

Minnesota's Red-tailed Hawks: Probabilistic Origins of *B.j. abieticola* and Dark-morph Migrants

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Project Overview:

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 (Figure 1). 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.

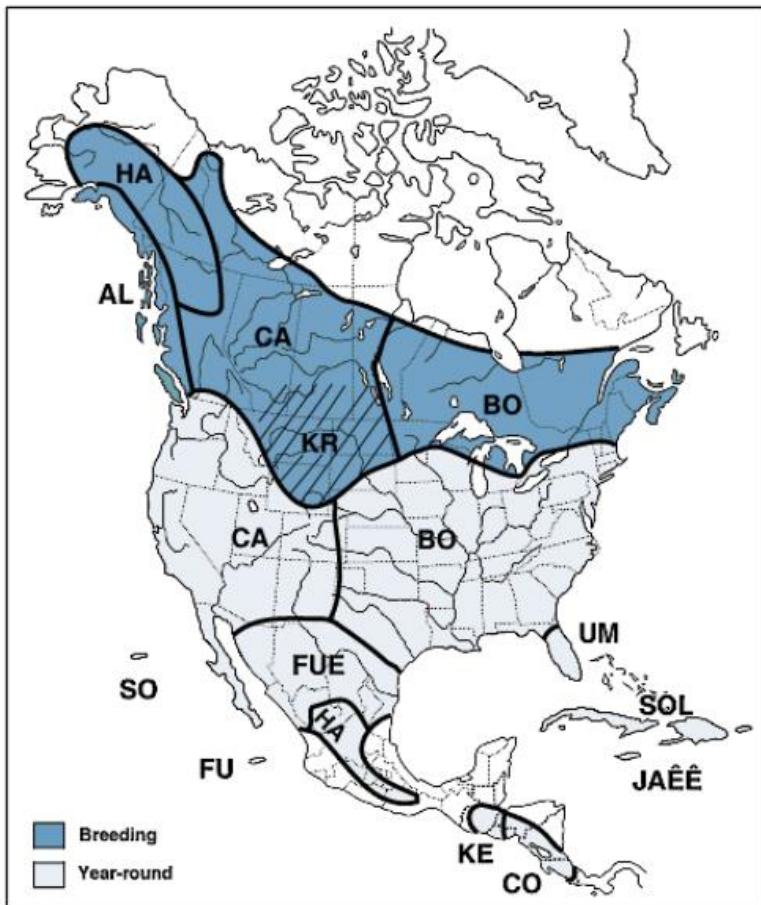


Figure 1. Breeding and Year-round Distributions of Red-tailed Hawk Subspecies. The approximate breeding and year-round distributions of Red-tailed Hawk subspecies and races cover the majority of North America, Central America, and neighboring islands. Figure by Preston and Beane (2020), adapted from Johnsgard (1990).

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).

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 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.

Objectives:

- 1) Investigate these topics of interest in Minnesota by sampling migratory birds with *B.j. abieticola* and dark-morph phenotypes.
- 2) Compare their genetic data to those of known *B.j. borealis* and *B.j. calurus* breeding populations.
- 3) Investigate the breeding locations of presumed adult dark-morph *B.j. abieticola* individuals using satellite transmitters to provide high-resolution supplementary data.

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). Fall migration samples were collected from free-flying adults and juveniles captured with mist-net, 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 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. 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. All breeding season samples were collected during 2021 apart from archived samples donated from Milliken University, IL, USA that were collected between 2015 and 2020.

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)
<i>B.j. borealis</i>	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
<i>B.j. calurus</i>	Breeding	California	7
		Idaho	3
		Oregon	6
		Washington	7
<i>Buteo jamaicensis</i> 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.

Microsatellite Data Collection & Analysis

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). DNA was amplified via PCR, then microsatellite visualization of PCR products was performed. GeneMarker 3.0.1 was used to visualize PCR products and score alleles. 12 of the 17 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.

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.

Results:

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* (Table 2).

Table 2. 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 <i>B.j. borealis</i>	Breeding <i>B.j. calurus</i>	Mig <i>B.j. abieticola</i>	Mig Dark-morphs
Breeding <i>B.j. borealis</i>	-			
Breeding <i>B.j. calurus</i>	0.016***	-		
Mig <i>B.j. abieticola</i>	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.

Population assignment probabilities supported that these migratory individuals were more closely related to *B.j. borealis* than *B.j. calurus* (Figure 2). 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 (Figure 3). 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*.

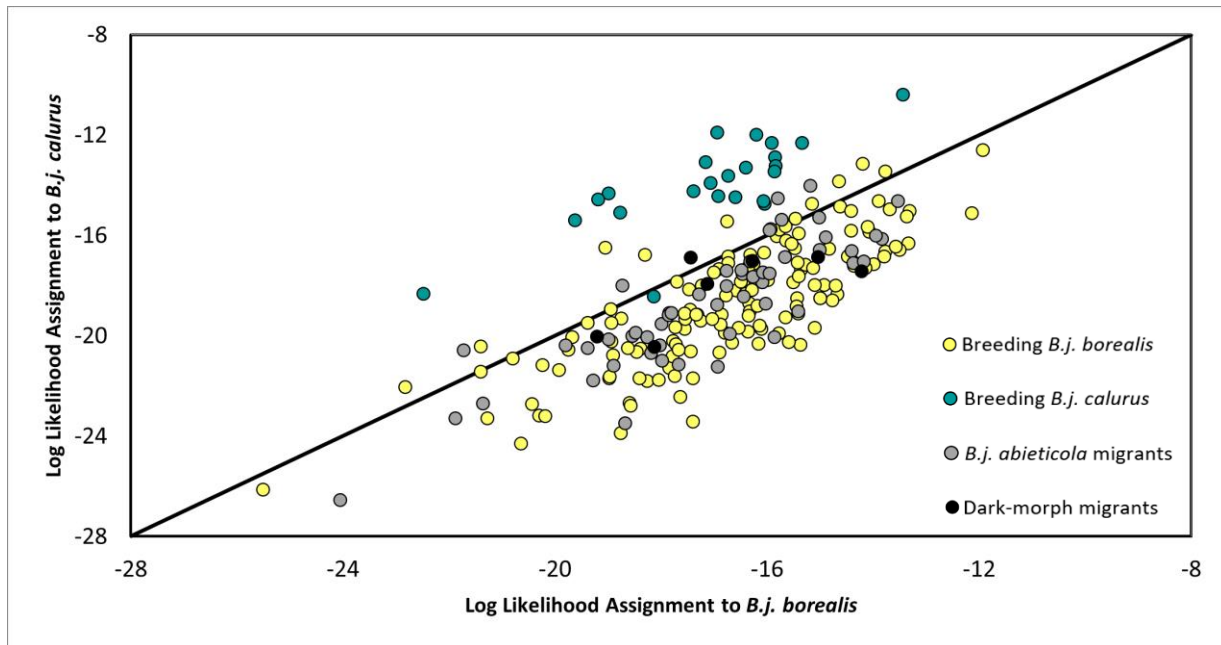


Figure 2. Population Assignment of Individuals to Subspecies. Population assignment is based on log likelihood values of *B.j. abieticola* migrants and dark-morph migrants to *B.j. borealis* and *B.j. calurus*. If an individual point is above the 1:1 line, it has a higher log likelihood of being assigned 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 that fall closer to the 1:1 line indicates they have a higher probability of being assigned to either population.

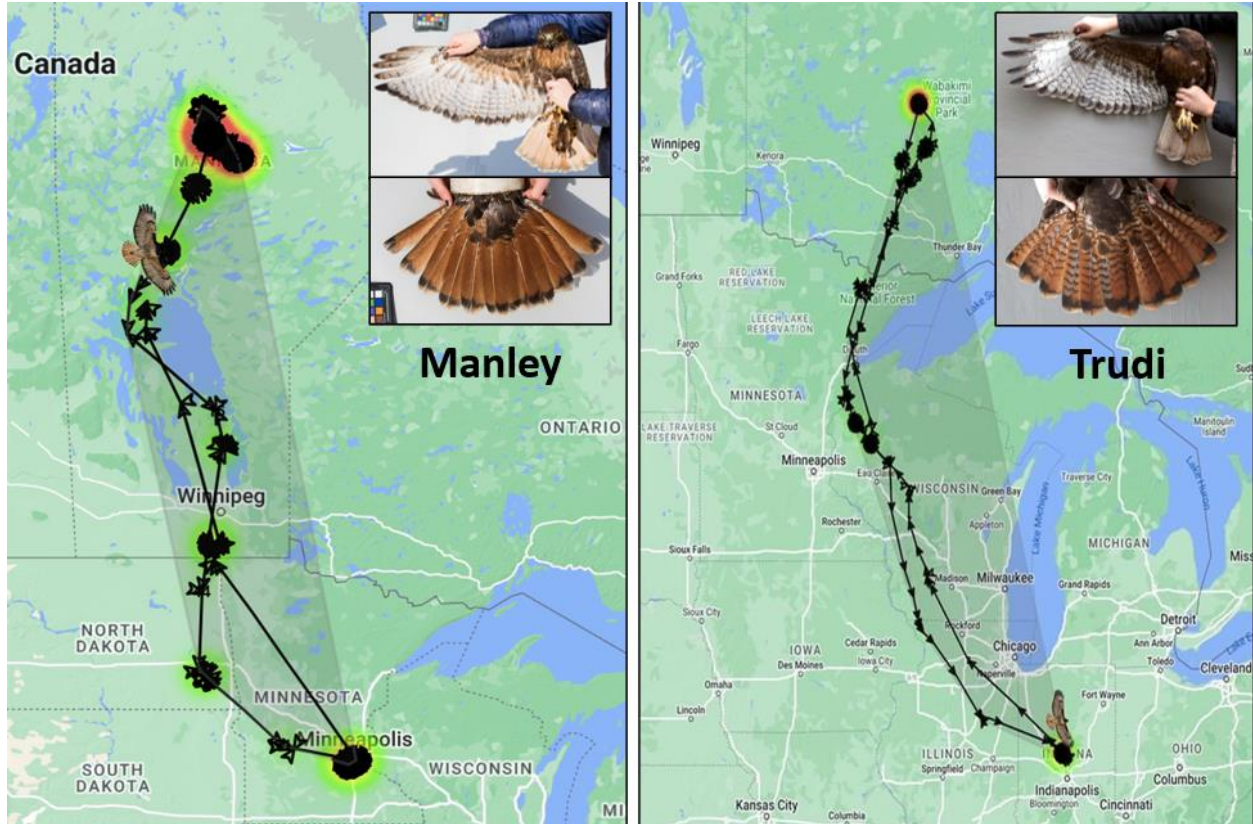


Figure 3. Movement Patterns of presumed Adult Dark-morph *B.j. abieticola* with Satellite Transmitters. Manley was fitted with a transmitter in Scott County, MN in February 2021, migrated north to summer in northern Manitoba, then returned to the same wintering grounds in 2022. Manley summered in Manitoba in Spring of 2022. Trudi was fitted with a transmitter in Duluth, MN in Fall of 2021, then migrated south to winter in Indiana. Trudi began migrating north in Spring of 2022 and spent the summer season in Ontario. Trudi has since returned to the same wintering grounds from the previous year.

Discussion:

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*. 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.

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 and Trudi, two presumed dark-morph *B.j. abieticola*, suggest summering locations east of the Rocky Mountains. In 2021, Manley summered in northern Manitoba and Trudi in Ontario; two regions currently recognized as part of the *B.j. borealis* breeding range (Figure 1) and presumed *B.j. abieticola* breeding range.

Budget Report:

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