genetic variation. Revising subspecies limits in light of an improved understanding of geographic variation among populations promotes a more accurate and functional taxonomic classification of birds, which has broad implications across ornithology.

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