Title

Genome-wide microsatellite marker development from next-generation sequencing of two non-model bat species impacted by wind turbine mortality: *Lasiurus borealis* and *L. cinereus* (Vespertilionidae)

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Introduction

Although promoted as a sustainable energy resource, an unanticipated impact of wind-energy development has been widespread mortality of bats and birds throughout the world (National Research Council 2007; Kunz et al. 2007; Calvert et al. 2013). In North America, migratory tree-roosting species in the genus *Lasiurus* (Vespertilionidae) consistently comprise a large percentage of bat mortalities at turbine sites (Barclay et al. 2007; Kunz et al. 2007; Arnett et al. 2008), with the eastern red bat (*Lasiurus borealis*) and the hoary bat (*L. cinereus*) together representing up to 74% and 82% of fatalities in the eastern and central U.S., respectively (Arnett et al. 2008). Because of their reclusiveness, low roosting densities, frequent dispersal, and seasonal migration habits, migratory bats are not amenable to traditional methods (e.g., mark-recapture) to estimate demographic variables, such as effective population size (N_e), evidence of temporal change in N_e, and degree of population structure (Moussy et al. 2012). In such cases, highly polymorphic molecular markers can provide critically important data for assessing bat conservation status and impacts of wind energy development.

Development of genetic resources has been meager for bats in the *Lasiurus* genus, and there remains a lack of a large, robust set of highly polymorphic nuclear markers necessary for population genetic studies of this important bat tribe (Table 1). Transferability of microsatellite markers from distantly related Vespertilionid species with greater genetic resources is often poor, resulting in only a few usable loci (Vonhof et al. 2002). Thus, there is an immediate need for developing genetic-marker resources for Lasiurine bats to allow assessment of their conservation status.

With the advent of high-throughput next generation sequencing platforms, the development and deployment of a large number of genomic microsatellite loci is now possible in non-model species, and on timescales rapid enough to be responsive to emerging conservation threats (Ekblom and Galindo 2010; Gardner et al. 2011; Fernandez-Silva et al. 2013). Here, we use whole-genome sequencing and simple sequence repeat (SSR) bioinformatics search algorithms to develop the first genome-wide set of microsatellite loci for bats in the genus *Lasiurus.* These markers greatly extend the genetic resources available for North American tree bats, and should be invaluable to conservation biologists studying environmental impacts on bat populations (Jones et al. 2003).

Data Access

- *Scripts* – Shell script outlining pipeline for sequence alignment and consensus sequence calling is available as File S1 from DRYAD entry doi:10.5061/dryad.70254.
- *Sequence files* – Raw sequence files with quality scores (.fastq) are available for each species from the NCBI Sequence Read Archive: PRJNA225945
- *Reference file* – Myotis_lucifugus.Myoluc2.0.64.dna.toplevel.fa, available from ftp://ftp.ensembl.org/pub/release-73/fasta/myotis_lucifugus/dna/.
- *Sequence alignment files* – One aligned sequence file (.bam) for each individual is available from NCBI Sequence Read Archive: PRJNA225945.

Meta Information

- *Sequencing center* –University of Minnesota Genomics Center (http://www.bmgc.umn.edu/index.htm).
- *Platform and model* – Sequencing was done on an Illumina (San Diego, California, USA) HiSeq 2000.
- *Design Description* - As part of a larger study on wind turbine mortality to migratory bats, we obtained wing tissue samples of *L. borealis* and *L. cinereus* from multiple wind energy sites in the Central Appalachian region of Pennsylvania, Maryland, and West Virginia of the United States. To develop genomic resources for marker discovery, we selected a single individual from both *L. borealis* and *L. cinereus* (both samples from the Backbone Mountain wind facility, MD, USA) for whole-genome shotgun sequencing. Amplification testing of candidate SSR loci and polymorphism screening used DNA samples from an additional 23 individuals per species.
- *Run date* – Run began on July 11, 2012 and ended July 24, 2012.

Library

- *Strategy* – Whole genomic DNA
- *Taxon* – Sequencing libraries were prepared for a single individual of each species, *Lasiurus borealis* and *Lasiurus cinereus* (family Vespertilionidae; subfamily Vespertilioninae; tribe Lasiurini). To determine levels of polymorphism at candidate SSR loci, we genotyped an additional sample of 23 individuals of each species (Table 2).
- *Sex* – For the genomic sequencing libraries, the *L. borealis* sample was from a male, and the sex of the *L. cinereus* sample was unknown.

- *Location* – Both samples used in constructing the genomic sequencing libraries came from the Criterion Wind Project on top of Backbone Mountain, near Oakland Maryland. Information on samples for polymorphism screening is detailed in Table 2.
- *Sample handling* – Sample tissue was stored in 95% ethanol and sampled with a sterile wing punch prior to DNA extraction.
- *Layout* – We sequenced paired-end reads (2 x 100 bp).
- *Library Construction Protocol* -- From each individual, we extracted total genomic DNA from wing punches using Qiagen (Germantown, Maryland, USA) DNeasy Blood and Tissue kits. DNA was quantified using a Qubit fluorometer (Life Technologies, Carlsbad, California, USA), and normalized to a concentration of 10 ng/µl. Samples were then shipped to the University of Minnesota Genomics Center (http://www.bmgc.umn.edu/index.htm) for library construction and sequencing. Briefly, 1.3 – 2.6 µg of DNA was sheared using a Covaris (Woburn, Massachusetts, USA) ultrasonicator, followed by fragment purification, end polishing, and ligation of indexed adaptors. The two indexed libraries were then pooled, clustered on a single flow cell lane of an Illumina HiSeq2000, and sequenced to a target depth of 160 million 2 x 100 bp paired-end reads.
- *Nominal size (paired) of fragments sequenced* -- The mean insert size of libraries was 250 bp (*L. borealis*) and 280 bp (*L. cinereus*).

Processing

- *Pipeline* -- Raw reads were demultiplexed, trimmed of adaptors, and fastq files generated conforming to the Illumina CASAVA 1.8 standard. Assessment of read quality was conducted using FASTQC (written by Simon Andrews; available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We performed reference-guided read mapping using the *Myotis lucifugus* (little brown bat) reference genome, sequenced by the Broad Institute. We used version 2.0.64 of the unmasked *Myotis* reference genome, available from Ensembl (http://useast.ensembl.org/Myotis_lucifugus/Info/Index), which consists of 72,784 contigs totaling 1.97 Gb in size. Reads were mapped against the indexed *Myotis* reference genome using the '*aln'* and '*sampe'* commands in bwa v0.6.2 (Li and Durbin 2010). Resulting sam alignment files were then converted to bam format and used to generate read pileups using the '*mpileup*' command in samtools v0.1.18, with the '-C50' flag to downgrade mapping quality of reads showing excessive mismatches to the reference genome (Li et al. 2009). Pileups were then used to generate a consensus sequence for each species. Prior to generating the consensus sequence, we noticed some genomic regions with extremely high read depth and an abundance of SNP variants, probably reflecting mapping of reads to paralogous gene copies. Therefore, we filtered our consensus sequence for just those bases where coverage was <100 reads, using the vcf2fq function with the flag '–D 100' in the vcfutils.pl script available with samtools.

Consensus sequences were used in downstream analyses to locate potential SSRs within the genome of each species and design associated primers. We used

MSATCOMMANDER v1.0.8 (Faircloth 2008) to search for di, tri- and tetra-nucleotide repeats, with minimum repeat values of 6, 6, and 4, respectively. We implemented the Primer3 wrapper within MSATCOMMANDER to design primers from the flanking sequence of each locus, setting the target Tm = 62 °C.

Forty eight candidate SSR loci were selected among the total set of loci identified by MSATCOMMANDER for cross-species amplification testing and polymorphism screening. Candidate loci were selected based on (i) presence in the consensus sequence of both species, and (ii) minimizing the distance between species in the corresponding genomic position of the forward and reverse primers. Candidate loci and their primer sequences were then manually checked against the merged bam genome alignments across species, using the Tablet genome viewer (Milne et al. 2013). Primer regions were screened for potential polymorphisms among species and degenerate primers synthesized as needed to facilitate cross-species amplification. Both forward and reverse primer sequences were checked for paralogy against the *Myotis* reference genome using BLASTN adjusted for short input sequences. All forward primers were synthesized with a 5' "tail" which corresponded to one of four fluorescently labeled universal primers (Blacket et al. 2012) (Table 3).

All primer pairs were first tested as single plex reactions on two individuals of *L. cinereus* and one individual of *L. borealis*, which included the two individuals used for genome sequencing. Reactions were carried out in 10 µl volumes using the Qiagen Multiplex PCR kit. Final concentrations of reactants were: 1x Qiagen Multiplex PCR Master Mix, 0.5x Q-Solution, 0.1 µM of tailed forward primer, 0.1 µM of universal

147 forward primer labeled with 1 of 4 fluorophores (6FAM, NED, PET, or VIC), 0.2 µM of
148 reverse primer, 2 µl of DNA (approximately 4.0 ng/reaction) and brought up to volume
149 with water. PCR reactions were run on an Eppendorf Mastercycler Pro under the
150 following conditions: 95 °C for 15min followed by 30 cycles of 94 °C for 30s; 57 °C for
151 90s; 72 °C for 90s; a final extension of 72 °C for 30 min then a 4 °C hold. Each reaction
152 product was checked for amplification on agarose gels, and PCR products exhibiting a
153 band at the desired size were sent to the Penn State Huck Genomics Core Facility for
154 genotyping on an Applied Biosystems 3730xl and genotypes sized against LIZ500 size
155 standard using Applied Biosystems Peak Scanner v1.0 software (available from:
156 http://www.lifetechnologies.com/order/catalog/product/4381867). Loci were
157 eliminated from further development if they failed to amplify (LAS5891AC, LAS9598AG),
158 produced multiple bands on agarose gels (LAS2537AG, LAS6925AC), or if genotype
159 scoring was deemed unreliable (LAS9351AC), leaving a total of 43 candidate loci for
160 characterizing polymorphism (Table 3).
161 Promising primer pairs (i.e., those amplifying potentially polymorphic loci) were
162 grouped for testing in multiplex reactions. The multiplex reactions consisted of 4, 6, or 8
163 primer pairs. The groupings were based on product size and fluorophore so that a
164 minimum of approximately 100 bp existed between products labeled with the same
165 fluorophore. Multiplex combinations were tested on the same individuals as for the
166 single plex reactions and under the same PCR conditions and reactant concentrations.
167 To screen for polymorphism in the final set of candidate loci, we genotyped 23 *L.*
168 *cinereus* and 23 *L. borealis* individuals. Genotyping samples were processed as above,

and fluorescently labeled fragments were sized with PeakScanner, scored manually by 2 independent observers, and the resulting alleles binned into discrete size classes using TANDEM (Matschiner and Salzburger 2009). Levels of allelic polymorphism, heterozygosity, and Hardy-Weinberg and genotypic linkage equilibrium were determined with FSTAT (Goudet 1995), and estimation of the presence of null alleles and their frequencies was performed using MICROCHECKER (Van Oosterhout et al. 2004).

- *Runs* – Two runs were submitted to NCBI's SRA as .fastq files. Each run contains two files for each paired-end sequence.

Results

- *Quality scoring system* – Quality scores were based on the Illumina CASAVA 1.8 standard, corresponding to Phread+33.
- *Quality scoring ASCII character range* – from "!" to "J"
- *Mean / Median coverage per contig* -- Illumina sequencing returned 87,085,923 (*L. borealis*) and 79,861,641 (*L. cinereus*) reads passing filter, with average Q-scores of 36.27 and 35.96, respectively, and high Q-score values across the length of the reads. Alignment of *Lasiurus* paired-end reads to the *Myotis* reference genome resulted in a low proportion of mapped reads for both *L. borealis* (23,332,716 or 13.4%) and *L. cinereus* (23,285,951 or 14.6%), probably reflecting the relatively high divergence between *Myotis* and *Lasiurus.* However, among the reads that did map successfully, the majority were mapped in their proper pairing (86% for *L. borealis* and 82% for *L.*

cinereus), and covered 10,822 of the 11,654 scaffolds in the *Myotis* reference genome. Coverage, estimated as reads per kilobase of reference genome sequence (RPK), averaged 5.9 across species.

- *Polymorphism rate* -- Using the consensus sequences from each species, we identified a total of 35,077 candidate genomic SSR regions in *L. borealis* and 27,690 SSR regions in *L. cinereus* (File S2, DRYAD doi:10.5061/dryad.70254). The majority of SSR's consisted of di-nucleotide repeats (69.7%), followed by tetra- (22.7%) and tri-nucleotide repeats (7.6%). At least one SSR region was identified in 1,791 scaffolds (*L. borealis*) and 1,593 scaffolds (*L. cinereus*). Given the large numbers of scaffolds in the *Myotis* reference genome and the wide distribution in scaffold size (range 0.003 – 65 Mb), the number of identified SSR regions per scaffold varied from 1 to 1,423 (*L. borealis*) and 1 to 1,094 (*L. cinereus*), averaging 19.6 and 17.4 SSR regions/scaffold per species, respectively. Primers were developed from flanking sequence in each these regions, and 69 SSR's contained primer sequences that differed by <10 bp in their start position within the genome for either the forward or reverse primers, or both. These loci were targeted as the most likely candidates for robust cross-species amplification. Of these, 21 primer pairs were eliminated due to low read coverage in the SSR region or flanking sequence, frequency of imperfect repeats, and/or polymorphism in the primer sequence region, leaving 48 SSR regions for further testing and development. Of these, 43 loci produced reliable amplification of single bands and genotype profiles that could be reliably scored. Multiplexing development with these loci produced a total of 6 multiplexes that together contained 34 of the 43 loci, with plex level ranging from 4 – 8 loci per plex,

while the remaining loci (N=8) were scored in single plex (Table 3). All multiplex genotypes were independently confirmed to be identical in their allele sizes against the corresponding single plex genotypes for each locus.

Polymorphism screening in a panel of 46 individuals (23 per species) showed these loci to be highly variable (Table 4), with 42 of 43 loci polymorphic in one or both of these two *Lasiurus* species (LAS9084AC). In *L. borealis,* 40 of 43 loci amplified and 38 were polymorphic, with an average of 12.0 alleles per locus (range 1-26). In *L. cinereus,* 40 of 43 loci amplified and all were polymorphic, although polymorphism was slightly lower than in *L. borealis,* averaging 9.8 alleles per locus (range: 2-20). The effective number of alleles and expected heterozygosity were also high in these loci, although some loci showed high polymorphism in one species but low or fixed allele number in the other (Table 4).

In *L. borealis,* 12 of 38 polymorphic loci showed significant departures from Hardy-Weinberg equilibrium, caused by an excess of homozygous genotypes (mean F_{IS} = 0.167). In contrast, these same loci showed relatively few instances of homozygote excess in *L. cinereus* (mean F_{IS} = 0.028), with only a single locus (LAS7468AC) displaying significant departure from H-W equilibrium (Table 4). Analysis of homozygosity and allele size distributions using MICROCHECKER suggested that 14 loci may contain null alleles in *L. borealis*, while only 2 loci show evidence of null alleles in *L. cinereus* (Table 4). There were no significant tests of pairwise genotypic linkage disequilibrium among any of the 42 loci in either species.

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292 **Tables**

293

294 **Table 1.** Microsatellite development in Vespertilionid bats, showing a lack of genetic resource development in genus *Lasiurus*.
295 Studies were found through searching Google Scholar and ISI Web of Science, and by searching public databases of sequence data
296 (NCBI Genbank and the European Nucleotide Archive). SSR loci that were successfully cross-amplified in *Lasiurus* spp. are also noted.
297

Species	SSR loci (published)	SSR loci (database)	x-amplified in *Lasiurus*	Reference
Chalinolobus tuberculatus	11			DeKrout et al. (2009) Microsatellite markers for the endemic New Zealand long-tailed bat (*Chalinolobus tuberculatus*). Molecular Ecology Resources 9:616-618.
Corynorhinus rafinesquii (=Plecotus rafinesquii)	15			Piaggio et al. (2009) Development and characterization of 15 polymorphic microsatellite loci isolated from Rafinesque's big-eared bat, *Corynorhinus rafinesquii*. Molecular Ecology Resources 9:1191-1193.
Corynorhinus townsendii	8			Piaggio et al. (2009) Eight polymorphic microsatellite loci developed and characterized from Townsend's big-eared bat, *Corynorhinus townsendii*. Molecular Ecology Resources 9:258-260.
Eptesicus fuscus	9		yes	Vonhof et al. (2002) Characterization of dinucleotide microsatellite loci in big brown bats (*Eptesicus fuscus*), and their use in other North American vespertilionid bats. Molecular Ecology Notes 2:167-169.
Kerivoula papillosa (=K. pellicida)	22	35		Struebig et al. (2008) Isolation and characterisation of microsatellite loci in the papillose woolly bat, *Kerivoula papillosa* (Chiroptera: Vespertilionidae). Conservation Genetics 9:751-756.
Lasiurus borealis	2		yes	Korstian (2012) High genetic diversity and lack of structure in eastern red bats L. borealis. Rice University Master's thesis.
Lasiurus borealis			yes	Wills (2000) Population genetics of eastern red bats, Lasiurus borealis. University of Tennessee undergraduate thesis.
Miniopterus fuliginosus	12			Han et al. (2008) Isolation and characterization of microsatellite loci in the long-fingered bat *Miniopterus fuliginosus*. Molecular Ecology Resources 8:799-801.

Miniopterus schreibersii	5			Miller-Butterworth et al. (2002) Isolation and characterization of highly polymorphic microsatellite loci in Schreibers' long-fingered bat, *Miniopterus schreibersii* (Chiroptera: Vespertilionidae). Molecular Ecology Resources 2:139-141.
Miniopterus schreibersii	20			Wood et al. (2011) Development and characterisation of 20 microsatellite loci isolated from the large bent-wing bat, *Miniopterus schreibersii* (Chiroptera: Miniopteridae) and their cross-taxa utility in the family Miniopteridae. Molecular Ecology Resources 11:675-685.
Murina gracilis	24			Kuo and Rossiter (accessed October 2013) . Source: European Nucleotide Archive.
Murina ussuriensis		12		Ishibashi et al. (accessed October 2013) Development of novel microsatellite DNA markers in the Ussurian tube-nosed bat, *Murina ussuriensis*, by using existing markers. Source: European Nucleotide Archive.
Myotis bechsteinii	4			Kerth et al. (2002) Mean colony relatedness is a poor predictor of colony structure and female philopatry in the communally breeding Bechstein's bat (*Myotis bechsteinii*). Behav Ecol Sociobiol 52:203-210.
Myotis grisescens	16	17		Lindsay et al. (2013) Novel microsatellite loci to investigate population structure in the endangered gray bat (*Myotis grisescens*). Conservation Genetics Resources doi: 10.1007%2Fs12686-013-9969-6.
Myotis lucifugus	4			Jan et al. (2012) Development of conserved microsatellite markers of high cross-species utility in bat species (Vespertilionidae, Chiroptera, Mammalia). Molecular Ecology Resources 12:532-548.
Myotis macropus		5		Campbell and Guay (2009) Genetic differentiation among populations of a specialist fishing bat suggests lack of suitable habitat connectivity. Biological Conservation 142:2657-2664.
Myotis myotis	2		yes	Petri et al. (1997) Paternity assessment and population subdivision in a natural population of the larger mouse-eared bat *Myotis myotis*. Molecular Ecology 6:235-242.
Myotis myotis	13	41		Castella and Ruedi (2000) Characterization of highly variable microsatellite loci in the bat *Myotis myotis* (Chiroptera: Vespertilionidae). Molecular Ecology 9:993-1011.
Myotis nattereri	8			Scott et al. (2013) Isolation and characterization of eight polymorphic microsatellite loci for Natterer's bat, *Myotis nattereri* (Vespertilionidae, Chiroptera). Conservation Genetics Resources 5:643-645.
Myotis sodalis	10			Oyler-McCance and Fike (2012) Characterization of small microsatellite loci isolated in endangered Indiana bat (*Myotis sodalis*) for use in non-invasive sampling. Conservation Genetics Resources 3:243-245.
Myotis sodalis	9			Trujillo and Amelon (2012) Development of microsatellite markers in *Myotis sodalis* and cross-species amplification in *M. gricescens, M. leibii, M. lucifugus*, and *M. septentrionalis*. Conservation Genetics 10:1965-1968.

Myotis velifer		6	Parlos and Forstner (accessed October 2013) Microsatellite cross-species amplified products among *Myotis velifer*. Source: European Nucleotide Archive.
Nyctalus leisleri	11		Boston et al. (2009) Development and characterization of 11 polymorphic compound tetranucleotide microsatellite loci for the Leisler's bat, *Nyctalus leisleri* (Vespertilionidae, Chiroptera). Conservation Genetics 10:1501-1504.
Nyctalus noctula	10		Mayer et al. (2000) Polymorphic microsatellite loci in vespertilionid bats isolated from the noctule bat *Nyctalus noctula*. Molecular Ecology 9:2208-2212.
Nyctalus noctula	2		Petri et al. (1997) Paternity assessment and population subdivision in a natural population of the larger mouse-eared bat *Myotis myotis*. Molecular Ecology 6:235-242.
Nyctophilus gouldi		16	Fuller et al. (accessed October 2013) Isolation and characterisation of 16 microsatellite markers for the endangered *Nyctophilus gouldi* and cross-amplification in *N. geoffroyi* using 454-pyrosequencing. Source: European Nucleotide Archive.
Pipistrellus abramus	10		Wei et al. (2009) Isolation and characterization of microsatellite loci in the Japanese pipistrelle (*Pipistrellus abramus*). Conservation Genetics 10:677-679.
Pipistrellus pipistrellus	6		Racey et al. (2007) Microsatellite DNA polymorphism confirms reproductive isolation and reveals differences in population genetic structure of cryptic pipistrelle bat species. Biological Journal of the Linnean Society 90:539-550.
Pipistrellus pygmeaus (=P. pipistrellus)	6		Racey et al. (2007) Microsatellite DNA polymorphism confirms reproductive isolation and reveals differences in population genetic structure of cryptic pipistrelle bat species. Biological Journal of the Linnean Society 90:539-550.
Plecotus auritus	5	6	Burland et al. (1998) Isolation and characterization of microsatellite loci in the brown long-eared bat, *Plecotus auritus*, and cross-species amplification within the family Vespertilionidae. Molecular Ecology 7:136-138.
Plecotus austriacus	150		Razgour et al. (accessed October 2013) Loss and gain of genetic diversity in edge populations under climate change. Source: European Nucleotide Archive.
Scotophilus kuhlii	11		Sun et al. (2009) Isolation and characterization of 11 microsatellite loci in *Scotophilus kuhlii* (Lesser Asiatic Yellow House Bat). Conservation Genetics 10:1857-1859.
Tylonycteris pachypus	9	19	Hua et al. (2007) Isolation and characterization of microsatellite loci in the flat-headed bat (*Tylonycteris pachypus*). Molecular Ecology Notes 7:486-488.
Tylonycteris pachypus	13		Yin et al. (2009) Development and characterization of 13 novel microsatellite loci from the flat-headed bat (*Tylonycteris pachypus*) with cross-species amplification in closely related taxa. Conservation Genetics 10:1061-1063.

298 **Table 2.** Genomic location, multiplexing groups, and primer sequences for 43 microsatellite loci successfully amplified and
299 characterized in *Lasiurus borealis* and *L. cinereus*. Degenerate bases follow IUPAC ambiguity codes. Locus name has the repeat motif
300 appended. Contig and Genomic position follow the *Myotis lucifugus* reference genome (v2.0.64). Size is the expected amplicon size
301 based on the genomic sequence from which primers were developed. Plex designates the multiplex combination that a locus
302 belongs to (MP#), or if used in single plex (SP). Forward primers were designed with 5' tails that are matched with a corresponding
303 fluorophore-labeled primer tail (see Methods).

Locus	Contig	Genomic position	Size	Plex	Fluorophore and Tail (5' - 3')	Tail and Forward Primer (5' - 3')	Reverse Primer (5' - 3')
LAS8965AC	GL430520	2362	403	MP1	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGACTCGCCACCTTGTTCTCAC	AGTCCACACCAGCTGCACAG
LAS9263AG	GL430215	40324	309	MP1	6FAM:GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCATCTGTTGGGAGGAGGTGCTG	GGGAATTATCAGCGGTGCAGG
LAS9284AG	GL430191	32219	444	MP1	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGTGTCACCCTGGACTTGTCCC	GAGGTTTGGTGGCTGATGGG
LAS9367AC	GL430106	120327	236	MP1	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCGGCTGACACGTTACTTGGG	TAACACTCCTCTACCCGGCG
LAS6266AG	AAPE02064088	11725	294	MP2	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGTCTGACACTTGGGTCCTGGC	TGGCCCTCATCCCATAATCAC
LAS7831AC	AAPE02059876	31204	413	MP2	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCAGGTGGACAGGGAGATTTGAC	TGTCAGCCTGGAAGTACTGC
LAS8539AC	GL430892	25623	195	MP2	6FAM:GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCACCAGTTCCAGCCTTGCACAG	TGCTTTGGTGCCTCGAACAG
LAS8953AC	GL430536	160848	350	MP2	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGCCAGGGAGTAGGCCAGTGG	CCAGGGAGTAGGCCAGTGG
LAS9555AG	GL429928	234608	452	MP2	PET;CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGTGCCTGCATTCCTTCATCCATG	CCCAAACCCTTGACACTTCC
LAS9618AC	GL429863	259102	178	MP2	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCATACCACCTGCTGACCTTGG	GACAGCTGCTCACCCAAATC
LAS1436AG	AAPE02070691	453	216	MP3	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCAAGGTGAAAGGGAGAGCAGG	CAAAGTGCTTGGGTCACAGTG

LAS6594AC	AAPE02063262	9169	143	MP3	6FAM:GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCAGCAAATGGGCACACACATG	TGCCTAAACATTACAACTGCAC
LAS7468AC	GL431833	25500	322	MP3	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCAGTGAGGTCTGAAGYGGGAG	GACCTCGAACACCAGAGCAG
LAS8425AC	GL431017	30182	178	MP3	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGACAGTGGGAATGGAGTGAAAGC	GCCAGATTCCATTGCCATGC
LAS8644AC	GL430807	53851	241	MP3	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGTGTTYGCATGTCAGTGTAGGC	ACATTCCAAAAATGTAAGGTGC
LAS9074AC	GL430396	236446	364	MP3	6FAM:GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCATTGCCAACGCCATCTGTCAC	GATGCATAGCCACCACACCC
LAS9435AC	GL430049	205959	401	MP3	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGGGTAGAAGCAACCCAAGTGWC	TGTGCAACCATCATCACTGTCC
LAS2282AC	AAPE02069544	2944	147	MP4	6FAM;GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCACCRCACCCACTCCAGAGGC	TCCCAGTATTTCTTCCAGCTGC
LAS5368AC	AAPE02065459	6965	184	MP4	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGGCTCTGATTTGGAGGATGTGGC	TCCTCACCTGCMCTTCCTT
LAS8982AG	GL430480	162341	376	MP4	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCCCCGTGATGACCATGCAGC	TGCGGTTTGTGCCAAGAGTC
LAS9279ACCT	GL430212	76763	408	MP4	NEDCAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGAGCTTTCAGGGTGGAAGCAC	CATGAGCACAAACAAACCCTCC
LAS9594AC	GL429888	300652	300	MP4	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGAGAACTTGACCTCAGCCATGTC	CCCAGCAAGACATTCCTGACC
LAS9621AC	GL429859	461768	333	MP4	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGACCACTTGCCCRCCTCACCC	GCCTCTGATTCACCACCTGC
LAS4206AC	AAPE02067024	5612	308	MP5	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGGRGAATTCTGCCTTGACTGGG	GGACCCAGTGCCAATCCAAC
LAS8830AC	GL430516	84871	266	MP5	6FAM:GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCAGATGGGAATAAGGACTAGAGTG	CCAATTTGGCACCATTCCCAG
LAS8843AC	GL430506	89721	167	MP5	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCTCAGACAAAGAAGGCCTGTC	CTGAGGACGGGTGYGTTCCC
LAS9084AC	GL430382	16087	462	MP5	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGCATTCCTGTCCTCGGTTCATCC	TACTGGTGGACGGGTGCTAC
LAS9141AC	GL430323	136139	307	MP5	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGGTCGCCCAGTGTCCATGATG	CGACCTCCTTGTTCAATACC
LAS9290AC	GL430174	511717	391	MP5	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCCAACAGAGAGCTCYATTACC	GRGGCTGTGCTTGTAGTGCC
LAS9524AC	GL429961	421013	419	MP5	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGCACACAGAAGGCAGCCAATG	GCACCTGGGCAAGAAGTACG

LAS9613AC	GL429870	233801	191	MP5	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGTCCTAACACCTGTCCCTGCC	AACACTCCCAATCCAAGCAC
LAS4052AC	AAPE02067234	3646	343	MP6	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGTACAGTRTGTGGGCAGGTGG	TGGGAGATGGGACCACAGTG
LAS9151AC	GL430320	76595	267	MP6	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCCAGCGTTGTGCCTACAAATG	GCAGCCAGGATTCATGTCGG
LAS9361AC	GL430123	373506	352	MP6	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGCAGCTGCTTCCACTGAGCTC	TTCCCARCCATGTGCACTGC
LAS9405AC	GL430078	36692	226	MP6	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGACAGGTGCTGGGTTCCCAGC	CTGCTTACACMTCTTTACCTGG
LAS1045AAT	AAPE02071242	481	468	SP	NED:CAGGACCAGGCTACCGTG	CAGGACCAGGCTACCGTGAGGAGGCACCCAATCACTGTG	GGTCCGATTCCAGTCAAAGG
LAS2547AG	AAPE02069201	582	465	SP	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGGCCTAAAGTGCACATGTCTG	CAGARCTATGAGGAACATTGGC
LAS9114AG	GL430356	12489	458	SP	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCTCCAGGAAGCTCAGAGATCACC	TCTTGCTCGGACGGTTTCTA
LAS9126AG	GL430350	328845	327	SP	VIC:GCCTTGCCAGCCCGC	GCCTTGCCAGCCCGCTGCTGCAGGTGTCCTTCAAG	AGAGAGTGAACAGCCCAGCC
LAS9462ACGC	GL430012	548335	406	SP	6FAM:GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCAAAACGCGCCGACTTACTCTG	GGCCGCGATGTTGTACCTAG
LAS9498AC	GL429983	21401	434	SP	PET:CGGAGAGCCGAGAGGTG	CGGAGAGCCGAGAGGTGAGCTCTCCTGACACTGCGTG	CCATGTCCTGTTTGCTGCTG
LAS9606AC	GL429875	154423	449	SP	6FAM:GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCATCTGCCTGGCTCACTTCCTC	CACCAGCAATGACACATGGATC
LAS9673AC	GL429809	131601	362	SP	6FAM:GCCTCCCTCGCGCCA	GCCTCCCTCGCGCCACATGCTGGCTGGGCTACTTC	AGCTCCACCTGCAGCTRGG

Table 3. Wind turbine projects providing bat tissue samples for genetic analysis. Numbers indicate sample size in the polymorphism screening panel (N=23 samples per species).

Site	*L. borealis*	*L. cinereus*
Backbone, MD	16	15
Mountaineer, WV		3
Mt Storm, WV	7	3
Pennsylvania Game Commission (PAGC), PA		2

Table 4. Levels of polymorphism in 42 microsatellite loci genotyped in 23 *L. borealis* and 23 *L. cinereus*. The effective number of alleles (A_E) is rarefied to a sample size of N=10. An '*' after F_{IS} indicates presence of possible null alleles. A value of 'na' indicates a locus for which no amplification occurred in that species.

	L. borealis					*L. cinereus*				
Locus	***A***	A_E	H_o	H_e	F_{IS}	***A***	A_E	H_o	H_e	F_{IS}
LAS1045AAT	6	5.544	0.826	0.798	-0.035	6	5.998	0.783	0.793	0.014
LAS1436AG	na	na	na	na	na	13	12.696	0.870	0.869	-0.001
LAS2282AC	16	12.040	0.500	0.951	0.474*	13	12.782	0.870	0.906	0.040
LAS2547AG	15	11.145	0.909	0.934	0.027	16	16.000	0.864	0.938	0.080
LAS4052AC	16	11.948	0.826	0.943	0.124	14	13.781	0.913	0.915	0.002
LAS4206AC	18	12.818	0.870	0.954	0.088	3	2.957	0.435	0.505	0.139
LAS5368AC	26	15.344	0.913	0.971	0.060	13	12.825	0.913	0.910	-0.003
LAS6266AG	20	14.018	0.682	0.970	0.297*	16	15.737	0.957	0.934	-0.024
LAS6594AC	7	6.077	0.739	0.811	0.089	10	9.869	0.913	0.846	-0.079
LAS7468AC	24	14.480	0.913	0.964	0.053	14	13.823	0.696	0.927	0.249*
LAS7831AC	15	9.784	0.478	0.885	0.460*	17	16.694	0.870	0.921	0.056
LAS8425AC	17	11.555	0.522	0.938	0.444*	12	11.824	1.000	0.884	-0.131
LAS8539AC	4	2.857	0.348	0.336	-0.035	10	9.870	0.957	0.861	-0.111
LAS8644AC	10	8.313	0.826	0.866	0.046	3	2.957	0.348	0.299	-0.162
LAS8830AC	15	10.853	0.955	0.922	-0.035	10	9.911	0.783	0.847	0.076
LAS8843AC	20	13.646	0.957	0.958	0.001	8	7.955	0.565	0.790	0.284
LAS8953AC	14	9.890	0.864	0.897	0.037	8	7.957	0.783	0.847	0.076
LAS8965AC	1	1.000	0.000	0.000	--	8	8.000	0.773	0.725	-0.066
LAS8982AG	9	6.749	0.591	0.837	0.294*	6	5.956	0.522	0.704	0.258
LAS9074AC	9	7.031	0.870	0.823	-0.056	7	6.869	0.696	0.676	-0.029
LAS9114AG	13	9.939	0.421	0.920	0.542*	na	na	na	na	na
LAS9126AG	10	7.774	0.864	0.844	-0.023	6	5.912	0.478	0.610	0.216
LAS9141AC	2	1.686	0.000	0.087	1.000	10	9.955	0.783	0.861	0.091

LAS9151AC	11	7.505	0.609	0.809	0.248	11	10.825	0.957	0.874	-0.095
LAS9263AG	1	1.000	0.000	0.000	---	2	2.000	0.045	0.045	0.000
LAS9279ACCT	2	1.435	0.043	0.043	0.000	4	4.000	0.739	0.634	-0.165
LAS9284AG	6	4.759	0.522	0.693	0.247	4	6.000	0.227	0.461	0.507
LAS9290AC	13	9.838	0.522	0.919	0.432*	16	15.650	1.000	0.903	-0.107
LAS9361AC	14	10.993	0.957	0.928	-0.031	2	1.957	0.043	0.043	0.000
LAS9367AC	22	13.827	0.870	0.956	0.090	20	20.000	0.955	0.959	0.005
LAS9405AC	6	5.153	0.864	0.788	-0.096	6	5.999	0.696	0.764	0.089
LAS9435AC	na	na	na	na	na	9	8.910	0.826	0.821	-0.006
LAS9462ACGC	3	3.000	0.700	0.611	-0.145	na	Na	na	na	na
LAS9498AC	16	11.681	0.455	0.945	0.519*	2	2.000	0.565	0.447	-0.265
LAS9524AC	14	10.875	0.696	0.933	0.254*	13	12.910	0.957	0.899	-0.064
LAS9555AG	21	13.135	0.696	0.952	0.269*	19	18.520	0.826	0.921	0.103
LAS9594AC	8	6.675	0.682	0.829	0.178	5	4.999	0.609	0.780	0.219
LAS9606AC	13	8.655	0.857	0.851	-0.007	12	11.868	0.913	0.900	-0.014
LAS9613AC	11	9.326	0.870	0.884	0.017	14	13.782	0.913	0.887	-0.029
LAS9618AC	11	8.427	0.609	0.915	0.335*	12	12.000	0.864	0.870	0.007
LAS9621AC	11	8.947	0.545	0.904	0.396*	9	8.955	0.870	0.853	-0.020
LAS9673AC	11	7.894	0.818	0.847	0.034	9	9.000	0.682	0.837	0.185

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Additional supplementary files available from DRYAD doi:10.5061/dryad.70254

File S1. UNIX bash script containing informatics pipeline used for read mapping and generation of the consensus sequence.

File S2. SSR identification output from MSATCOMMANDER with accompanying candidate primers.