MINNESOTA'S RED-TAILED HAWKS: PROBABILISTIC ORIGINS OF *B.J. ABIETICOLA* AND DARK-MORPH MIGRANTS

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Table of Contents

List of Tables

List of Figures

Abstract

The Red-tailed Hawk (*Buteo jamaicensis*) is one of North and Central America's most common, polymorphic raptor species, with an extensive geographic range divided into 12 putative subspecies ranges. In North America, plumage polymorphism occurs along a clinal gradient with dark-morph individuals becoming less prevalent east of the Rocky Mountains. Polymorphism and other plumage traits can be used to identify individuals to a subspecies, but high levels of intergradation and individual variation can complicate identification. Duluth, Minnesota, USA is a migratory hotspot well known for phenotypically diverse Red-tailed Hawks, including *B.j. abieticola* and dark-morphs plumages. Due to atypical plumage traits of *B.j. abieticola* and dark-morphs, subspecific origins of Minnesota's migratory individuals are not always resolved. Genetic data was collected from Duluth's migratory Red-tailed Hawk population and known subspecies populations, *B.j. calurus* and *B.j. borealis*, to determine the probabilistic subspecific origins of *B.j. abieticola* and dark-morph migrants. Twelve microsatellite markers were used to analyze and compare genetic diversity and population structure within and among breeding populations and the migratory individuals. Bayesian statistics were also performed to determine probabilistic assignments of migratory individuals to putative subspecies. Supplemental spatial data was also collected from two presumed adult dark-morph *B.j. abieticola*. Pairwise F_{ST} revealed the *B.j. abieticola* and dark-morph migrants were both more genetically similar to *B.j. borealis* than *B.j. calurus*. Population assignment probabilities supported that these migratory individuals were more closely related to *B.j. borealis* than *B.j. calurus*. Furthermore, preliminary satellite transmitter

data from one presumed adult dark-morph *B.j. abieticola* migrant revealed the individual spent at least one summer east of the Rocky Mountains. These findings suggest Minnesota's *B.j. abieticola* and dark-morph migrants have a higher probability of originating from *B.j. borealis,* a Red-tailed Hawk subspecies historically known to only present light-morph plumage, than *B.j. calurus.*

Introduction

Background

The Red-tailed Hawk (*Buteo jamaicensis*) is one of the most widespread raptor species in the western hemisphere, well-known for its polymorphic plumage that varies throughout its geographic range. The distribution of the Red-tailed Hawk spans from the northern edge of the Boreal Forest in Canada to the Caribbean and southern Central America, reaching as far west as Alaska and the eastern Atlantic seaboard (Liguori and Sullivan 2014). The species geographic range occurs across deciduous woodlands, coniferous and boreal forests, plains, scrub deserts, and tropical rainforests (Preston and Beane 1993). Like other widespread avian species, geographic variation occurs across the Red-tailed Hawk's range, resulting in 12 generally recognized subspecies, and several subspecific races (Gillham 1956, Liguori and Sullivan 2014, Preston and Beane 2020). Additionally, Red-tailed Hawks have a high degree of territory fidelity, resulting in regional populations (Janes 1984). *B.j. borealis* and *B.j. calurus* are two of the most well-known subspecies, as their geographic ranges are widespread and are adjacent to each other at the Rocky Mountains; *B.j. borealis* extends eastward to the Atlantic Ocean and *B.j. calurus* extends westward to the Pacific Ocean (Figure 1). Furthermore, experts support the recognition of another subspecies, *B.j. abieticola* (Todd 1950), which is currently classified by the American Ornithological Society (Chesser et al. 2020) as a race of *B.j. borealis* (Preston and Beane 2020). In addition to individual variation within subspecies, variation in size and color occurs as a clinal gradient and has assisted in delineating subspecies boundaries and taxonomic status (Ridgway 1914, Gillham 1956, Mendel 1965).

Climate and habitat are two of the most likely predictors as to why morphological and plumage differences occur in Red-tailed Hawks and occur in a clinal pattern. Subtle relationships in size variation and topographic features are likely adaptations to minor climatic gradients (James 1970). Contradictory to Bergmann's ecogeographic rule, there is evidence that Red-tailed Hawks are smaller overall in the northern part of the species range and increase in body size southward (James 1970, Hull et al. 2010). Furthermore, overall body size of subspecies has been found to decrease from East to West (Pearlstine and Thompson 2004). Plumage melanin predictably seems to correspond with geographic variation as well (Mattison and Witt 2021). Like other temperate migrant buteos, color polymorphism is expressed ventrally in Red-tailed Hawks, appearing in three main color morphs – light, intermediate (rufous), and dark (Preston 1980, Rohwer and Paulson 1987, Liguori 2004). Intermediate- and dark-morphs are difficult to tell apart, therefore any non-light bird is commonly referred to as a dark-morph (Liguori 2004). All three morphs occur in the western regions of the USA and Canada, but intermediate- and dark-morphs rarely occur east of the Rocky Mountains (Liguori 2004). Therefore, subspecies in the western part of the range are considered polymorphic (i.e., *B.j. calurus, B.j. harlani*), and eastern subspecies are thought to be monomorphic. Nevertheless, dark-morphs are observed, albeit rarely, breeding, migrating, and wintering considerably east of the known polymorphic subspecies ranges, in locations such as Ontario, Minnesota, and New York (Iron 2012).

Historically, the taxonomy of Red-tailed Hawk subspecies has followed one set of criteria established by O'Brien and Mayr (1991) in which members of a subspecies share a geographic range or habitat and have concordant phenotypic characteristics. *B.j. calurus* has been described as having the most variable plumage, possibly due to the extremely heterogenous environment it occupies, from scrub desert to boreal forest (Taverner 1936, Preston 1980). Overall, adult *B.j. calurus* are typically distinguished from other subspecies by their paler red tails with narrow cross-barring, a throat that is never pure white, thigh barring (Dickerman and Parkes 1989), and less extensive buff markings on the upper wing coverts and flight feathers (Liguori 2004) (Figure 2). *B.j. borealis* is well-known for its bright tail with a single subterminal band, lightly marked or pure white throat and thighs, and a belly-band of fine dark streaks (Dickerman and Parkes 1987) (Figure 3A). While these traits are commonly associated with the respective subspecies, substantial individual variation in relation to interbreeding of subspecies can complicate subspecific identification, sometimes making it impossible (Liguori 2001). Birds showing traits of multiple subspecific ancestries are known as intergrades, likely natal to an area where subspecies breeding ranges are adjacent and could overlap. Potential intergradation, phylogenetic relatedness to other subspecies, knowledge gaps in the geographic range across the USA and Canada, and unknown polymorphic status are some reasons why *B.j. abieticola* is not yet officially recognized as a subspecies, but rather just a race of *B.j. borealis*.

The natural history of *B.j. abieticola* populations in the USA and Canada has led to long-standing debates regarding its taxonomic status as a Red-tailed Hawk subspecies. The

northern population of Red-tailed Hawks breeding in the spruce-fir belt of Canada, later named *B.j. abieticola*, was first described by Todd in 1950, then acknowledged as a subspecies by Parkes in 1952 (Dickerman and Parkes 1987, Liguori and Sullivan 2014). Plumage traits unique to *B.j. abieticola* include bold and heavy belly-band streaks and rich buff/rufous coloration of the underparts (Dickerman and Parkes 1987) (Figure 3B). *B.j. abieticola* is sometimes considered an intergrade between *B.j. borealis* and *B.j. calurus* because of a mixed suite of plumage traits, such as an unmarked tail with a single black subterminal band (similar to *B.j. borealis*), and a heavily streaked throat and thigh feathers (similar to *B.j. calurus*) (Dickerman and Parkes 1987, Liguori and Sullivan 2014). The extent of *B.j. abieticola*'s breeding range has yet to be fully described, but is assumed to overlap with *B.j. borealis* and *B.j. calurus* in the southern and western boreal forest, respectively (Figure 4) (Liguori and Sullivan 2014, Robinson unpublished data). In 1986, Godfrey declared that insufficient information about taxonomic status and geographic range is reason to invalidate *B.j. abieticola* as a subspecies (Dickerman and Parkes 1987). Presently, *B.j. abieticola* is not listed on the American Ornithological Society Checklist (Iron 2012, Chesser et al. 2020, Liguori pers. comm).

Resolving the taxonomic status of *B.j. abieticola* in relation to other Red-tailed Hawk subspecies will greatly improve our understanding of phenotypic variation within the species. Birders and ornithologists are becoming more aware of *B.j. abieticola* as a distinct phenotype that is at least treated as a more heavily marked race of *B.j. borealis*, but there is still strong support to assign it subspecific status (Liguori 2001, 2005, Sullivan 2011, Crossley et al. 2013, Liguori and Sullivan 2014). Conversely, *B.j.*

abieticola is still sometimes recognized as *B.j. calurus* (Liguori and Sullivan 2014)*;* one driving factor to this identification being the undetermined polymorphic state of *B.j. abieticola*. Many believe *B.j. abieticola* could also be polymorphic (Liguori, Sullivan, Nicoletti, pers. comm.) and are the source of many dark-morph individuals observed in historic monomorphic eastern regions. Preliminary field observations consider potential dark-morph *B.j. abieticola* different from dark-morph *B.j. harlani* because of their tail patterns (S. Figure 1) but are harder to distinguish from dark-morph *B.j. calurus*. Despite challenging plumage differences, geographic locations and migratory patterns of putative *B.j. abieticola* dark-morphs are not believed to align with *B.j. calurus*. Evidence of migrating and wintering light-morph *B.j. calurus* east of the Great Plains is lacking (Sullivan pers. comm.). Furthermore, about one in 50 *B.j. calurus* phenotypes (Sullivan pers. com.) are dark-morphs, supporting the idea that *B.j. calurus* are likely not the dark-morphs observed in the eastern extent of the species range.

Hundreds of *B.j. abieticola* are observed migrating and wintering through the Great Plains, Great Lakes, and northeast Atlantic region, but not west of the Rocky Mountains south of Canada (Iron 2012, Liguori and Sullivan 2014, Nicoletti pers. comm.). Duluth, Minnesota is a Red-tailed Hawk migratory hotspot located along the southwest edge of Lake Superior, with about 2% of birds being dark-morphs (Nicoletti pers. comm.). Furthermore, Red-tailed Hawk sampling performed at Hawk Ridge Bird Observatory in Duluth, MN, USA in fall of 2020 (N = 81) documented at least 50% of light-morph Red-tailed hawks expressing *B.j. abieticola* phenotypes (Pesano unpublished data). Despite the numerous contemporary and historical observations of Red-tailed Hawk plumage,

breeding, and movement ecology across all subspecies, genetic data should be collected to determine the probabilistic origins of both *B.j. abieticola* and dark-morph migrants.

Genetic differentiation of Red-tailed Hawk subspecies has previously been analyzed using mitochondrial DNA (mtDNA) and microsatellite (MSAT) markers. Because Red-tailed Hawks are highly mobile raptors, high levels of gene flow between subspecies (Arguedas and Parker 2000), may result in low levels of genetic differentiation (Kimura et al. 2002). Nevertheless, Red-tailed Hawks subspecies exhibit natal philopatry (Newton 1979) and still maintain various levels of genetic differentiation. Neutral mtDNA markers detected slight yet consistent population structure between eastern and western populations, with one unique haplotype in the eastern population and two unique haplotypes in the western populations (Pearlstine 2004). While mtDNA is able to capture genetic differentiation between Red-tailed Hawk populations, mtDNA is not sensitive enough to detect cryptic levels of population structure and gene flow (McDonald and Potts 1997, Arguedas and Parker 2000, Baker 2000, Milot et al. 2000), so microsatellites have been employed to study these aspects of population genetics in Red-tailed Hawk subspecies (Hull et al. 2008, 2010). Using 17 microsatellite loci developed specifically for buteo species, population structure was detected between and within breeding populations of *B.j. borealis* and *B.j. calurus;* one cluster identified with *B.j. borealis* and two clusters identified with northern and southern populations of *B.j. calurus* (Hull et al. 2008, 2010). Low, but significant genetic differentiation was also detected between the two subspecies ($F_{ST} = 0.031$, $P < 0.001$) with population boundaries generally aligning with the Rocky Mountains (Hull et al. 2008). Significant genetic differentiation and

population structure was also detected between *B.j. calurus* and both *B.j. borealis* and *B.j. harlani*, but not between *B.j. borealis* and *B.j. harlani* (Hull et al. 2010).

Main Questions and Hypotheses

While Red-tailed Hawks are not species of conservation concern, understanding how plumage polymorphism varies geographically can help provide information regarding the evolutionary processes that maintain biodiversity and which subspecies have evolutionary potential (Moritz 1994, Smith and Wayne 1996). With a wide breeding range across various climates and habitats, it is expected that phenotypic traits vary within and among subspecies. Currently, *B.j. abieticola*'s genetic relatedness to other subspecies, and the status of polymorphism in eastern breeding populations, are of high interest. Our two main research objectives are to, 1) investigate these topics of interest in Minnesota by sampling migratory birds with *B.j. abieticola* and dark-morph phenotypes, and 2) compare their genetic data to those of known *B.j. borealis* and *B.j. calurus* breeding populations. An additional third research objective is to investigate the breeding locations of presumed adult dark-morph *B.j. abieticola* individuals using satellite transmitters to provide high-resolution supplementary data. Ultimately, the evidence found in Minnesota will aid larger-scale research concerning Red-tailed Hawk population genetics and movement ecology conducted by The Red-tailed Hawk Project at Cornell University. Our objectives lead us to ask these three main questions:

1) Are Minnesota's *B.j. abieticola* migrants, more genetically similar to *B.j. borealis* than *B.j. calurus*?

2) Are Minnesota's dark-morph migrants more genetically similar to *B.j. borealis* than *B.j. calurus*?
3) Are Minnesota's presumed adult dark-morph *B.j. abieticola* migrants returning to breeding grounds east or west of the Rocky Mountains?

Based on the current knowledge of genetic differentiation and migratory patterns of Red-tailed Hawk subspecies, our hypotheses to our main questions are as follows:

1) Minnesota's *B.j. abieticola* migrants are more genetically similar to *B.j. borealis* than *B.j. calurus*.
2) Minnesota's dark-morph migrants are more genetically similar to *B.j. borealis* than *B.j. calurus*.
3) Minnesota's presumed adult dark-morph *B.j. abieticola* migrants are returning to breeding grounds east of the Rocky Mountains.

Methods

Sample Collection

Whole blood or red blood cells were collected from 309 total Red-tailed Hawks across 17 states during fall migration (August 15 – December 15), winter (February), and the breeding season (May through August) (Table 1, Figure 5). Fall migration samples were collected from free-flying adults and juveniles captured with mist-nest, dho-gazas, and bow nets at Hawk Ridge Bird Observatory, Duluth, Minnesota, USA (46.846919, -92.031802) between 2019 and 2021. Winter sampling for free-flying dark-morph adults on established territories occurred in Scott County, Minnesota, USA (S. Table 1) using bal-chatris in 2021. 170 total migration/winter samples were collected, including 52 light-morphs confidently identified as *B.j. abieticola* and seven dark-morphs; the other 80 individuals were light-morphs identified as *B.j. borealis* or were unidentifiable to subspecies. Breeding season samples were collected from nestlings, juveniles, and adults admitted to permitted raptor rehabilitation facilities and animal hospitals (S. Table 1). Samples were collected from admitted birds that were likely natal or held a territory in either the *B.j. borealis* or *B.j. calurus* breeding range (Figure 5). Samples were not collected from regions along the recognized subspecies border to avoid sampling potential intergrades (Figure 5). All breeding season samples were collected during 2021 with the exception of archived samples donated from Milliken University, IL, USA that were collected between 2015 and 2020.

Approximately 0.1 mL – 1 mL of whole blood was drawn via jugular venipuncture or brachial venipuncture using heparinized needles. Whole blood samples exceeding 0.4 mL collected during fall migration underwent centrifugation for other research projects, leaving the remaining red blood cells for this research. Approval for the collection, possession, and transport was approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol ID: 2105-39137A) and permitted by the U.S. Fish and Wildlife Service (Permit Number: MBPER0014234). Blood samples were stored on ice in the field, or on at least 5 kg of dry ice if shipped, prior to storing in -20^{o}C freezers at the laboratory. Nuclear DNA was extracted from a 200 µL blood/Phosphate Buffer Solution using QIAGEN DNeasy 96 Blood & Tissue Kits (QIAGEN, ID: 69504). Final extraction product was stored at -20^{o}C while in use and at -80^{o}C thereafter. Quantification of 2 µL of DNA with 98 µL of TE buffer and 100 µL of Quanit-IT Picogreen dsDNA stain (Thermo Fisher Scientific, ID: P11496) was then performed using a Biotek Synergy HTX Multi-Mode Reader (Agilent Technologies, ID: S1LFA). The amount of DNA (ng) per µL of ddH_2O was normalized to create stock plates. Stock plates were stored in a -80^{o}C freezer.

In addition to genetic data, plumage photos were collected. Plumage photos were taken against a neutral background and captured a bird's ventral and dorsal sides, upper tail and under tail spread, and head profile. All birds sampled during migration and winter were leg-banded with U.S. Geological Survey aluminum bands. Trapping, banding, and handling of birds was permitted by the Bird Banding Laboratory (Permit Number: 23927) and State of Minnesota Department of Natural Resources (Permit Number: 29208).

Plumage photos were used to identify migratory individuals as *B.j. abieticola* (Liguori and Sullivan 2014, Nicoletti pers. comm.) or as dark-morphs. Individuals that could not be confidently assigned as having characteristic *B.j. abieticola* plumage, could confidently be assigned as *B.j. borealis*, or were not able to be identified to one subspecies, were grouped as "other."

Microsatellite Data Collection

Each individual was genotyped at 17 polymorphic microsatellite loci (BswA110w, BswD122w, BswA204w, BswA317w, BswD210w, BswD220w, BswA303w, BswB111aw, BswD234w, BswD310w, BswD313w, BswB220w, BswB221w, BswD327w, BswA302w, BswD127w, BswA312w), developed by Hull et al. 2007, in seven multiplex polymerase chain reactions (PCR) (S. Table 2). Amplification of DNA via PCR was performed using a PCRmax Alpha Cycler 2 Thermocycler (Cole-Parmer Instrument Company, ID: EW-93945-22) and set to conditions previously described by Hull et al. 2007. Microsatellite visualization of PCR products was performed using an Applied Biosystems 3730XL DNA Analyzer (Thermo Fisher Scientific, ID: 3730XL). GeneMarker 3.0.1 was used to visualize PCR products and score alleles (SoftGenetics, State College, PA, USA). Across the 17 loci, only 9.06% of samples failed to amplify.

Analysis of Genetic Diversity

All 17 microsatellite loci were tested for deviations from Hardy-Weinberg equilibrium (HWE) using Genepop 1.1.7 software (Rousset 2008) in RStudio 4.1.1 (RStudio Team

2020) using exact conditional testing with 10,000 dememorization Markov chain steps, 100 batches, and 1,000 iterations per batch (Hull et al. 2010). Five of the 17 loci deviated from HWE and were dropped from subsequent analyses (S. Table 2). Genotypic linkage disequilibrium was assessed in Genepop using exact testing, 10,000 dememorization Markov chain steps, 100 batches, and 1,000 iterations per batch (Hull et al. 2010) between each pair of loci across the entire sampled population. Holm-Bonferroni correction tests for multiple comparisons were performed to determine significance of all HWE and genotypic linkage disequilibrium tests (Rice 1989). The number of alleles per locus in HWE were also determined. Null allele frequencies for each locus were determined using Genepop and the average null allele frequencies were calculated. Corresponding genotyping failure rates were also calculated for each locus in Genepop then averaged for the entire sample population.

Genetic diversity indices were calculated using migratory individuals (*B.j. abieticola*, breeding *B.j. borealis*, and breeding *B.j. calurus* as the main units of analyses, hereafter referred to as "subunits". The number of expected and observed homozygotes and heterozygotes for each locus, per subunit, were determined by Genepop, and were then used to calculate the levels of observed and expected heterozygosity for the entire population and each subunit. The program GenAlEx 6.503 (Peakall and Smouse 2006, 2012) was used to detect the number of private alleles within each subunit. The total number of alleles per locus for each subunit were determined using Genepop and were then used to calculate the average number of alleles per locus. Allelic richness for each

locus corrected for sample size or smallest subunit (n = 7) was computed using adegenet package in RStudio (Jombart 2008) then averaged for each subunit.

Genetic Differentiation Analyses

Probabilistic Bayesian clustering analysis of genotypes was performed using all samples without *a priori* information in the program Structure 2.3.4 (Pritchard et al. 2000). The analysis used an admixture model with a uniform prior, autocorrelated assumed allele frequencies, and a run length of 100,000 Markov chain iterations with 10,000 burn-in iterations (Hull et al. 2008) for K = 1 through 5. K represents the number of estimated subpopulations. By testing varying levels of K, we wish to find the number of subpopulations detected by the program based on genotype patterns rather than assigning populations by locality, phenotype, etc. The analysis for each level of K was repeated 10 times, resulting in 50 total analyses for the dataset (Hull et al. 2010). Structure Harvester 0.6.94 was implemented to visualize the Structure output and calculate the most likely number of genetic clusters (delta$K$) using the Evanno method (Earl and vonHoldt 2012). A delta$K$ of 2 was estimated as the minimum value of K that did not sacrifice any explanatory power. To visualize admixture proportion, structure output files were visualized using K = 2 via the pophelperShiny GitHub app (Francis 2017).

Admixture within the total sample population was tested in RStudio using the rstatix package (Kassambara 2021). Prior to admixture analysis, Structure output files for K = 2 were run in *CLUMPP* 1.1.2 to permute the cluster coefficient matrices of the 10 iterations

so they match as closely as possible (Jakobsson and Rosenberg 2007). *A priori* individual data was randomly inputted via the Greedy algorithm, weighted by the number of individuals, over 1,000 repeats. The *CLUMPP* analysis calculated cluster frequencies for each individual based genotype. A linear model was created using the cluster 1 genetic frequencies as the response variable and subunit as the predictor variable. An ANOVA was performed to detect a significant explanation of variation in cluster 1 genetic frequencies in response to phenotype. Non-significant results would indicate contemporary admixture between the sample population.

Pairwise *G*-statistical Analysis for F_{ST}, based on 999 permutations, were performed in GenAlEx to estimate the variance in allele frequencies of the 12 loci among *B.j. calurus*, *B.j. borealis*, migratory *B.j. abieticola*, migratory dark-morphs, and other migrants (Peakall and Smouse 2006, 2012). Holm-Bonferroni corrections for multiple tests were applied to the *P*-values calculated by GenAlEx.

Log likelihood assignment tests were performed to determine the probabilistic subspecies origins of migratory individuals. Log likelihood assignment values for each sample and mean log likelihood values for each subunit comparison were calculated in GenAlEx. Pairwise biplots of likelihoods for the entire sample population and comparisons of interest (breeding *B.j. borealis*/breeding *B.j. calurus*, breeding *B.j. borealis/B.j. abieticola* migrants, breeding *B.j. calurus/B.j. abieticola* migrants, breeding *B.j. borealis*/dark-morph migrants, and breeding *B.j. calurus*/dark-morph migrants) were created with a

standard 1:1 line. R^2 values and corresponding p-values were calculated for each subunit comparison.

Spatial Data Collection

Two 25g ES 500 Wildlife Tracker GSM+Argos Satellite units (Cellular Tracking Technologies, Rio Grande, New Jersey, USA) were deployed on adult dark-morph Red-tailed Hawks that are presumed to be *B.j. abieticola* based on plumage traits. One unit was deployed on an individual captured in February 2021 in Scott County, Minnesota, USA and the other was deployed on a migratory individual captured in October 2021 in Duluth, Minnesota, USA. GPS fixes collect every 15 min, 7 days per week across the Argos satellite network that is available with or without access to a cellular network. Fixes upload to the Cellular Tracking Technologies User Interface once a day, if the unit has access to the cellular network, or once the bird returns to an area with cellular coverage. Approval and authorization to attach backpack transmitters on Red-tailed Hawks were provided by the University of Minnesota Institutional Animal Care and Use Committee (Protocol ID: 1904-36977A) and Bird Banding Laboratory (Permit Number: 23927), respectively.

Results

Analysis of Genetic Diversity

Of the 12 microsatellite loci used, deviation from Hardy-Weinberg equilibrium was detected at one locus ($P \leq 0.001$). Subsequent testing revealed deviation occurred only at BswD127w when migratory individuals were included in the analysis. When migratory individuals were removed from analysis, BswD127w did not deviate from HWE. Because migratory individuals may not originate from the same reproducing population, we believe this one deviation will not interfere with the interpretation of our results. Out of 198 comparisons, 5 cases of linkage disequilibrium (LD) were detected, indicating regular recombination of alleles in the population is occurring. There was a high degree of variation in the number of alleles per locus (S. Table 2) and per subunit (Table 2). No apparent null alleles were detected, based on an average null allele frequency of 0.02 with a genotyping failure rate of 0.003 across all 12 loci. Table 2 summarizes the sample size (N), expected heterozygosity (H_E), observed heterozygosity (H_o), average number of alleles (A_a), allelic richness (AR_C), and private alleles (A_P) for the subunits. The average observed heterozygosity (0.75 ± 0.04) was comparable to the average expected heterozygosity (0.74 ± 0.02), and average allelic richness corrected for sample size (17.58 ± 13.43) were similar across the subunits, demonstrating genetic diversity is prevalent among each subunit and the entire population.

Genetic Differentiation Analyses

Probabilistic Bayesian clustering analysis ($K = 2$) in Structure did not reveal any apparent population structure or distinct populations (Figure 6). Genotype frequencies did not significantly differ among the phenotype groups indicating there is genetic admixture among populations (P = 0.91). Pairwise *G*-statistical Analysis for F_{ST} revealed low levels of genetic differentiation between all subunit comparisons but did indicate statistically significant levels of F_{ST} between several comparisons. Significant genetic differentiation was detected between *B.j. borealis* and *B.j. calurus* ($F_{ST} = 0.016$, $P \leq 0.001$). Both *B.j. abieticola* migrants and dark-morph migrants were more genetically similar to *B.j. borealis* than *B.j. calurus*, with only the comparisons to *B.j. calurus* being statistically significant (Table 3).

Population assignment results determined the subspecies to which migrants have the highest probability of being assigned. For reference, both *B.j. borealis* and *B.j. calurus* individuals had higher average log likelihoods of assigning to themselves compared to the other subspecies (Table 4). The mean log likelihood value of *B.j. abieticola* migrants is higher for *B.j. borealis* (-17.12) than *B.j. calurus* (-18.72), meaning *B.j. abieticola* migrants have a higher probability of being assigned to the *B.j. borealis* population. The mean log likelihood values of dark-morph migrants assigned to *B.j. borealis* (-16.79) is also higher than the mean log likelihood value of their assignment to *B.j. calurus* (-18.08). Overall, there is some correlation between *B.j. calurus* and *B.j. borealis* ($R^2 = 0.37$, $P < 0.001$). Pairwise biplots of log likelihoods (Figure 7) revealed *B.j. abieticola* migrants are more correlated with *B.j. borealis* ($R^2 = 0.77$, $P < 0.001$) than *B.j. calurus*

($R^2 = 0.14$, $P = 0.24$). Furthermore, dark-morph migrants were more similar to *B.j. borealis* ($R^2 = 0.29$, $P < 0.001$) than *B.j. calurus* ($R^2 = 0.16$, $P = 0.38$).

Spatial Data Collection

Results concerning spatial data are preliminary but suggest interesting migratory patterns of both presumed adult dark-morph *B.j. abieticola* migrants sampled in Minnesota. Our first target bird, hereafter known as Manley, was tagged in February 2021 while wintering in Scott County, MN, USA from approximately January through mid-April. Manley's dark-brown ventral coloration, rufous breast, and unbanded tail with well-defined subterminal band (classically seen in *B.j. borealis* and alleged dark-morph *B.j. abieticola*) was an ideal combination for investigating potential dark-morph eastern Red-tailed Hawks (Figure 8A). Satellite data revealed Manley spent the summer of 2021 east of the Rocky Mountains in northern Manitoba (Figure 8B). Upon capture, Manley was entering its third cycle and likely did not breed in 2021. Manley returned to the same wintering territory in 2022 and begun migrating north, out of cellular service, into Manitoba.

The second target bird, hereafter referred to as Trudi, was fitted with a transmitter in the fall of 2021 at Hawk Ridge Bird Observatory. Trudi presents plumage possible for alleged dark-morph *B.j. abieticola*, but perhaps more typical for a potential intergrade, with a reddish tail with multiple bands and a smudgy sub-terminal band (Figure 8C). While Trudi's subspecific origins are not known, adult *B.j. harlani* is not likely due to the

tail pattern. Satellite data revealed Trudi wintered near Indianapolis, IN, USA (Figure 8D). As of mid-April 2022, Trudi began migrating north, but was only detected as far as northern Minnesota before leaving cellular range. More information about both Trudi's and Manley's breeding ranges is required before comparing spatial use to other subspecies ranges.

Discussion

Main Questions and Hypotheses

Understanding the subspecific origins of Minnesota's *B.j. abieticola* migrants and dark-morph migrants has captured the curiosity of raptor researchers over the last several decades. *B.j. abieticola* is currently considered a race of *B.j. borealis*, but individuals with the *B.j. abieticola* phenotype have also been deemed intergrades of the western and eastern subspecies. Furthermore, *B.j. abieticola* is plausibly polymorphic. Over time, two main theories have been posited regarding Minnesota's migratory dark-morphs of unknown subspecific origin; 1) dark-morphs are *B.j. calurus* that have migrated outside of their typical geographic range, 2) dark-morphs are *B.j. abieticola* migrating from their presumed range in Canada's Boreal Forest. Our main research objective was to establish the probabilistic subspecific origins of Minnesota's *B.j. abieticola* migrants and dark-morph migrants, relative to putative subspecies, *B.j. calurus* and *B.j. borealis*. This objective was accomplished by answering and supporting the following questions and hypotheses:

1) Are Minnesota's *B.j. abieticola* migrants more genetically similar to *B.j. borealis* than *B.j. calurus*?

   Hypothesis 1: Minnesota's *B.j. abieticola* migrants are more genetically similar to *B.j. borealis* than *B.j. calurus*.

2) Are Minnesota's dark-morph migrants more genetically similar to *B.j. borealis* than *B.j. calurus*?

Hypothesis 2: Minnesota's dark-morph migrants are more genetically similar to *B.j. borealis* than *B.j. calurus*.

3) Are Minnesota's presumed adult dark-morph *B.j. abieticola* migrants returning to breeding grounds east or west of the Rocky Mountains?
   Hypothesis 3: Minnesota's presumed adult dark-morph *B.j. abieticola* migrants are returning to breeding grounds east of the Rocky Mountains.

Pairwise F_{ST} indicated both *B.j. abieticola* and dark-morph migrants are more genetically similar to *B.j. borealis* and were significantly less similar to *B.j calurus*, therefore we fail to reject Hypothesis 1 and 2. Similar to other studies (Hull et al. 2008, 2010), our data revealed low but significant levels of genetic differentiation between *B.j. borealis* and *B.j. calurus*, indicating our Pairwise F_{ST} values for migrant to breeding comparisons should hold stock. However, *a priori* population structure did not reveal any subspecific clustering within the entire sample population, which was supported by a high level of admixture. Schwartz and McKelvey (2008) have suggested population structure is not detected when individuals and their alleles have not been evenly sampled across a distribution on the landscape. Depending on the genetic gradient in which samples were collected, the composition of clustering will not reflect accurately. The results of our clustering analysis may not have been portrayed accurately considering only 23 breeding *B.j. calurus* were sampled compared to 147 breeding *B.j. borealis*. Furthermore, mean log likelihoods for both *B.j. abieticola* and dark-morph migrants were lower for *B.j. calurus* than *B.j. borealis*, indicating they are not as likely to be assigned as *B.j. calurus*. Log

likelihood assignment tests have also strongly supported geographic and racial composition assignments of newly colonized populations of Canada Geese (*Branta canadensis*) in Greenland (Scribner et al. 2003). Posterior probabilities calculated using a Bayesian model estimate statistical confidence in individual assignments, accurately resulting in genetic identities being assigned to the most closely associated reference breeding population provided (Scribner et al. 2003).

Preliminary spatial data collected from Manley, a presumed dark-morph *B.j. abieticola*, suggests summering locations east of the Rocky Mountains, thus we fail to reject Hypothesis 3. In 2021, Manley summered in northern Manitoba, a region currently recognized as part of the *B.j. borealis* breeding range (Figure 1) and presumed *B.j. abieticola* breeding range (Figure 5). Manley currently appears to have headed towards the same summering territory in May of 2022 and will likely breed. Specific summering locations should be available upon Manley's migration south into a region with cellular service. More data about Trudi's summer location is needed before we can further support or refute Hypothesis 3. Scribner et al. (2003) found Canada Geese of unknown breeding origins had microsatellite allele frequencies that corresponded to breeding locations detected by satellite telemetry. So, while more information is needed to make sufficient conclusions with satellite spatial data, it is likely genetic data will continue support our current conclusions regarding these birds' summering locations.

We conclude that Minnesota's *B.j. abieticola* migrants and dark-morph migrants are more genetically similar to *B.j. borealis* than *B.j. calurus*, and preliminary spatial data of presumed dark-morph *B.j. abieticola* are returning to summering grounds east of the Rocky Mountains. Ultimately, this data indicates polymorphism is highly likely in the eastern clade of Red-tailed Hawk. Polymorphism has not historically been considered in *B.j. borealis*, but has been in *B.j. abieticola*. If future research is able to confirm dark-morph plumage in breeding *B.j. abieticola*, several mechanisms could be maintaining a low ratio of polymorphism in the population.

Mechanisms Maintaining Color Polymorphism

Color polymorphism occurs in a wide variety of taxa, but its purpose in certain groups, like Red-tailed Hawks, could be maintained by several different ecological mechanisms. Color polymorphism has been observed in both ancestral and modern bird taxa, likely having evolved this trait independently over evolutionary time due to apostatic, disruptive, or sexual selection (Galeotti et al. 2003). The majority of polymorphic raptors are buteos, suggesting polymorphism may have evolved in a common ancestor. However, polymorphism does not always occur throughout an entire buteo species, as seen in Red-tailed Hawks. Polymorphism in some buteo populations could be maintained as a result of disruptive selection (i.e., crypsis, thermoregulatory adaptations) when populations exist over a heterogeneous environment (Kettlewell 1956, Murton 1971, Johnson and Brush 1972, Otte and William 1972). Heterogenous environments are composed of various climatic and habitat breaks which may influence habitat-biased dispersal and population structure within a species (Hull et al. 2008). For example, population structure

was detected within northern and southern populations of *B.j. calurus*. Genotypic differences corresponded with the habitat differences north and south of the Sierra Nevada Mountains (Hull et al. 2008). Furthermore, *B.j. calurus* is polymorphic, possibly indicating the ratio of light- and dark-morph plumages in its geographic range is maintained by frequency dependent selection. These genetic and phenotypic differences associated across and within putative Red-tailed Hawk subspecies could have evolved as a result of disruptive selection and are now maintained by contemporary environmental factors.

While disruptive selective theories have not been proven, color-morph tends to correlate with cryptic camouflage and thermoregulation. Disruptive selection assumes color-morph is associated with crypsis or physiological adaptations to the environment (Galeotti et al. 2003). Variable light conditions in association with habitat, may be a strong selective mechanism maintaining color polymorphism (Galeotti et al. 2003). For example, significant differences in perch-site selection were discovered between light- and dark-morph Red-tailed Hawks ($P \leq 0.001$) (Preston 1980). Light-morph birds were more likely to use perches in open habitat, whereas dark-morph birds were more likely to use perches in dense wooded habitat (Preston 1980). Selective pressures for color-morph are likely linked to bird detectability within a habitat. Habitat is not as heterogenous in the eastern portion of the Red-tailed Hawk range, perhaps indicating why *B.j. borealis* may be monomorphic. On the other hand, according to two ecogeographic rules, disruptive selection may correlate with physiological advantages. Gloger's geographic rule predicts darker plumage associating with wetter and warmer environments (Mattison and Witt

2021). Bogert's geographic rule is contradictory to Gloger's rule and predicts darker plumage is associated with colder environments (Mattison and Witt 2021). Crypsis and Bogert's rule could both suggest the of maintenance of rare polymorphism in *B.j. abieticola* because the presumed geographic range is in northern latitudes with dense wooded habitat.

Broader Impacts

The genetic and phenotypic variation within and across Red-tailed Hawk subspecies provides a great model system for understanding evolutionary processes in a widespread, migratory species. Maintenance of genotypic and phenotypic variability in the Red-tailed Hawk has likely helped and will continue to help the species adapt to novel conditions (Lande and Shannon 1996). Presently, the North American Red-tailed Hawk population is stable and not currently of great conservation concern (Hoffman and Smith 2003, Farmer et al. 2007, Craighead et al. 2016). Nevertheless, identifying levels of genetic diversity, population differentiation, and locations of breeding grounds are critical tasks. Subspecies population levels could change over time in response to habitat fragmentation, climate change, or prey availability across the entire species range. By upholding surveys of genetic variation within a species, researchers will be able to keep track of genetic variability as it changes over time as a result of anthropogenic change (Moritz 1994, Moritz et al. 1996). Ultimately, these observations can help maintain biodiversity at a larger scale (Moritz 1994, Moritz et al. 1996).

Future Directions

While we can say with more confidence Minnesota's *B.j. abieticola* migrants and dark-morphs migrants are more genetically similar to *B.j. borealis* than *B.j. calurus*, we need to elucidate more information regarding genetic similarity of our migratory individuals to other breeding populations (i.e., *B.j. abieticola* and *B.j. harlani*). *B.j. borealis* is not necessarily polymorphic, but one of its races, *B.j. abieticola*, may be. By understanding the genetic relatedness of breeding *B.j. abieticola* to other subspecies, we should be able to more confidently assign Minnesota's *B.j. abieticola* and dark-morph migrants to more than just *B.j. borealis* and *B.j. calurus*. Furthermore, *B.j. harlani* are known to migrate east of the Rocky Mountains and occasionally through Duluth, and can typically be identified in the field. However, juvenile dark-morphs that do not obviously present *B.j. harlani* plumage still present a caveat to truly understanding the subspecific origins of all dark-morph migrants in Duluth. Even though we do know *B.j. harlani* is genetically different from *B.j. calurus*, based on the microsatellites currently being used, we do not know the full extent in which *B.j. harlani* and *B.j. borealis* differentiate (Hull et al. 2010). As a result, the subspecific composition of Minnesota's migratory dark-morphs still needs to deduced between the relative amount of *B.j. harlani* and *B.j. borealis*. Furthermore, deploying more satellite transmitters on unique dark-morph Red-tailed Hawks and light-morph *B.j. abieticola* will provide more evidence that can either support of refute polymorphism in the eastern breeding range of the Red-tailed Hawk.

Tables and Figures

Table 1. Summary of Red-tailed Hawk Genetic Samples. Genetic samples were obtained from subspecies of known breeding locations during the breeding season, and individuals of unknown subspecies and breeding locality during the migration/winter seasons.

Subspecies	**Season**	**State**	**Sample Size (n)**
B.j. borealis	Breeding	Delaware	1
		Iowa	6
		Illinois	30
		Minnesota	35
		Missouri	27
		North Carolina	8
		New Jersey	3
		Ohio	7
		Pennsylvania	4
		South Carolina	14
		Virginia	6
		Vermont	5
		Wisconsin	1
B.j. calurus	Breeding	California	7
		Idaho	3
		Oregon	6
		Washington	7
Buteo jamaicensis spp.	Fall migration/winter	Minnesota	139*
Total (N)	-	-	309

*Seven individuals are dark-morphs, 52 are light-morphs with *B.j. abieticola* phenotype, and 80 were light-morphs identified as *B.j. borealis* or unidentifiable to subspecies.

Table 2. Red-tailed Hawk Genetic Diversity Indices Across Subunits. 309 individuals were genotyped across 12 microsatellite loci. Migratory individuals were grouped by phenotype to assess genetic diversity within this subunit. "Other" migrants include those that could not easily be identified to subspecies phenotype or have a *B.j. borealis* phenotype.

Subunits	**N**	**H_E ± sd**	**H_o ± sd**	**A_a ± sd**	**AR_C ± sd**	**A_P**
Breeding *B.j. borealis*	147	0.76 ± 0.19	0.76 ± 0.19	14.75 ± 11.77	5.98 ± 2.16	35
Breeding *B.j. calurus*	23	0.71 ± 0.21	0.71 ± 0.21	8.25 ± 5.07	5.52 ± 2.34	2
Mig *B.j. abieticola*	52	0.74 ± 0.21	0.71 ± 0.20	11.58 ± 7.19	5.95 ± 2.26	12
Mig Dark-morphs	7	0.75 ± 0.19	0.79 ± 0.20	5.5 ± 2.47	7.58 ± 5.18	1
Mig Other	80	0.76 ± 0.19	0.76 ± 0.18	12.17 ± 8.73	5.99 ± 2.21	13
Total	309	0.74 ± 0.02	0.75 ± 0.04	10.45 ± 3.61	17.58 ± 13.43	-

N = the number of samples, H_E = expected heterozygosity, H_o = observed heterozygosity, A_a = average number of alleles per locus, AR_C = allelic richness corrected for smallest population sample size (n = 14), A_P = number of private alleles.

Table 3. Pairwise F_{ST} Comparisons of Red-tailed Hawk Subspecies and Migrants. Pairwise F_{ST} values, calculated from 12 microsatellite loci, represent relative level of genetic differentiation between compared subunits.

	Breeding *B.j. borealis*	**Breeding *B.j. calurus***	**Mig *B.j. abieticola***	**Mig Dark-morphs**
Breeding *B.j. borealis*	-			
Breeding *B.j. calurus*	0.016***	-		
Mig *B.j. abieticola*	0.004	0.021***	-	
Mig Dark-morphs	0.025	0.040**	0.022	-

**Indicates significance of $P \leq 0.01$ and ***indicates significance of $P \leq 0.001$ following a Holm-Bonferroni correction.

Table 4. Population Assignment of Individuals. Mean log likelihood values of migratory individuals were calculated to determine which subspecies the migrants are more likely to assign to. The mean log likelihood was also determined for the breeding populations. Log likelihoods were calculate using allele frequencies from all 12 loci. Statistical significance was determined based on R^2 values.

	Breeding ***B.j. borealis***	**Breeding** ***B.j. calurus***	**Mig** ***B.j. abieticola***	**Mig** **Dark-morphs**
Breeding *B.j. borealis*	-16.65***	-17.09	-17.12***	-16.79***
Breeding *B.j. calurus*	-18.61***	-13.95	-18.72	-18.08

**Indicates significance of $P \leq 0.01$ and ***indicates significance of $P \leq 0.001$ following a Holm-Bonferroni correction.

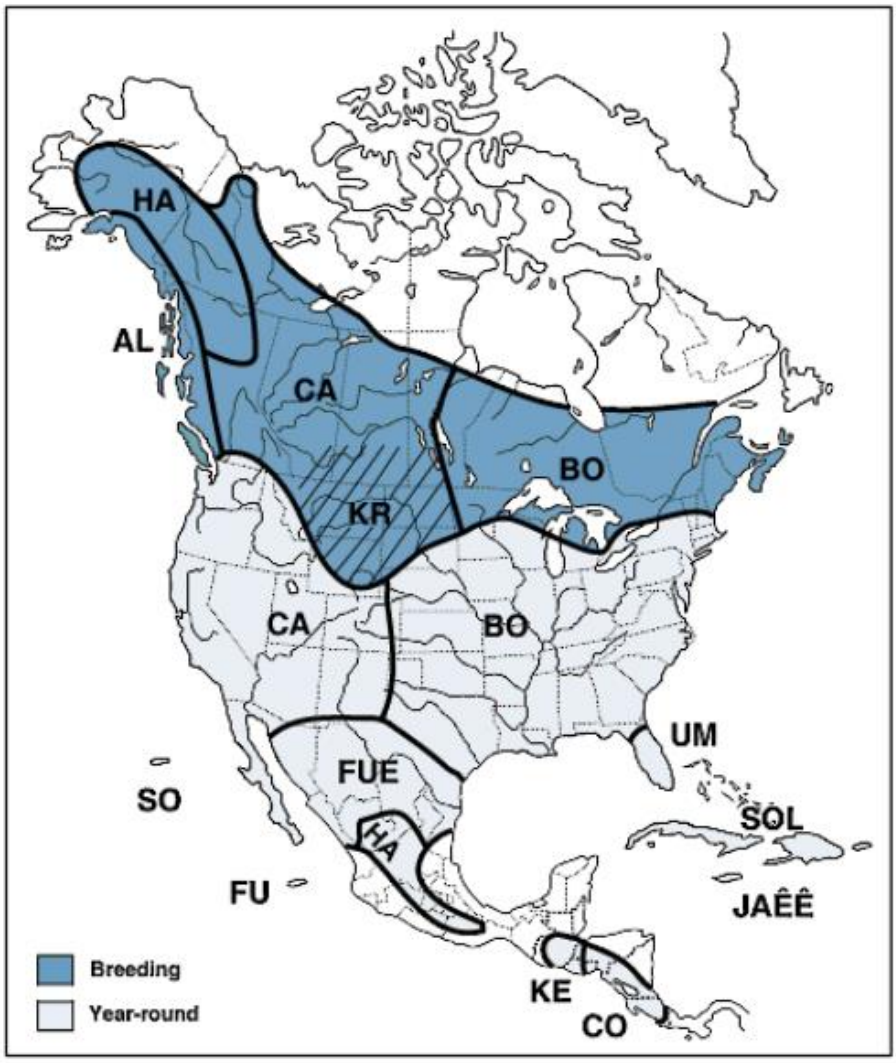

Figure 1. Breeding and Year-round Distributions of Red-tailed Hawk Subspecies. The approximate breeding and year-round distributions of 14 Red-tailed Hawk subspecies and races cover the majority of North America, Central America, and neighboring islands. HA (northeast North America) = *harlani*, AL = *alascensis*, CA = *calurus*, KR = *kriderii*, BO = *borealis*, FUE = *fuertesi*, SO = *soccorroensis*, HA (Mexico) = *hardopus*, FU = *fumosus*, UM = *umbrinus*, SOL = *solitudinus*, JAÊÊ = *jamaicensis*, KE = *kemsiesi*, CO = *costaricensis*. Figure by Preston and Beane (2020), adapted from Johnsgard (1990).

Figure 2. Adult *B.j. calurus* Plumage Characteristics. *B.j. calurus*, also known as the "western Red-tailed Hawk," is polymorphic with light being the predominant color-morph. Light-morphs generally present whitish throats, narrow tail stripes, and cross-barring and belly-band streaks on the ventral side. Dark-morphs are similar to light, but have uniform dark-brown to black ventral sides. Intermediate-morphs are also presented in this subspecies and tend to be more mottled with rufous than the dark-morphs. Illustrations by Emma Regnier (2022).

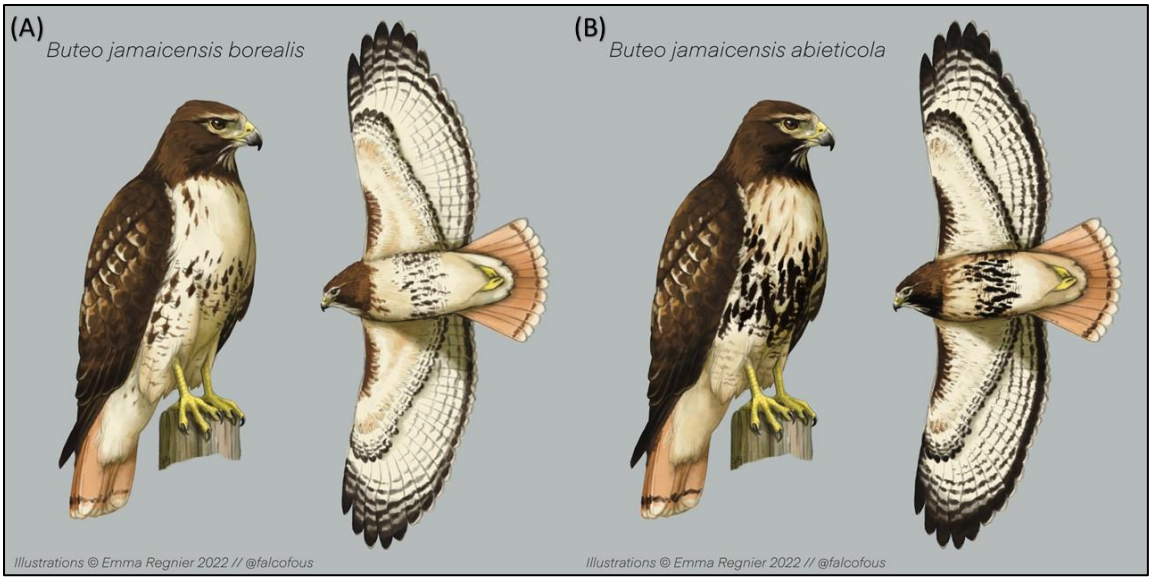

Figure 3. Adult *B.j. borealis* and *B.j. abieticola* Plumage Characteristics. (A) *B.j. borealis*, also known as "eastern Red-tailed Hawk," is monomorphic, presenting only a light-morph. Overall, *B.j. borealis* has sparse belly-band markings and a plainly marked tail with a defined subterminal band. (B) *B.j. abieticola*, also known as the "northern Red-tailed Hawk," is currently only known to be monomorphic, but may present a dark-morph. *B.j. abieticola* is technically identified as a heavily marked race of *B.j. borealis* and is not officially acknowledged as a subspecies due partly to current knowledge gaps regarding its polymorphic status. Its tail pattern ranges from plain to lightly marked with a defined subterminal band. Illustrations by Emma Regnier (2022).

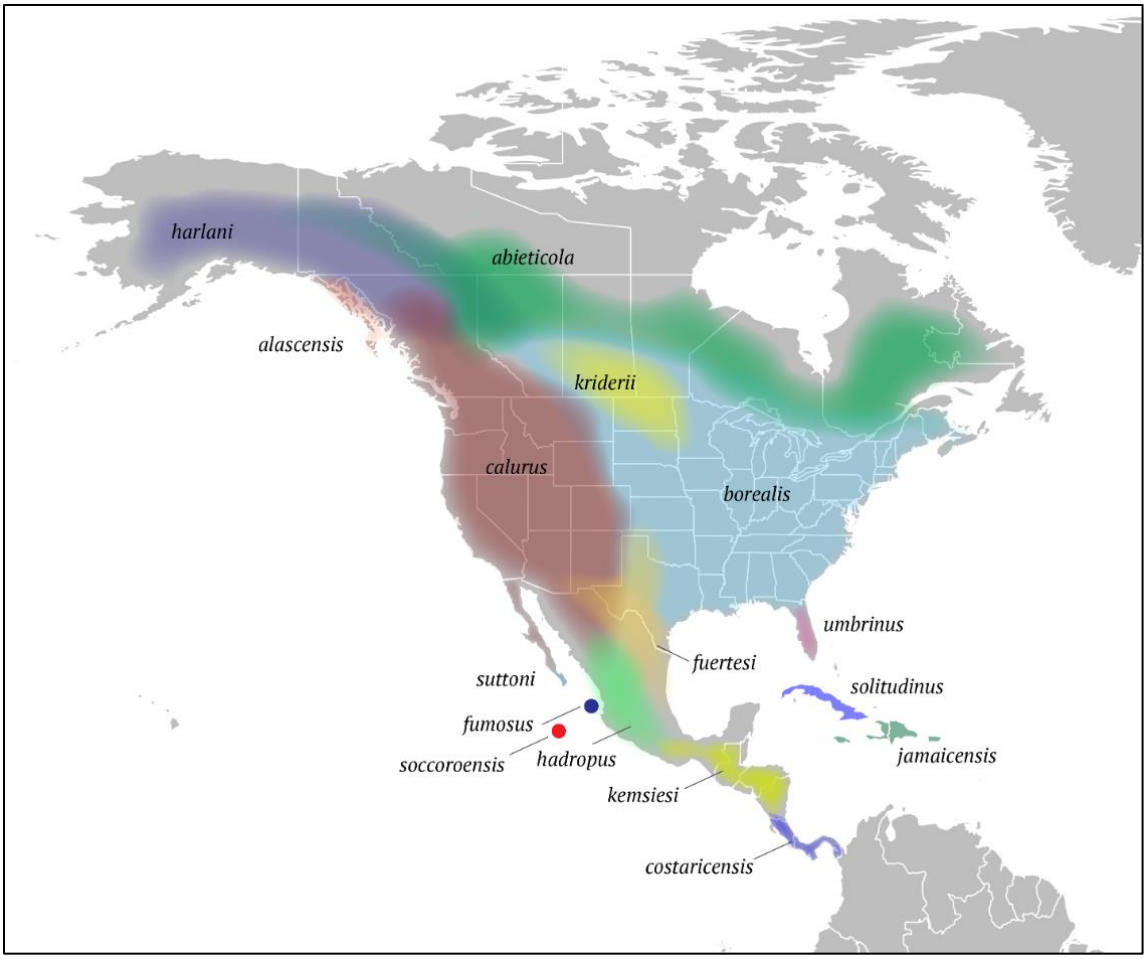

Figure 4. Contemporary Interpretation of Red-tailed Hawk Subspecies Ranges. Based on more current photographic and spatial data collected from Red-tailed Hawk subspecies, modified geographic ranges have been depicted by B. Robinson (unpublished data). This map identifies the likely geographic range of *B.j. abieticola* stretching across the Boreal Forest, from the Atlantic seaboard through northwest Canada. *B.j. abieticola*'s presumed breeding range may overlap with other subspecies ranges, such as *B.j. borealis*, *B.j. calurus*, and *B.j. harlani*.

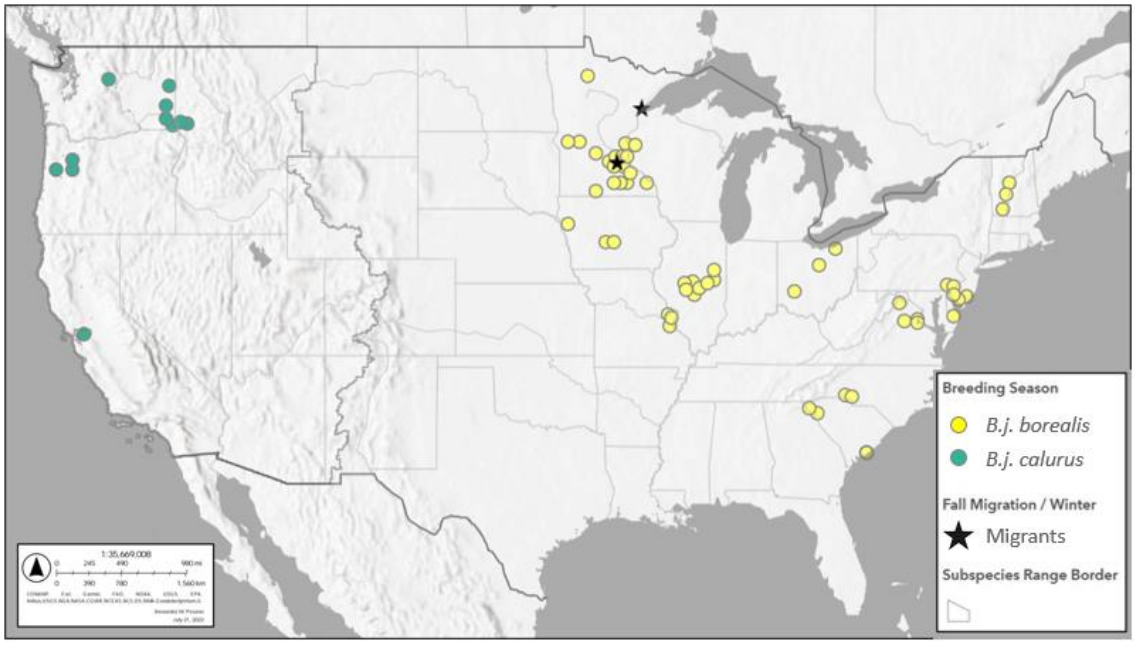

Figure 5. Red-tailed Hawk Sampling Locations. Genetic sampling of 309 Red-tailed Hawks occurred from 2019 – 2021. Individuals from unknown breeding locations were sampled during fall migration and winter in Duluth, MN and Savage, MN, respectively. Samples collected from subspecies during the breeding season are strictly within their respective breeding ranges, not along the border where breeding populations may intermix. Breeding season points represent the counties where injured birds were located before being brought to rehabilitation facilities.

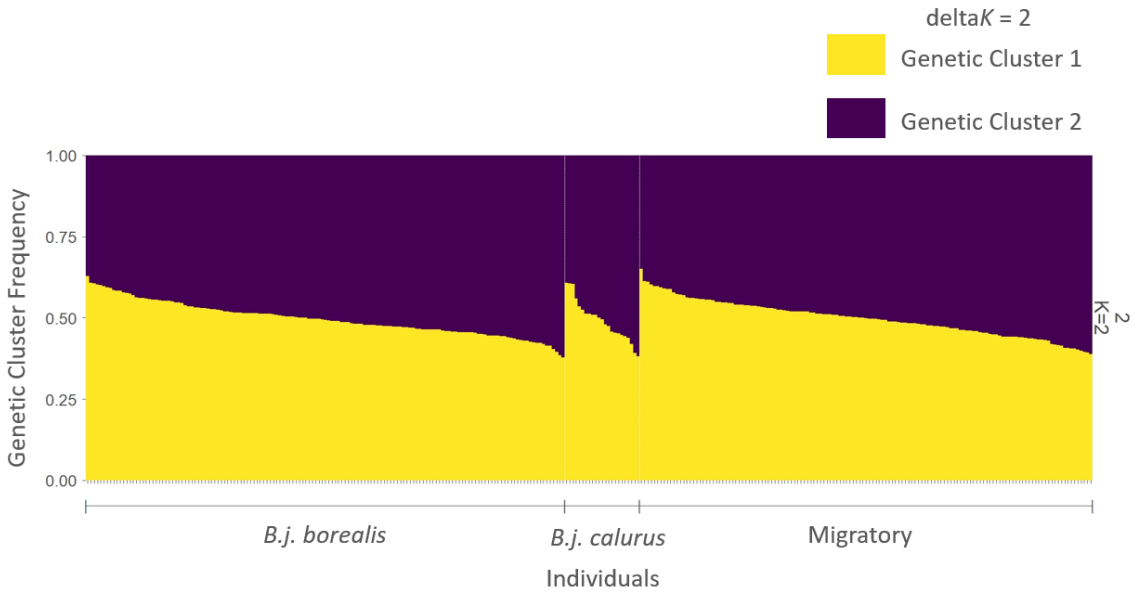

Figure 6. Probabilistic Bayesian Clustering Bar Plot of Genetic Cluster Frequencies. A Bayesian clustering analysis was performed using the program Structure to infer population structure, identify distinct populations, and assign migratory individuals of unknown genetic origin to breeding populations (*B.j. borealis* and *B.j. calurus*). An Evanno analysis performed with no *a priori* information from individuals revealed the highest probability of two genetic clusters (delta*K* = 2) within the sample population. Each individual's genetic information is comprised of a proportion of genetic cluster 1 and genetic cluster 2. Individuals were then arranged into their corresponding subspecies or migratory group.

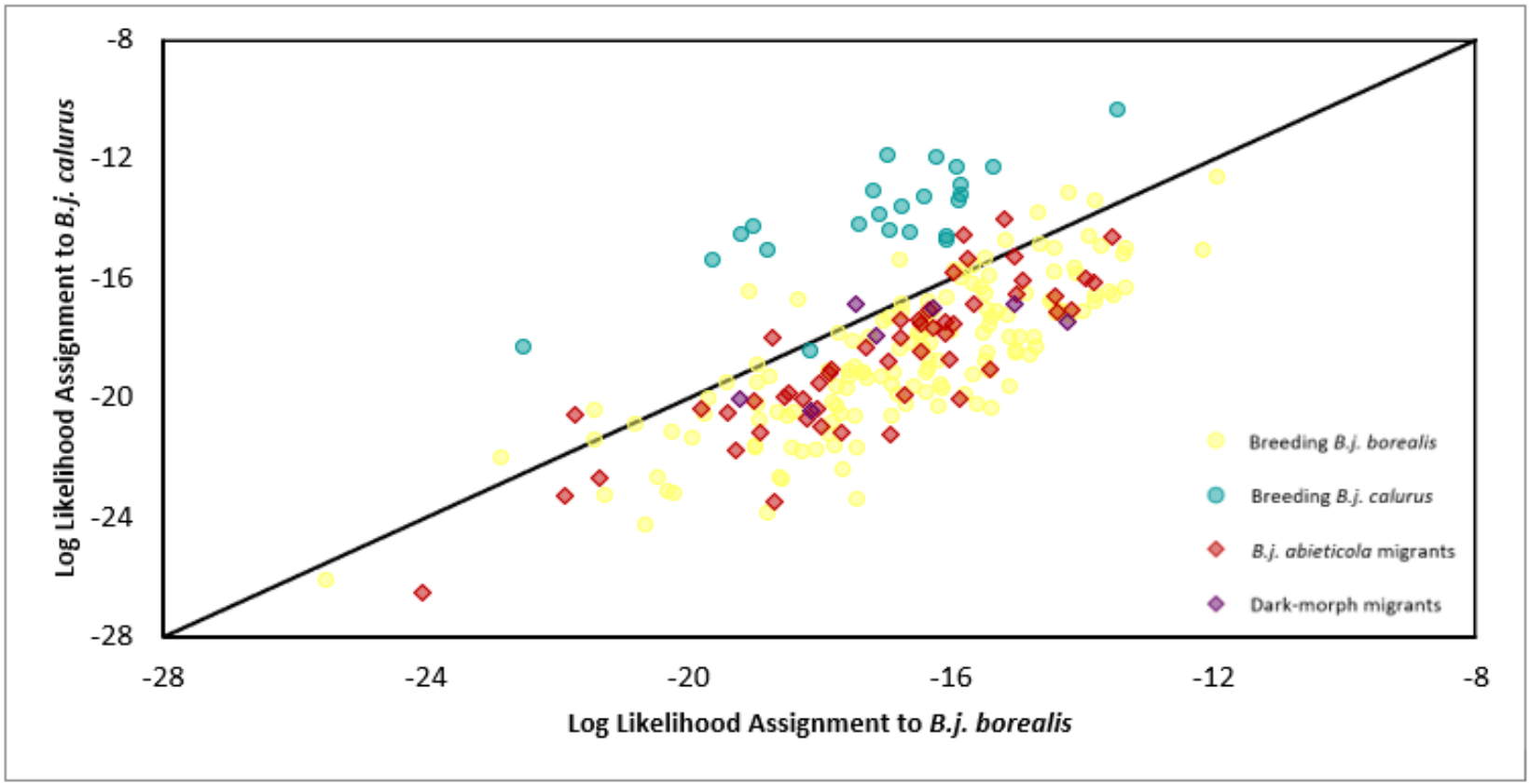

Figure 7. Population Assignment of Individuals to Subspecies. Population assignment is based on log likelihood values of *B.j. abieticola* migrants and dark-morph migrants to *B.j. borealis* and *B.j. calurus*. If an individual point is above the 1:1 line, it has a higher log likelihood of being assigned to *B.j. calurus*, but if it is below the line, it has a higher log likelihood of being assigned to *B.j. borealis*. Points the fall closer to the 1:1 line indicates they have a higher probability of being assigned to either population.

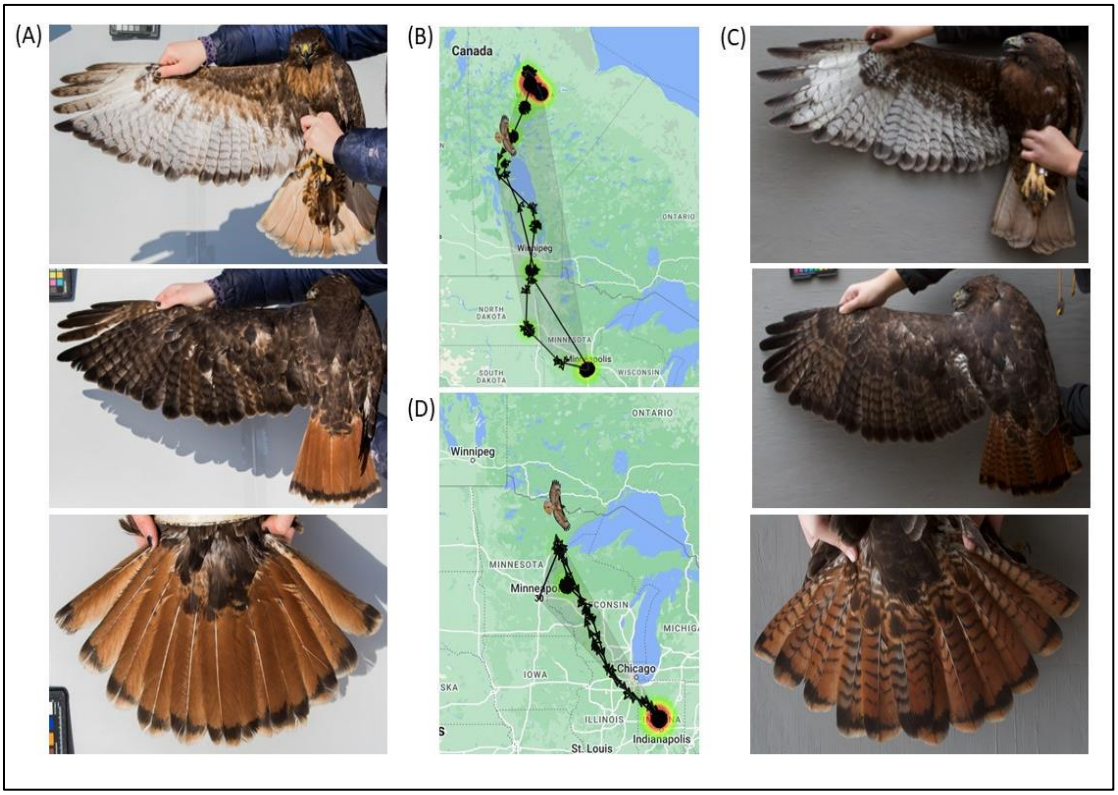

Figure 8. Presumed Adult Dark-morph *B.j. abieticola* with Satellite Transmitters. (A) Manley was fitted with a transmitter in Scott County, MN in February 2021, and represents dark brown /rufous ventral plumage and a typical *B.j. borealis/B.j. abieticola* tail pattern. (B) Manley summered in northern Manitoba, and is potentially using the same migratory routes and destinations as the previous year. (C) Trudi was fitted with a transmitter in Duluth, MN in fall of 2021, and has dark brown plumage and a tail pattern possible for *B.j. abieticola*. (D) Trudi wintered in Indiana, then began migrating north until leaving cellular range.

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Appendix

Supplemental Tables and Figures

Supplementary Table 1. Breeding Sample Metadata. Injured Red-tailed Hawks admitted to permitted rehabilitation facilities or animal hospitals came from various locations throughout the state, or neighboring state, in which the facility is located. Latitude and longitude are provided for each county an injured bird was found in during the breeding season.

Facility	State	County	Lat, Long	Sample Size (n)
Blue Ridge Wildlife Center	VA	Culpepper	38.49, -77.96	1
		Loudon	39.11, -77.62	2
		Frederick	39.20, -78.26	1
		Prince William	38.57, -77.28	1
		Stafford	38.38, -77.33	1
Carolina Raptor Center	NC	Gaston	35.29, -81.18	1
		Mecklenburg	35.25, -80.83	7
	SC	Anderson	34.52, -82.64	2
		Oconee	34.75, -83.07	1
		Spartanburg	34.95, -82.01	1
Chintimini Wildlife Center	OR	Benton	44.49, -123.43	3
		Linn	44.49, -122.53	2
		Marion	44.90, -122.58	1
Glen Helen Raptor Center	OH	Greene	39.69, -83.89	4
Lindsay Wildlife Experience	CA	Contra Costa	37.92, -121.93	7
Milliken University / Illinois Raptor Center	IA	Champaign	40.14, -88.20	1
		Christian	39.55, -89.28	1
		Ford	40.60, -88.22	1
		Logan	40.12, -89.37	1
		Macon	39.86, -88.96	19
		Menard	40.03, -89.80	1
		Piatt	40.01, -88.59	1
		Sangamon	39.76, -89.66	5
Ohio Bird Sanctuary	OH	Cuyahoga	41.42, -81.66	1
		Richland	40.77, -82.54	2
Saving Our Avian Resources	IA	Dallas	41.68, -94.04	2
		Polk	41.69, -93.57	3
		Woodbury	42.39, -96.04	1

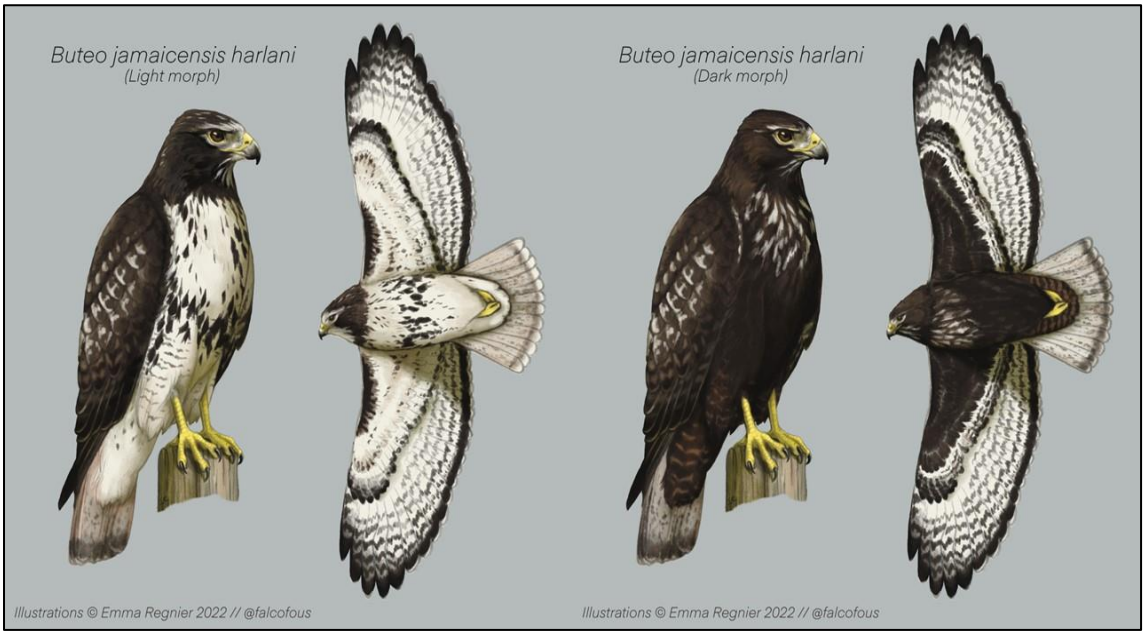
The Center for Birds of Prey	SC	Charleston	32.84, -79.96	10
The Raptor Center	MN	Beltrami	47.97, -94.94	1
		Carver	44.82, -93.80	2
		Chisago	45.50, -92.91	2
		Dakota	44.67, -93.07	3
		Dodge	44.02, -92.86	1
		Goodhue	44.41, -92.72	1
		Hennepin	45.00, -93.48	10
		Martin	43.67, -94.55	1
		Meeker	45.12, -94.53	1
		Pope	45.59, -95.44	1
		Ramsey	45.02, -93.10	3
		Scott	44.65, -93.54	2
		Steele	44.02, -93.23	1
		Stevens	45.59, -96.00	1
		Waseca	44.02, -93.59	1
		Washington	45.04, -92.88	1
		Wright	43.99, -91.78	3
	WI	Polk	45.46, -92.44	1
Tri-State Bird Rescue	DE	Sussex	38.66, -75.40	1
& Rescue	NJ	Atlantic	39.48, -74.68	1
		Cumberland	39.37, -75.11	1
		Salem	39.59, -75.35	1
	PA	Chester	39.97, -75.75	3
		Delaware	39.92, -75.40	1
Vermont Institute of Natural	VT	Orange	44.01, -72.38	1
Science		Windsor	43.58, -72.59	2
		Windham	42.99, -72.71	2
Washington State University	ID	Nez Perce	46.33, -116.75	2
		Lewis	46.24, -116.43	1
	WA	Asotin	46.19, -117.20	2
		Chelan	47.87, -120.62	2
		Garfield	46.43, -117.55	1
		Spokane	47.62, -117.40	1
		Whitman	46.90, -117.52	1
World Bird Sanctuary	MO	Jefferson	38.26, -90.54	1
		St. Charles	47.62, -90.68	5
		St. Louis	38.64, -90.44	21
Total (N)	**-**	**-**	**-**	**170**

Supplementary Table 2. Microsatellite Loci used for Genotypic Analysis. 17 microsatellite loci developed by Hull et al. 2007 were used in PCR and amplified across 7 multiplex panels. Seven templates and panels containing dye label and repeat motif were created in GeneMarker 3.0.1 to visualize alleles amplified at each locus. Loci were amplified with GS600 LIZ standard.

Locus	Dye Label	Primer Sequence (5'-3')	Repeat Motif	*M*	*N*	GenBank Accession #
BswA110w[*]	6-FAM	F: ATTTTGAGAGGTGAAGGTCACG R: CAGGTCAGTGAAGGACTCTGC	$(CA)_{18}$	1	-	DQ985707
BswD122w[†]	PET	F: GTCAGGCAGTTGGACTAGATGA R: GATGGGGAACTGCTCTAAACAT	$(GAGAA)_{10}$	1	38	DQ985717
BswA204w[*]	NED	F: GCAGAAGGAAATGTGTTTGGTT R: TAAGAAACCAGGTGGCATTAGG	$(CA)_{16}$	2	-	DQ985708
BswA317w	PET	F: CTGAAAATGTCACCACAACAAA R: TGAGTAAGCACAGGAGATGGAT	$(CA)_{17}$	2	6	DQ985712
BswD210w[†*]	VIC	F: TTAACAAGTCCAAATGCTGGAT R: TTGGAATAAATGGTCATTGTAGGT	$(GAGAA)_{14}$	2	-	DQ985721
BswD220w	6-FAM	F: TAACTTTTGGTCAGCCCTGAAT R: TCTGTGGCACTGCAATGAAT	$(GAGAA)_{11}$	2	11	DQ985722
BswA303w	PET	F: ACTGAATAAGCAGAGGGCAAAA R: TGGCACTTCCATAGTCAATCAG	$(CA)_{15}$	3	11	DQ985710
BswB111aw	NED	F: TCATCCCAATGCAGTTCTCA R: CACTGGCATGAATGGACAGA	$(CATC)_{7}$	3	6	DQ985713
BswD234w	VIC	F: GGAATTGCATAGGTCAAACACA R: CTGTGCAACATATTATTTCCCTTG	$(GAGAA)_{17}$	3	11	DQ988163
BswD310w[†*]	PET	F: GAACAATTTGGGATACACTGA R: TAATGCCATGATGTTATCAGAC	$(GAGAA)_{24}$	4	-	DQ985725
BswD313w[†]	6-FAM	F: CTGCACCTTTCTTCTTATGC R: GCTGAGGTCTGAATTTTTACC	$(GAGAA)_{19}$	4	19	DQ985727
BswB220w	NED	F: GGCTTTTCTGATTGAATTAGGG R: CACAAACTGTTGCCTGAACTTT	$(AAT)_{9}$	5	9	DQ985714
BswB221w	PET	F: TAACTTCGACACAGGGTAGCAA R: TGGGAGAGTGTTTGTGCTCTTA	$(AAT)_{4}$	5	4	DQ985715
BswD327w	6-FAM	F: ATGGTCCACTAGAATGTTTGAC R: TCTCCCTATGTTACGTTAGCAT	$(GAGAA)_{9}$	5	11	DQ985729

BswA302w	NED	F: CGAAGTTGTGCAATCTCATTTC R: CTTGCTTTCACAATTTGCAGTC	$(CA)_{16}$	6	11	DQ985709
BswD127w†	PET	F: CAGGGTGGACAGACAGGTAG R: GTGAGGCAGTTGGACTTGAT	$(GAGAA)_9$	6	12	DQ985719
BswA312w*	NED	F: GGCAGAAATCAGCAGCATAAAT R: CCACTCCCTCATGAAACAGATT	$(CA)_{21}$	7	-	DQ985711

†Irregular repeats detected; violating stepwise mutation model (Hull et al. 2007). *Microsatellite locus removed from further analyses due to deviations from HWE. *M*, multiplex panel. *N*, number of alleles.



Supplementary Figure 1. Adult *B.j. harlani* Plumage Characteristics. *B.j. harlani*, also referred to as "Harlan's Hawk," is polymorphic with dark being the predominant color-morph. Light-morphs have blackish streaking on the belly, a whitish throat, wavy banding on the underside of flight feathers, and white mottling on upper wing coverts. Dark-morphs are similar to light but with a dark ventral side with white mottling. Overall, tails are marbled with various colors, such as gray, white, black, and red. Intermediate-morphs are also present in *B.j. harlani* and tend to present more white mottling on the ventral side compared to dark-morphs. Illustrations by Emma Regnier (2022).

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Ethics Statement

The ethics and methodology of this research were reviewed and approved by several organizations, while also being followed appropriately by those performing the research. Federal Bird Banding Permit (Permit Number: 23927); United States Department of the Interior U.S. Geological Survey, Patuxent Wildlife Research Center, Bird Banding

Laboratory provided authorization to band Red-tailed Hawks in the state of Minnesota, with special authorization to a) take, possess, and transport blood samples (not to exceed 1% body mass), b) hand capture, c) use Bal-chatris, d) use Bow nets, e) use Dho-gazas, f) use Mist nets, g) mark Red-tailed Hawks with Satellite/ Cell/ GPS Transmitter backpacks and attachment materials that do not exceed 3% total body weight. Institutional Animal Care and Use Committee (Protocol ID: 1904-36977A); University of Minnesota, Research Animal Resources, Environmental Health & Safety approved to attach backpack transmitters on Red-tailed Hawks admitted. Capture & release for research and Salvage Special Permit (Permit Number: 29208); State of Minnesota Department of Natural Resources, Division of Ecological and Water Resources provided permission to collect blood from Red-tailed Hawks, possess and transport carcasses and samples of raptors at Hawk Ridge Bird Observatory for genetic research. Institutional Animal Care and Use Committee (Protocol ID: 2105-39137A); University of Minnesota, Research Animal Resources, Environmental Health & Safety approved to collect blood from breeding Red-tailed Hawks admitted to rehabilitation facilities. Scientific Collecting Permit (Permit Number: MBPER0014234); Department of the Interior U.S. Fish and Wildlife Service, Migratory Bird Permit Office permitted authorization to take, transport, possess, and receive blood samples from Red-tailed Hawks from Federal migratory bird rehabilitators.

Intellectual Contributions

Several professionals have conceived, performed, designed, wrote, and reviewed various aspects of this research and are acknowledged for their contributions.

1) Conceived the idea, design, experiment (supervised research, formulated question or hypothesis) – Frank Nicoletti, Alexandra M. Pesano, Dr. Matthew Etterson, Dr. Eric Waits.
2) Performed the experiments (collected data, conducted the research) – Alexandra M. Pesano, Frank Nicoletti, Dr. Matthew Etterson, Dr. Eric Waits, Luke Smith.
3) Wrote the paper (or substantially edited the paper) – Alexandra M. Pesano, Dr. Matthew Etterson, Dr. Eric Waits, Dr. Briana Gross, Dr. Mark Clark, Frank Nicoletti.
4) Developed or designed methods – Alexandra M. Pesano, Dr. Eric Waits, Dr. Briana Gross.
5) Analyzed the data – Alexandra M. Pesano, Dr. Eric Waits, Dr. Matthew Etterson, Dr. Briana Gross.
6) Contributed substantial materials, resources, or funding – Frank Nicoletti, Dr. Matthew Etterson, Dr. Eric Waits, Dr. Briana Gross.