THE DIET OF WIND FARM BATS

by

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Submitted in partial fulfillment of the
requirements for Departmental Honors in
the Department of Biology
Texas Christian University
Fort Worth, Texas

May 3, 2013

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INTRODUCTION

Globally, wind power has become the fastest-growing source of renewable energy (National Renewable Energy Lab – www.nrel.gov). However, despite environmental benefits, wind energy facilities have become increasingly detrimental to bird and bat populations (Drewitt and Langston 2006, Kunz et al. 2007a,b, Kuvlesky et al. 2007). Exemplified by the Wolf Ridge Wind, LLC site in north-central Texas, migratory tree-roosting bats in particular are being killed by wind turbines at an alarming rate (Arnett et al. 2008, Baerwald and Barclay 2011). Most fatalities occur during relatively low wind speed conditions during late summer and early fall, which coincide with when the bats are most active during the fall migratory season. Experimental curtailment involving reduction of wind turbine operation when wind speeds are low and the potential for collision is greatest have been shown to significantly reduce bat fatalities (Arnett et al. 2011). However, additional efforts to reduce fatalities are ongoing.

Fatality searches have identified five species at Wolf Ridge Wind, LLC, the large majority of which were eastern red (Lasiurus borealis) and hoary bats (L. cinereus). (Amanda Hale, pers. comm.). While the bats may be encountering wind turbines incidentally due to geographical convergence of their migratory paths with wind energy facilities, they may also be specifically targeting the turbines for use as foraging sites (Cryan and Barclay, 2009). Bats have been observed foraging close to wind turbines and stomach content analyses suggests they are foraging prior to being hit and killed by wind turbines (Horn et al. 2008, McGuire and Guglielmo 2009). There is limited data, however, about the diet of tree-roosting species at wind energy sites, and comparisons between consumed prey and local insect populations have not been conducted.

Utilizing DNA bar-coding, this study seeks to genetically sequence bat fecal samples and captured moths found at Wolf Ridge Wind, LLC to obtain a representative understanding of these bats' diets at a wind energy site. Past applications of DNA bar-coding to dietary studies have successfully identified arthropod prey through genetic sequencing of bat feces, notably including the eastern red bat (Clare et al. 2009, Zeale et al. 2011). Assuming the moth DNA barcode region is sufficiently variable to distinguish individual species types within the fecal matter, prey taxa may be identified with greater resolution than is possible through direct examination of insect exoskeletons in fecal material (Clare et al. 2009). With this information, it can be determined whether attraction to wind turbine-localized insect populations may be contributing to the high number of migratory bat fatalities at wind energy sites.

METHODS AND MATERIALS

Study Area and Population

Bat fecal samples and moth specimens were collected from the Wolf Ridge, LLC wind energy site in north-central Texas in July - October 2012 and stored dried as part of a graduate student's thesis work (Cochran 2013) and ongoing research on bat mortality (Amanda Hale and Victoria Bennett). Some moth specimens were preserved in 100% ethanol. DNA was previously extracted from some of the fecal samples found near the wind turbines using the QIAamp DNA Stool Mini Kit (Qiagen), and five samples were identified as belonging to the eastern red bat (*Lasiurus borealis*) via DNA bar-coding by another student (Ali Schildt). These five samples were used in this study.

DNA Extraction and PCR

DNA was extracted by removing the legs from individual moth specimens and grinding them in 200 µl lysis buffer and 10 µl *Proteinase K*, then incubating overnight at 55°C. 100 µl of 7.5 M ammonium acetate was added to the samples; they were incubated on ice for 10 minutes, and centrifuged to pellet proteins. Isopropanol (210 µl) was then added to the supernatant and mixed before centrifuging again, to pellet the DNA. The pellet was washed with 70% ethanol and resuspended in 50 µl Tris pH 8.5.

For the moth specimens an approximately 650 base pair mitochondrial segment of the *cytochrome oxidase I* gene was amplified using LCO1490 and HCO2198 (Folmer et al. 1994). Each 50 µL polymerase chain reaction (PCR) reaction contained 2.5 mM $MgCl_2$, 1X PCR buffer, 0.2 mM dNTPs, 0.2 U *Taq* DNA polymerase, ~70 ng DNA, and 0.5 µM of forward and reverse primers. The following cycling parameters were used: one cycle at 95°C for 2 min, then 30 cycles of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C, followed by a final 5 min hold at 72°C in an ABI 2720 thermal cycler.

Eastern red bat fecal samples were amplified at a small segment of the *cytochrome oxidase I gene* (157 bp) using the ZBJ-ArtF1c (5'-AGATATTGGAACWTTATATTTTATTTTTGG-3') and ZBJ-ArtR2c (5'-WACTAATCAATTWCCAAATCCTCC-3') primers (Zeale et al. 2011). DNA in fecal material is often highly degraded, necessitating the use of "mini-barcodes". PCR reactions (10 µl) contained 4 µl DNA, 0.5 µM of each primer, 1X QIAGEN Multiplex PCR Master Mix with HotStarTaq, Multiplex PCR buffer with 3 mM MgCl2 pH 8.7, and dNTPs. The cycling parameters were one cycle at 95°C for 15 min, followed by 40 cycles

of 30 s at 94°C, 90 s at 55°C, 90 s at 72°C, then a final extension at 60°C for 30 min on an ABI 2720 thermal cycler. These PCR products were then gel-purified, inserted into pGEM TA vectors, and transformed into JM109 competent cells. Eight clones were then amplified for each fecal sample using vector primers. Each 20 μL PCR reaction contained 1.5 mM $MgCl_2$, 1X PCR buffer, 0.2 mM dNTPs, 0.2 U *Taq* DNA polymerase, one colony, and 0.5 μM of forward and reverse primers. The following cycling parameters were used: one cycle at 95°C for 5 min, then 30 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, followed by a final 5 min hold at 72°C in an ABI 2720 thermal cycler.

Sequencing

PCR samples were electrophoresed and analyzed via UV light to ensure amplification had occurred. PCR reactions were then enzymatically cleaned before sequencing using *Antarctic Phosphatase* (New England Biolabs) and *ExoI* (New England Biolabs) as per manufacturer's instructions. Samples were sequenced in both forward and reverse directions using the ABI Big Dye Terminator Cycle Sequencing v 3.1 chemistry kit (Applied Biosystems USA). The samples were then electrophoresed on an ABI 3130XL Genetic Analyzer (Applied Biosystems USA).

Analysis

Sequences were trimmed, edited, and organized into contigs using Sequencher v 4.8 (GENECODES). All sequences were submitted to GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify species. Sequences were considered a match if they were 99% identical to specific identified sequences in GenBank. Sequences were then aligned in MEGA v. 5.0 (Tamura et al. 2011). The Kimura 2-parameter genetic

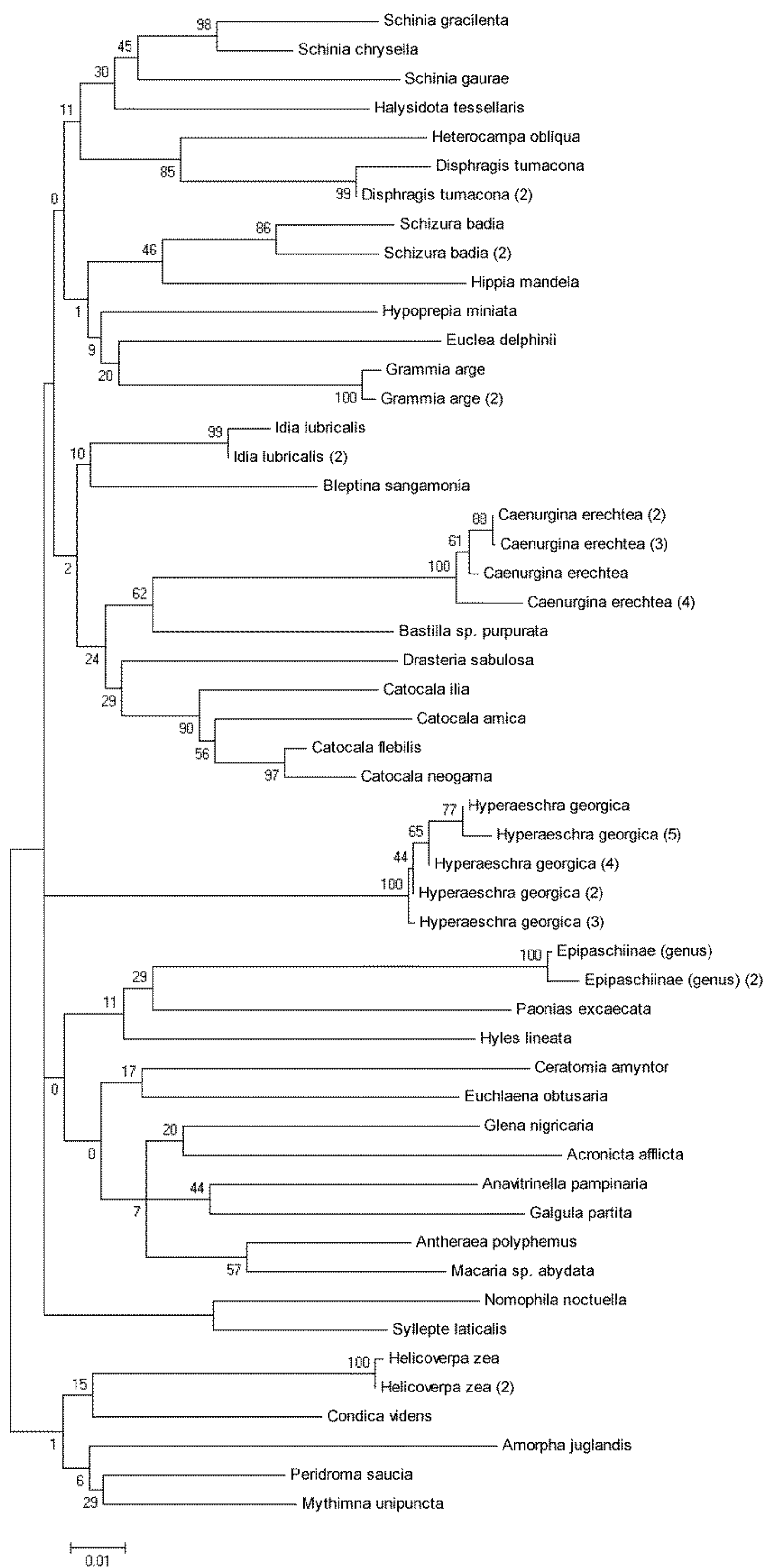
distance was calculated between all sequences, and these distances were used to construct a neighbor-joining tree to cluster the various moth species in MEGA. Bootstrap values were calculated using 1000 replicates.

RESULTS

Moth Barcoding

66 moth specimens were sequenced at the *cytochrome oxidase I gene*. Of these specimens, 57 were identified to species using GenBank, while 9 failed to sequence properly. 52 of the identified sequences were unique and were incorporated into the neighbor-joining tree (Fig. 1).

Figure 1. (following page). Neighbor-joining tree of moth species collected at Wolf Ridge Wind, LLC. Bootstrap values are next to the branches.


Schinia gracilenta
Schinia chrysella
Schinia gaurae
Halysidota tessellaris
Heterocampa obliqua
Disphragis tumacona
Disphragis tumacona (2)
Schizura badia
Schizura badia (2)
Hippia mandela
Hypoprepia miniata
Euclea delphinii
Grammia arge
Grammia arge (2)
Idia lubricalis
Idia lubricalis (2)
Bleptina sangamonia
Caenurgina erechtea (2)
Caenurgina erechtea (3)
Caenurgina erechtea
Caenurgina erechtea (4)
Bastilla sp. purpurata
Drasteria sabulosa
Catocala ilia
Catocala amica
Catocala flebilis
Catocala neogama
Hyperaeschra georgica
Hyperaeschra georgica (5)
Hyperaeschra georgica (4)
Hyperaeschra georgica (2)
Hyperaeschra georgica (3)
Epipaschiinae (genus)
Epipaschiinae (genus) (2)
Paonias excaecata
Hyles lineata
Ceratomia amyntor
Euchlaena obtusaria
Glena nigricaria
Acronicta afflicta
Anavitrinella pampinaria
Galgula partita
Antheraea polyphemus
Macaria sp. abydata
Nomophila noctuella
Syllepte laticalis
Helicoverpa zea
Helicoverpa zea (2)
Condica videns
Amorpha juglandis
Peridroma saucia
Mythimna unipuncta
0.01

The long lengths of the tree branches and clustering of the same species indicate a good ability to distinguish between moth species at Wolf Ridge on the basis of the mitochondrial barcode region.

Bat Feces Barcoding

The five fecal samples identified as belonging to eastern red bats contained the following insect DNA:

- Bat 1: *Pterostichus rostratus* (beetle species); *Metrioptera bonneti* (grasshopper species)
- Bat 2: *Ixylasia ciarana* (moth species); *Teratura geniculata* (katydid species)
- Bat 3: *Peridroma saucia* (moth species)
- Bat 4: *Gryllus sp.* (cricket species)
- Bat 5: *Cyclotrachelus constrictus* (beetle species); *Dalcerides sofia* (moth species)

None of the identified insect species were found in more than one fecal sample and a maximum of two insect species were found in any one fecal sample. The bar-coded sequences for these species were clustered in a neighbor-joining tree (Fig. 2). Based on these results these species were easily distinguished using the mini-barcode region. Multiple unique sequences for each species represent either small base pair changes due to cloning and PCR error or to multiple individuals of the same species in that bat's feces.

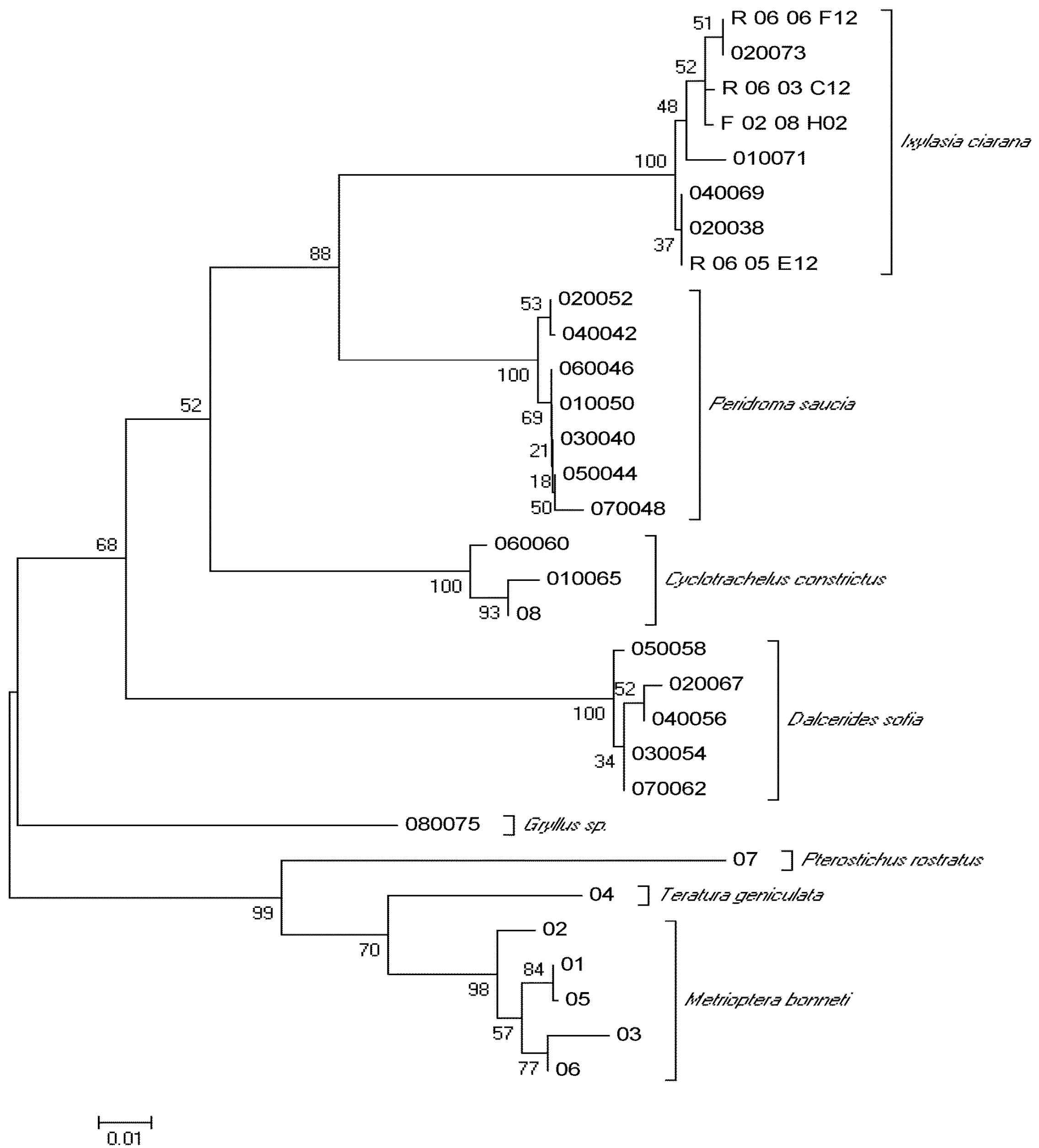


Figure 2. Neighbor-joining tree of insect species detected in eastern red bat fecal samples. Numbers next to branch tips are clone numbers; bootstrap values are next to the branch points.

DISCUSSION AND CONCLUSIONS

The potential of DNA barcoding to distinguish ingested insect species in bat fecal samples was corroborated by this study. I initially barcoded a number of moth specimens from Wolf Ridge because previous studies of eastern red bats suggest that moths make up the largest percentage of their diets (Whitaker 2004, Clare et al. 2009, Feldhamer et al. 2009). Three of the five fecal-identified insects were moth species and one moth species *Peridroma saucia,* was identified in both the initial moth specimen pool and in one sample of analyzed bat fecal matter, which helps to indicate that bats are actually feeding on insect specimens found within the Wolf Ridge Wind, LLC site. Three moth, two beetle, one cricket, one grasshopper, and one katydid species were found in the fecal samples. Also, none of the identified insect species overlapped between fecal samples. This signifies that the eastern red bat's diet is relatively diverse, although the degree of variation between individual diets remains speculative. Previous studies of eastern red bats have also indicated that they have a very diverse diet compared to other migratory tree roosting bats (Carter et al. 2004, Whitaker 2004). Future red bat dietary studies at Wolf Ridge should include greater insect diversity in barcoding studies, especially Coleoptera and Orthoptera.

The presence of crickets, grasshoppers, and katydids in the bats' diets supports the hypothesis that eastern red bats are specifically targeting wind turbines as foraging sites. While moths tend to be free-flying, these other insect species typically aggregate on wind turbine side surfaces (Victoria Bennett pers. comm.); hence, it is likely that these species were eaten directly off of the turbine structure, drawing bats near the turbines. Recent studies at the Wolf Ridge Wind, LLC site have also produced video footage of bats

swooping toward wind turbine surfaces in what appears to be gleaning foraging behavior. These two lines of evidence suggest bats are utilizing turbines for foraging.

Future dietary studies should focus on increasing the diversity of insects analyzed in representative specimen samples as well as increasing the number of bat fecal samples analyzed. Sampling and identifying insects from the surface of the turbines and then matching these to insects found in the feces would be especially interesting. While individual insect species can be identified in fecal samples, the relative numbers of each species eaten cannot at present be identified via DNA bar-coding.

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ABSTRACT

Nationally, migratory tree-roosting bats are being killed by wind turbines at an alarming rate. A similar trend has been found at the Wolf Ridge Wind, LLC site in north-central Texas. While the bats may be encountering wind turbines incidentally due to geographical convergence of their migratory paths with wind energy facilities, the bats may also be specifically targeting the turbines for use as foraging sites. Due to limited data regarding the diet of tree-roosting bat species at wind energy sites such as Wolf Ridge, comparisons between preferred prey and local insect populations have not been conducted, and it remains unknown if the bats are being attracted by particular insect species. Utilizing DNA bar-coding, bat fecal samples and moth specimens found at Wolf Ridge Wind, LLC were sequenced and, through genetic comparison, the particular moths included in the bats' diets were identified.