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LONG-DISTANCE MOVEMENT IN A DUSKY GREAT HORNED OWL AND LIMITS TO PHYLOGEOGRAPHY FOR ESTABLISHING PROVENANCE

Robert W. Dickerman1, Sabrina M. McNew1, and Christopher C. Witt1,2

ABSTRACT.—The Dusky Great Horned Owl (Bubo virginianus saturatus) of the Pacific Northwest region of North America is generally considered to be nonmigratory. Here we report a specimen of a Dusky Great Horned Owl that was salvaged in New Mexico and identified based on comparison of its plumage with a large series of museum skins. We attempted to corroborate this identification by comparing mitochondrial DNA sequences between the specimen and a representative sample of 5 Great Horned Owl subspecies from western North America. This analysis revealed minimal mitochondrial genetic variation and no evidence of population genetic structure, suggesting that the marked plumage differences among subspecies evolved since the late Pleistocene. To evaluate the possibility that the salvaged specimen was transported after death, we analyzed its stomach contents. The stomach contained remains of a desert cottontail (Sylvilagus audubonii), a locally abundant rabbit species in central New Mexico. The mitochondrial haplotype of the rabbit was novel but closely related to haplotypes found in New Mexico and west Texas. This is the first report of long distance movement in the Pacific Northwest subspecies of Great Horned Owl, though we cannot rule out the possibility of anomalous melanism. Although there is tremendous potential for forensic-style phylogeographic investigation of animal movements, this study illustrates that sequence databases are not yet adequate to the task, even for common North American vertebrate species.

RESUMEN.—Por lo general, se considera que el Búho Americano (Bubo virginianus saturatus) de la región Noroeste del Pacífico de Norteamérica no tiene hábitos migratorios. En este estudio nos referiremos a un espécimen de Búho Americano que se rescató en Nuevo México y se identificó de acuerdo con comparaciones del plumaje con una gran cantidad de pieles. Intentamos corroborar esta identificación comparando secuencias de ADN mitocondrial del espécimen con una muestra representativa de cinco subspecies del Búho Americano del oeste de Norteamérica. Este análisis reveló una variación mitocondrial genética mínima y ninguna evidencia de estructura genética de la población, lo cual indica que las marcadas diferencias del plumaje entre subspecies se han ido desarrollando desde el final del Pleistoceno. Para evaluar la posibilidad de que el espécimen que se rescató haya sido transportado después de su muerte, analizamos el contenido del estómago, en el cual se halló una especie de conejo que abundaba en la zona, en el centro de Nuevo México, llamado conejo del desierto (Sylvilagus audubonii). El haplótipo mitocondrial del conejo era nuevo, pero se relacionaba estrechamente con los haplotipos que se encuentran en Nuevo México y en el oeste de Texas. Este es el primer informe de migración a larga distancia de las subspecies del Búho Americano del noroeste del Pacífico, sin embargo existe la posibilidad de melanismo anómalo. Existen grandes posibilidades de realizar investigaciones filogeográficas de estilo forense sobre la migración de los animales, este estudio demuestra que las bases de datos de las secuencias no son suficientes para realizar esa tarea, inclusive en los casos de especies comunes de vertebrados de Norteamérica.

The Great Horned Owl (Bubo virginianus) is a widespread and geographically variable New World species, with North American populations comprising at least 11 subspecies that differ in plumage, size, habitat, and vocalizations (Houston et al. 1998, Dickerman and Johnson 2008). In New Mexico, B. v. pallescens breeds at low elevations, whereas B. v. pinorum breeds in montane forests (Dickerman and Johnson 2008). Salvage efforts over the last ~25 years have yielded >300 specimens from New Mexico, including B. v. pallescens, B. v. pinorum, and many fall migrants or wintering individuals of subspecies from the northern Rockies (B. v. lagophonus), the northwestern Great Plains to the subarctic (B. v. subarcticus), and the eastern Great Plains (intergrades between B. v. pallescens and B. v. virginianus). Latitudinal migration or irruptive winter movement is clearly part of the annual cycle for at least the northernmost subspecies of Great Horned Owl; however, the nature of the owls’ movements is poorly known (Houston et al. 1998). The “Dusky” Great Horned Owl (B. v. saturatus) has been thought to be an exception. Its range is restricted to the

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rainforests of the Pacific coastal zone of the Northwest from Glacier Bay, Alaska, to approximately Santa Cruz, California (Grinnell and Miller 1944, AOU 1957). Grinnell and Miller (1944) note that *B. v. saturatus* occurs “in ‘diluted’ manifestation” at the southern and southeastern borders of its distribution. *Bubo v. saturatus* has long been considered to be essentially sedentary (Houston et al. 1998) or subject to only short-distance movements. For example, the 5th AOU checklist (1957) notes that it is a winter visitant to interior British Columbia. Grinnell and Miller (1944) also cite one specimen (MVZ 59111) from Grizzly Island, Solano Co., California, from 1 November 1941, as a “southward straggler.” A search of ORNIS revealed 5 additional, modestly extralimital specimens, identified as *B. v. saturatus*, which were collected between October and December from western Montana (MVZ 79183) or San Luis Obispo Co., California (ROM 86844, 86851, 86852; UCLA 7257). However, RWD examined the plumage of the western Montana specimen and determined it to be *B. v. lagophonus*.

We report the first specimen (MSB:Bird:37574) from New Mexico that is a typical *B. v. saturatus* by plumage. It was delivered dead to the gate of a rehabilitator in Corrales, Bernalillo Co., New Mexico, on 30 October 2011 and was said to be from the adjacent suburb of Rio Rancho. It obviously was not a captive bird, considering the absolute lack of feather abrasion and a small, partly digested desert cottontail (*Sylvalagus audubonii*) in its stomach.

DNA sequencing has recently facilitated our ability to identify the provenance of migrant (e.g., Kimura et al. 2002, Ruegg and Smith 2002, Webster et al. 2002, Wink 2006) and vagrant birds (e.g., Witt et al. 2010, Baumann et al. 2011, 2013, Engel et al. 2011, Johnson et al. 2011). The basis for this method is that populations that are geographically separated accrue unique genetic characteristics over time by genetic drift or natural selection acting on new or existing alleles. Geographically isolated populations are typically genetically divergent and diagnosable by molecular means, even if they have not diverged at all genetic loci (Avise 2000, Webster et al. 2002). The mitochondrial genome evolves much faster on average than nuclear DNA because of its higher mutation rate and smaller effective population size, and as a result, mitochondrial DNA sequence divergence is often a leading informative indicator of geographic provenance (Zink and Barrowclough 2008). The key limitation for using mitochondrial or other DNA sequences to efficiently infer avian movements or population connectivity is that reference databases must contain variable genetic sequences with known geographic structure.

Here we examine the identification and geographic origin of this anomalous New Mexico Great Horned Owl specimen by comparing its plumage with the extensive series of museum skins at the Museum of Southwestern Biology (MSB), as well as comparing its mitochondrial DNA with 11 other Great Horned Owl specimens representing 5 subspecies. We also attempt to establish the geographic location at which the owl consumed its last meal by analyzing mitochondrial DNA from the rabbit in its stomach.

**METHODS**

We extracted DNA from muscle tissue of MSB:Bird:37574 and 11 other Great Horned Owl specimens from the MSB Bird Collection using Qiagen DNEasy kits. In addition, we extracted DNA from the small mammal (MSB:Mamm:262536) found in the stomach of MSB:Bird:37574 and from one desert cottontail specimen collected in Rio Rancho, New Mexico, and held in the MSB Mammal Collection (MSB:Mamm:85844; Table 1).

We amplified the mitochondrial NADH dehydrogenase subunit 2 (ND2) gene for each owl specimen by using primers L5219 and H6313 (Sorenson et al. 1999), with reagent concentrations and thermalcycler protocols following Witt et al. (2010). We amplified and sequenced the mitochondrial cytochrome *b* (*cyt b*) gene of the 2 mammals by using primers L14724 and H15915 (Irwin et al. 1991) with the same PCR protocols as for the owls, except for a lower annealing temperature (47 °C). We sequenced with external primers, and with one additional internal primer for *cyt b* (L15171; Spradling et al. 2001), using BigDye 3.1 chemistry (ABI) and an ABI 3130 sequencer. We assembled and edited sequences manually using Sequencher 4.7 (GeneCodes, Ann Arbor, MI). We used the software package MUSCLE (Edgar 2004) to align ND2 and *cyt b* sequences and used
the program MEGA to calculate pairwise divergences and nucleotide diversity of ND2 sequences using the maximum composite likelihood model (Tamura et al. 2011). We used the programs DnaSP 5.10.01 (Librado and Rozas 2009) and Network 4.6.10 (Fluxus Technology, Ltd., Suffolk, England 2012) to make a minimum spanning tree of the haplotypes using the median-joining method (Bandelt et al. 1999).

We used the program Phyml (Guindon and Gascuel 2003) for phylogenetic analysis of mammalian cyt b sequences by maximum likelihood methods. We used the default parameters (HKY85 model with gamma-distributed rate variation among sites) and simultaneous estimation of model parameters with 100 bootstrap replicates to assess branch support.

**RESULTS**

The anomalous specimen, MSB: Bird: 37574, matched subspecies *B. v. saturatus* by plumage (Fig. 1). We compared the plumage to New Mexico specimens of *B. v. pinorum* (63 skins), *B. v. pallescens* (59 skins), *B. v. subarcticus* (2 skins), *B. v. lagophonus* (14 skins), *B. v. pallescens × B. v. virginianus* (11 skins), and *B. v. pallescens × B. v. pinorum* (11 skins). The head and dorsum of MSB: Bird: 37547 were substantially darker than any of the previously salvaged Great Horned Owl specimens at the MSB. The plumage was also darker than that of any North American Great Horned Owl subspecies other than *B. v. saturatus* (Fig. 1). The tawny colored feathers on the tarsi and toes also matched *B. v. saturatus*; all other subspecies known from New Mexico have whitish or buff tarsi and toes (Fig. 1).

Assembled sequences were 864 bp long for owl ND2 and 868 bp for rabbit cyt b. The sequence chromatograms were clean and unambiguous, without double peaks or any other evidence of nuclear DNA pseudogenes. Complete sequences are available on Genbank (Table 1).

We sequenced the ND2 gene for a total of 12 Great Horned Owls from 5 different localities and 5 different morphological subspecies (Table 1). We identified 6 unique haplotypes but found no geographic or taxonomic structure in the haplotype network (Fig. 2). As few as 11 nucleotide changes are sufficient to explain all of the observed variation across all 12 Great Horned Owl ND2 sequences (~0.013 substitutions per site). MSB: Bird: 37574 was a unique haplotype but differed by

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**Table 1. Museum of Southwestern Biology specimens and Genbank accession numbers for DNA sequences of Great Horned Owls (*Bubo virginianus*) and cottontail rabbits (*Sylvilagus* spp.) used in this study.**

<table>
<thead>
<tr>
<th>Catalogue no.</th>
<th>Species</th>
<th>Subspecies</th>
<th>Origin</th>
<th>Genbank accn. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSB: Bird: 28586</td>
<td><em>Bubo virginianus</em></td>
<td>saturatus</td>
<td>Oregon</td>
<td>JQ965161</td>
</tr>
<tr>
<td>MSB: Bird: 29188</td>
<td><em>Bubo virginianus</em></td>
<td>pinorum</td>
<td>Washington</td>
<td>JQ965162</td>
</tr>
<tr>
<td>MSB: Bird: 29190</td>
<td><em>Bubo virginianus</em></td>
<td>lagophonus</td>
<td>Oregon</td>
<td>JQ965163</td>
</tr>
<tr>
<td>MSB: Bird: 29189</td>
<td><em>Bubo virginianus</em></td>
<td>pinorum</td>
<td>Washington</td>
<td>JQ965164</td>
</tr>
<tr>
<td>MSB: Bird: 37574</td>
<td><em>Bubo virginianus</em></td>
<td>saturatus</td>
<td>New Mexico</td>
<td>JQ965165</td>
</tr>
<tr>
<td>MSB: Bird: 25642</td>
<td><em>Bubo virginianus</em></td>
<td>lagophonus</td>
<td>New Mexico</td>
<td>JQ965160</td>
</tr>
<tr>
<td>MSB: Bird: 20766</td>
<td><em>Bubo virginianus</em></td>
<td>pallescens</td>
<td>New Mexico</td>
<td>JQ965157</td>
</tr>
<tr>
<td>MSB: Bird: 21024</td>
<td><em>Bubo virginianus</em></td>
<td>pallescens</td>
<td>New Mexico</td>
<td>JQ965155</td>
</tr>
<tr>
<td>MSB: Bird: 21659</td>
<td><em>Bubo virginianus</em></td>
<td>pinorum</td>
<td>New Mexico</td>
<td>JQ965156</td>
</tr>
<tr>
<td>MSB: Bird: 23281</td>
<td><em>Bubo virginianus</em></td>
<td>pinorum</td>
<td>Utah</td>
<td>JQ965158</td>
</tr>
<tr>
<td>MSB: Bird: 23738</td>
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<td>pinorum</td>
<td>Texas</td>
<td>JQ965159</td>
</tr>
<tr>
<td>MSB: Bird: 18029</td>
<td><em>Bubo virginianus</em></td>
<td>subarcticus</td>
<td>New Mexico</td>
<td>JQ965154</td>
</tr>
<tr>
<td>MSB: Mamm: 55844</td>
<td><em>Sylvilagus audubonii</em></td>
<td>-</td>
<td>New Mexico</td>
<td>JQ965153</td>
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<tr>
<td>MSB: Mamm: 262536</td>
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<td>-</td>
<td>New Mexico</td>
<td>KF619076</td>
</tr>
<tr>
<td>Nalls et al. 2012</td>
<td><em>Sylvilagus audubonii</em></td>
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<td>HQ596488</td>
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<td>Halanych and Robinson 1999</td>
<td><em>Sylvilagus audubonii</em></td>
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<td>JQ965153</td>
</tr>
<tr>
<td>Matthee et al. 2004</td>
<td><em>Sylvilagus audubonii</em></td>
<td>-</td>
<td>Unknown</td>
<td>JQ965153</td>
</tr>
<tr>
<td>Matthee et al. 2004</td>
<td><em>Sylvilagus aquaticus</em></td>
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<td>Unknown</td>
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<tr>
<td>Matthee et al. 2004</td>
<td><em>Sylvilagus floridanus</em></td>
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<td>Unknown</td>
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<td>Matthee et al. 2004</td>
<td><em>Sylvilagus nuttallii</em></td>
<td>-</td>
<td>Unknown</td>
<td>JQ965153</td>
</tr>
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<td>Matthee et al. 2004</td>
<td><em>Sylvilagus obscurus</em></td>
<td>-</td>
<td>Unknown</td>
<td>JQ965153</td>
</tr>
<tr>
<td>Matthee et al. 2004</td>
<td><em>Sylvilagus palustris</em></td>
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<td>Unknown</td>
<td>JQ965153</td>
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<td>Matthee et al. 2004</td>
<td><em>Sylvilagus idahoensis</em></td>
<td>-</td>
<td>Unknown</td>
<td>JQ965153</td>
</tr>
</tbody>
</table>

We sequenced the ND2 gene for a total of 12 Great Horned Owls from 5 different localities and 5 different morphological subspecies (Table 1). We identified 6 unique haplotypes but found no geographic or taxonomic structure in the haplotype network (Fig. 2). As few as 11 nucleotide changes are sufficient to explain all of the observed variation across all 12 Great Horned Owl ND2 sequences (~0.013 substitutions per site). MSB: Bird: 37574 was a unique haplotype but differed by
only one base pair from the most common haplotype (Fig. 2).

We identified the animal found in the stomach of MSB:Mamm:37574 as a juvenile rabbit (Sylvilagus sp.) on the basis of morphological comparisons with MSB specimens. Comparisons of the rabbit’s cyt b sequence with previously published sequences indicated that it was a desert cottontail (Sylvilagus audubonii; Table 1, Fig. 3), a species that occurs
from central Montana south through the eastern Rocky Mountains and western Great Plains to western and central Texas, New Mexico, Arizona, southern Nevada, southern to north central California, and northwestern and north central Mexico south to Mexico City. The species is largely absent north of 41° latitude from northern Utah and eastern Idaho west to the Pacific coast (GBIF data), and it is therefore not expected to overlap the breeding distribution of the Dusky Great Horned Owl. The phylogenetic results placed the MSB:Mamm:37574 haplotype with other desert cottontail sequences, nested within a clade of haplotypes from west Texas and central New Mexico. Published sequences of desert
cottontail were diverse (mean within-group divergence 5.4%) and paraphyletic with respect to a published sequence of the mountain cottontail (S. nuttallii; Fig. 3). The specimen from the stomach of the owl was 0.4% divergent from the other central New Mexico desert cottontail specimen. By contrast, percent divergences among *Sylvilagus* species were substantially larger (6.3% to 10.9%; Table 2).

**DISCUSSION**

We conclude that the owl most likely represents subspecies *B. v. saturatus*, the Dusky Great Horned Owl, and that it arrived in central New Mexico from the coastal Pacific Northwest without having been transported after death. Plumage provides the sole evidence for identification until genetic techniques with higher resolution can be applied to Great Horned Owl phylogeography. We cannot rule out the possibility that this bird may be a melanistic individual from a local population, such as might be produced by a mutation at the MC1-R locus (Mundy et al. 2004). However, a mutant individual would not be expected to closely mimic *B. v. saturatus* because many possible mutations at different loci can increase melanism by different molecular mechanisms (Hoekstra 2006). Furthermore, there is no evidence of anomalous, melanistic individuals in the series of 411 skins and spread-wing specimens at the MSB.

Our molecular results for Great Horned Owls indicate that levels of mitochondrial diversity are extremely low and that subspecies have not been separated long enough to achieve monophyly. Despite our modest sampling, the current result is a strong preliminary indication that phenotypic divergence among North American Great Horned Owl subspecies occurred during the late Pleistocene, too recently to be accompanied by genetic divergence at loci that are effectively...
neutral. Phylogeographic inference of geographic provenance in North American Great Horned Owls will require the identification of genetic markers that are geographically structured. These may include rapidly evolving markers such as microsatellites (Wink 2006) or loci that are under selection for local adaptation to different environments (e.g., Steiner et al. 2009, Wilson et al. 2013). Emerging next-generation sequencing methods for phylogeography suggest that population-level genome scans for loci under selection will be eminently feasible in the near future (Mccormack et al. 2011). Once identified, any geographically structured locus could be used for inferring provenance via widely available and inexpensive Sanger sequencing methods.

The molecular evidence for the rabbit in the owl’s stomach suggests that the rabbit had been captured and consumed in central New Mexico where the owl was salvaged. The rabbit’s mitochondrial DNA was extremely closely related (<0.5% sequence divergence) to rabbits from west Texas and central New Mexico. Furthermore, the apparent deep genetic structure within the species suggests that desert cottontails from the northern or western parts of the species’ distribution would be more divergent. It is clear, however, that a definitive conclusion on the geographic origins of the rabbit must await detailed phylogeographic work on rabbits of the western United States. In this case, unlike the owls, the deep mitochondrial DNA structure indicates that the mitochondrial locus will be sufficient to identify provenance with at least moderate precision, but there are no sequences available for the majority of the species’ range. Furthermore, several of the S. audubonii sequences in Genbank currently contain no information about locality or museum specimen vouchers that allows the sequences to be applied to phylogeographic inference (Fig. 3).

Specimen salvage efforts and museum collections of skins and tissues continue to contribute to our understanding of distribution and movements of common North American vertebrate species. Reference databases, in combination with published studies and museum databases, document phylogeographic variation, potentially allowing for genetic identification of the geographic provenance of any sample (e.g., Witt et al. 2010, Baumann et al. 2011, 2013, Engel et al. 2011, Johnson et al. 2011). This study demonstrates 2 scenarios that can limit the phylogeographic inference of provenance. First, recent divergence among Great Horned Owl subspecies has resulted in insufficient genetic variation at an effectively neutral marker locus, mtDNA. Second, reference databases currently contain incomplete phylogeographic coverage for prey species, even for some of the most common and widespread North American vertebrates.

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