# GENETIC ANALYSIS OF THE SOUTHERN YELLOW BAT (*DASYPTERUS EGA*)

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ABSTRACT

For this research project I examined the genetic variation of the Southern yellow bat, *Dasypterus ega*. No previous genetic work has been done on this species of conservation concern even though genetic diversity is an important aspect of species conservation. I used a microsatellite analysis to examine the level of genetic variation of *D. ega* in southern Texas. I analyzed 75 bats from 15 counties using six loci that were used in a close relative, the Eastern red bat (*Lasiurus borealis).* There were 3-18 alleles at each locus and the level of heterozygosity was low (0.385). This value was lower than endangered species *Corynorhinus townsendii ingens* and much lower than that reported for a common, widespread species of bat, *L. borealis*. This indicates that *D. ega*, recognized as a "threatened" species in Texas, might have experienced a loss of genetic diversity due to being a leading-edge population, genetic drift, or a genetic bottleneck.

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# INTRODUCTION

*Dasypterus ega,* the Southern yellow bat, is found in the southern part of Texas, Mexico, and South America (Kurta and Lehr, 1995). *Dasypterus ega* belongs to the order Chiroptera and family Vespertilionidae (Kurta and Lehr, 1995). It is a tree-roosting bat known to roost in both wild and ornamental palms (Jimenez, 2016). *Dasypterus ega* is one of the rarest bats in Texas (Ammerman et al., 2012), and very little is known about it molecularly. There have been a few karyotype studies, but an in-depth genetic analysis has not been performed on the southern yellow bat. *Dasypterus ega* is currently threatened in Texas, but it is listed as "least concern" on the IUCN Red List (Barquez and Diaz, 2016) presumably because a large portion of its range is in Mexico where population declines have not been noted. Previously, *D. ega* was referred to as *Lasiurus ega* and was grouped with the other tree bats in the same genus as red and hoary bats. It has recently been shown that yellow bats should be their own genus (*Dasypterus*); however, the phylogenetic relationship with other *Lasiurus* bat species is close (Baird et al., 2015).

Ecologically, *D. ega* is an insectivore and may play a large part in the fight against crop pests in agriculture. Although not much is known about the feeding habits of *D. ega*, it is speculated that they feed on small to medium size flying insects (Ammerman et al., 2012). Texas has a large agricultural industry that produces cotton, hay, sorghum, wheat, and corn. Many crop pests can be regulated by insectivorous bats such as *D. ega*. It is estimated that the loss of bats would lead to losses of $3.7 billion a year to $53 billion a year in the United States (Boyles et al., 2011). While this amount is averaged over the entire United States, the importance of bats in agriculture is undeniable. *Dasypterus ega* likely contributes to the

consumption of crop pests. Agriculture in Texas plays a large role in the economy and averages $20 billion annually when including crops and cattle (Texas Department of Agriculture, 2018). One of every seven working Texas residents is in an agricultural related job (Texas Department of Agriculture, 2018). With the loss of even a small portion of crops, not only would the economy be impacted, but a large number of people would be without jobs. Insectivorous bats are important to agriculture (and to the ecosystem) because they reduce the number of insects that could damage crops.

Very little is known about the genetics of *D. ega*. A karyotype study from 1967 indicates that *D. ega* has a diploid number of 28 and a fundamental number of 46 (Baker and Patton, 1967). While this is similar to the rest of the lasiurine bats, the X chromosome of *D. ega* differs in being subtelocentric with a heterochromatic short arm (Bickham, 1987). Kurta and Lehr (1995) mentioned that *Lasiurus ega* belonged to the subgenus *Dasypterus,* however it continued to be referred to as *Lasiurus ega* until a study by Baird et al. (2015). *Dasypterus ega*, *D. intermedius*, and *D. xanthinus* are all yellow bats that can be found in Texas (Ammerman et al., 2012). All three species have several morphological characteristics that indicate they belong outside the subgenus *Lasiurus* including: lateral wings on the presternum that are broader than their body, a better developed sagittal crest, and a single upper premolar (Kurta and Lehr, 1995). *Dasypterus ega* is considerably smaller than its relative *D. intermedius* but is similar in size to *D. xanthinus.*

A systematic study was performed by Morales and Bickham (1995) that gathered genetic data of ribosomal RNA from the lasiurine bats, including *D. ega*. *Dasypterus ega* and *D. intermedius* were shown to have a closer relationship than the other yellow bats (Morales and Bickham, 1995). Morales and Bickham (1995) also mention that the yellow bats appear

to be separate from the red and hoary bats; however, no definitive conclusion could be made. The yellow bats displayed high divergence values in comparison to the red and hoary bats, indicating that the yellow bats may be older than the other bats in the lasiurine group (Morales and Bickham, 1995).

A study by Baird et al. (2015) indicated that there should be three separate genera of tree bats that include: *Lasiurus* (red bats), *Dasypterus* (yellow bats), and *Aeorestes* (hoary bats). This placed *Lasiurus ega* in a separate genus, *Dasypterus* (Baird et al., 2015). Their study examined 13 lasiurine species, by analyzing four loci from mitochondrial and Y-chromosomal DNA. Whether the different lineages should be recognized as different genera has been historically debated (Baird et al., 2015). Of the four loci examined, three (*ND1, Cytb,* and *DBY*) indicated that red and hoary bats group together as sister taxa, while the yellow bats are in a separate lineage. The remaining locus, *ND2,* weakly supported that hoary bats and yellow bats are sister taxa (Baird et al., 2015). It was also noted that there was a great degree of divergence between the North and South American lineages of *D. ega* (Baird et al., 2015). It is known that there is a larger population of *D. ega* in South America as opposed to the North American populations and that Texas is the far northern extent of the range for this species (Kurta and Lehr, 1995).

A study by Razgour et al. (2013) highlighted how populations tend to have lower genetic diversity on the edge of their range. This is an especially important aspect when considering climate change on a global scale. These edge populations are necessary for range shift and the spread of genetic diversity, as well as responses due to climate change (Razgour et al., 2013). Further, it is estimated that every 10 years there is a population decline of approximately 25% in threatened vertebrate species (Li et al., 2016). This trend began in the

19th century and has continued due to loss of biodiversity, industrialism, and the change in ecosystems globally (Li et al., 2016). The loss of populations can have an impact on overall genetic diversity of a species by reducing the number of alleles within the overall population. *Dasypterus ega* in Texas is located on the edge of the current distribution and is considered threatened by the state. These factors suggest that these populations might have low genetic diversity.

Heterozygosity can be calculated to estimate the amount of alleles within a population, which can give an indication of the genetic diversity. If the population size is small, inbreeding could occur within the population because of the small number of available mates. This process could lead to the loss of alleles. Heterozygosity can be used to determine if alleles in the population are at a normal and healthy level. This is an important aspect of conservation because a genetic bottleneck can have detrimental effects on a species as a whole. A loss of genetic diversity reduces the adaptive capacity of a population (Markert et al., 2010). The long-term viability of the population will decline with the loss of genetic diversity (Willoughby et al., 2015). These individuals will eventually be unable to continue adapting to the changing environment.

Microsatellite markers are non-coding sequences of DNA and are often used to provide estimates of population genetic diversity (Willoughby et al., 2015). Microsatellite alleles can be used to determine heterozygosity, which can be compared with other bat species of different conservation statuses (i.e. “threatened”, “endangered”) to give a better understanding of *D. ega*. The level of genetic diversity of a common, widespread species, the Eastern red bat (*L. borealis*), has been examined using microsatellites. The level of genetic diversity in Southern yellow bats is currently unknown despite its “threatened” status in the

state of Texas. The phylogenetic relationship of *D. ega* with other *Lasiurus* species is close (Baird et al., 2015), and I expected the microsatellite loci used for red bats to also work for Southern yellow bats. Cross-species amplification has been shown to work in the past with many organisms, including studies of African cichlid fish (Bezault et al., 2012) and Eastern spotted skunks (Shaffer, 2017). These studies showed that microsatellite primers can be used with closely related individuals to amplify the same loci from different species. Cross-species amplification is an important aspect pertaining to microsatellites and future studies that intend to test genetic diversity.

*Dasypterus ega* is a rare and understudied species that can potentially play a large role in the ecosystems it inhabits. Very little genetic work has been done on *D. ega*; the purpose of this project was to analyze microsatellite lengths of *D. ega* in order to obtain the genetic data for determining the genetic diversity within *D. ega.* I used the microsatellite data to determine the heterozygosity within the species. The heterozygosity of *D. ega* was then compared to the widespread, closely-related species *L. borealis*, along with other species of "endangered" and "least concern" bats. This allowed for a comparative method that highlights the different heterozygosity values between different species and populations.

## MATERIALS AND METHODS

*Sampling design* – I collected tissues (liver, muscle, heart, or kidney) from individuals of *D. ega* that were received from the Texas Department of State Health Services (DSHS) and prepared the bats as voucher specimens for the Angelo State Natural History Collections (ASNHC). The vouchered individuals, along with individuals previously belonging to the ASNHC, were loaned for this project. I was loaned 75 individuals from 15 Texas counties (Table 1). Most specimens were received by the DSHS for rabies testing and, other than county of collections, no specific locality information was available (Figure 1). An additional 19 individuals from Cameron County were represented by biopsied wing punches that were collected by Citlally Jimenez during her thesis work (Jimenez, 2016) on the roosting ecology of yellow bats in southern Texas.

*PCR amplification and primers* – DNA was extracted from its respective tissue using a QIAGEN DNeasy Blood & Tissue kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol, except, the elution of DNA was done into two elutions of 50 µl of AE buffer each. All DNA was quantified on a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). I identified primers from 10 microsatellite loci that were developed for studies working with *Corynorhinus townsendii, C. rafinesquii*, *Lasiurus borealis*, and *Plecotus auritus* (Table 2; Burland et al., 1998; Piaggio et al., 2008, 2009; Korstian et al., 2014; Vonhof and Russell, 2015), all which were shown to work with the *L. borealis* genome. These bats all belong to the same family, Vespertilionidae (Burland et al., 1998; Ammerman et al., 2012). Polymerase chain reaction (PCR) was performed in 12.5 µL reactions using QIAGEN's Type-it Microsatellite PCR Kit (QIAGEN Inc., Valencia, CA) that contained a master mixture of DNA polymerase, $MgCl_2$, and dNTPs. Each reaction

**Table 1.-**Vouchered specimens of *Dasypterus ega* examined in this study including the tissue and catalog number from each facility, a Department of State Health Services (DSHS) identification number if applicable, the tissue type used for DNA extraction, and collection information for each individual. Museum collection acronyms are as follows: ASNHC or ASK (Angelo State Natural History Collections), TK or TTU (Museum of Texas Tech University Genetic Resources Collection). Tissue abbreviations: H (heart), HK (heart or kidney), WP (wing punch from Citlally Jimenez), L (liver), S (skin), M (muscle), N/A (not available).

| Tissue Number | Catalog Number | DSHS Number | Tissue | TX County | Date Collected |
|---|---|---|---|---|---|
| TK171041 | TTU 113429 | 10VR4534 | HK | Aransas | 7-Jul-10 |
| ASK11627 | ASNHC 17404 | 14VR-0827 | H | Bandera | 12-Feb-14 |
| TK173067 | TTU 114567 | 11VR-3693 | H | Caldwell | 9-Jun-11 |
| TK171215 | TTU 113603 | 08VR-5691 | HK | Cameron | 26-Jul-08 |
| TK173083 | TTU 114583 | 11VR-4693 | H | Cameron | 30-Jun-11 |
| ASK9498 | ASNHC 16081 | 13VR-0563 | H | Cameron | 30-Jan-13 |
| ASK12714 | ASNHC 18214 | 14VR-8079 | H | Cameron | 24-Oct-14 |
| ASK13010 | N/A | N/A | WP | Cameron | 3-Jun-15 |
| ASK13011 | N/A | N/A | WP | Cameron | 3-Jun-15 |
| ASK13012 | N/A | N/A | WP | Cameron | 3-Jun-15 |
| ASK13013 | N/A | N/A | WP | Cameron | 3-Jun-15 |
| ASK13014 | N/A | N/A | WP | Cameron | 3-Jun-15 |
| ASK13015 | N/A | N/A | WP | Cameron | 3-Jun-15 |
| ASK13016 | N/A | N/A | WP | Cameron | 3-Jun-15 |
| ASK13018 | N/A | N/A | WP | Cameron | 4-Jun-15 |
| ASK13019 | N/A | N/A | WP | Cameron | 4-Jun-15 |
| ASK13020 | N/A | N/A | WP | Cameron | 4-Jun-15 |
| ASK13021 | N/A | N/A | WP | Cameron | 4-Jun-15 |
| ASK13022 | N/A | N/A | WP | Cameron | 4-Jun-15 |
| ASK13024 | N/A | N/A | WP | Cameron | 5-Jun-15 |
| ASK13026 | N/A | N/A | WP | Cameron | 25-Jul-15 |
| ASK13027 | N/A | N/A | WP | Cameron | 25-Jul-15 |
| ASK13029 | N/A | N/A | WP | Cameron | 28-Jul-15 |

**Table 1.**-Continued

| Tissue Number | Catalog Number | DSHS Number | Tissue | TX County | Date Collected |
|---|---|---|---|---|---|
| ASK10857 | ASNHC 17703 | N/A | H | Cameron | 28-Jul-15 |
| ASK13030 | N/A | N/A | WP | Cameron | 4-Sep-15 |
| ASK13031 | N/A | N/A | WP | Cameron | 4-Sep-15 |
| ASK13033 | N/A | N/A | WP | Cameron | 20-Nov-15 |
| ASK12715 | ASNHC 18215 | 17VR-4058 | HK | Cameron | 20-Jun-17 |
| ASK12718 | ASNHC 18218 | 17VR-4490 | L | Cameron | 6-Jul-17 |
| ASK9381 | ASNHC 15072 | 11VR-8411 | H | Comal | 1-Dec-11 |
| TK171121 | TTU 113509 | 09VR-7858 | H | Fayette | 17-Nov-09 |
| ASK11626 | ASNHC 17403 | 14VR-1336 | H | Hays | 5-Mar-14 |
| TK171204 | TTU 113592 | 08VR2880 | HK | Hidalgo | 3-Apr-08 |
| TK171206 | TTU 113594 | 08VR3199 | HK | Hidalgo | 4-May-08 |
| TK171044 | TTU 113432 | 09VR3861 | HK | Hidalgo | 9-Jun-09 |
| TK171049 | TTU 113437 | 09VR3864 | HK | Hidalgo | 9-Jul-09 |
| TK171045 | TTU 113433 | 10VR4595 | HK | Hidalgo | 8-Jul-10 |
| TK171046 | TTU 113434 | 10VR4375 | HK | Hidalgo | 30-Jun-10 |
| TK171048 | TTU 113436 | 10VR0908 | HK | Hidalgo | 18-Feb-10 |
| ASK10534 | ASNHC 15867 | 12VR-7823 | H | Hidalgo | 10-Dec-12 |
| ASK12702 | ASNHC 18202 | 14VR-5072 | L | Hidalgo | 2-Jul-14 |
| ASK12713 | ASNHC 18213 | 14VR-5303 | H | Hidalgo | 12-Jul-14 |
| ASK12711 | ASNHC 18211 | 16VR-0813 | L | Hidalgo | 17-Feb-16 |
| ASK12719 | ASNHC 18219 | 16VR-0701 | M | Hidalgo | 2-Nov-16 |
| TK171208 | TTU 113596 | 08VR5264 | HK | Kleberg | 12-Jul-08 |
| TK171160 | TTU 113548 | 08VR1629 | HK | Nueces | 18-Mar-08 |
| TK171040 | TTU 113428 | 08VR4759 | HK | Nueces | 26-Jun-08 |
| TK171042 | TTU 113430 | 08VR6354 | HK | Nueces | 22-Aug-08 |
| TK171159 | TTU 113547 | 08VR7107 | HK | Nueces | 20-Sep-08 |
| TK171043 | TTU 113431 | 10VR0218 | HK | Nueces | 14-Jan-10 |

**Table 1.**-Continued

| Tissue Number | Catalog Number | DSHS Number | Tissue | TX County | Date Collected |
|---|---|---|---|---|---|
| TK171038 | TTU 113426 | 10VR0715 | HK | Nueces | 9-Feb-10 |
| TK173072 | TTU 114572 | 11VR3872 | L | Nueces | 6/14/2011 |
| ASK11949 | N/A | N/A | H | Nueces | 29-Jun-11 |
| ASK11945 | N/A | N/A | H | Nueces | 3-Jul-11 |
| ASK12716 | ASNHC 18216 | 13VR-4518 | L | Nueces | 25-Jun-13 |
| ASK12706 | ASNHC 18206 | 13VR-5043 | L | Nueces | 11-Jul-13 |
| ASK12705 | ASNHC 18205 | 13VR-8722 | HK | Nueces | 3-Dec-13 |
| ASK12698 | ASNHC 18198 | 14VR-4965 | L | Nueces | 1-Jul-14 |
| ASK12699 | ASNHC 18199 | 14VR-8849 | L | Nueces | 30-Nov-14 |
| ASK12700 | ASNHC 18200 | 14VR-9134 | L | Nueces | 16-Dec-14 |
| ASK12701 | ASNHC 18201 | 16VR-0115 | L | Nueces | 8-Jan-16 |
| ASK12709 | ASNHC 18209 | 16VR-0818 | L | Nueces | 17-Feb-16 |
| ASK12712 | ASNHC 18212 | 16VR-7666 | HK | Nueces | 8-Nov-16 |
| ASK12710 | ASNHC 18210 | 17VR-0998 | H | Nueces | 23-Feb-17 |
| ASK12697 | ASNHC 18197 | 13VR-7883 | M | San Patricio | 24-Oct-13 |
| TK171187 | TTU 113575 | 08VR-5225 | H | Starr | 11-Jul-08 |
| ASK10521 | ASNHC 15835 | 12VR-4990 | H | Victoria | 29-Jun-12 |
| TK171495 | TTU 113610 | 08VR-5160 | S | Webb | 9-Jul-08 |
| TK171037 | TTU 113425 | 10VR-1605 | H | Webb | 18-Mar-10 |
| TK171020 | TTU 113408 | 10VR-1711 | H | Webb | 23-Mar-10 |
| ASK10535 | ASNHC 15868 | 12VR-4486 | H | Webb | 29-Jun-12 |
| ASK12703 | ASNHC 18203 | 13VR-4619 | M | Webb | 27-Jun-13 |
| ASK12708 | ASNHC 18208 | 16VR-0364 | L | Webb | 23-Jan-16 |
| ASK12717 | ASNHC 18217 | 17VR-0676 | L | Webb | 8-Feb-17 |
| TK171050 | TTU 113438 | 08VR8886 | HK | Willacy | 11-Dec-08 |

**Figure 1.-**A map of the counties of Texas that show the distributions of *Dasypterus ega* bats used in this study. The number of bats used from each county is as follows: Aransas – 1, Bandera – 1, Caldwell – 1, Cameron – 26, Comal – 1, Fayette – 1, Hays – 1, Hidalgo – 12, Kleberg – 1, Nueces – 19, San Patricio – 1, Starr – 1, Victoria – 1, Webb – 7, and Willacy – 1.

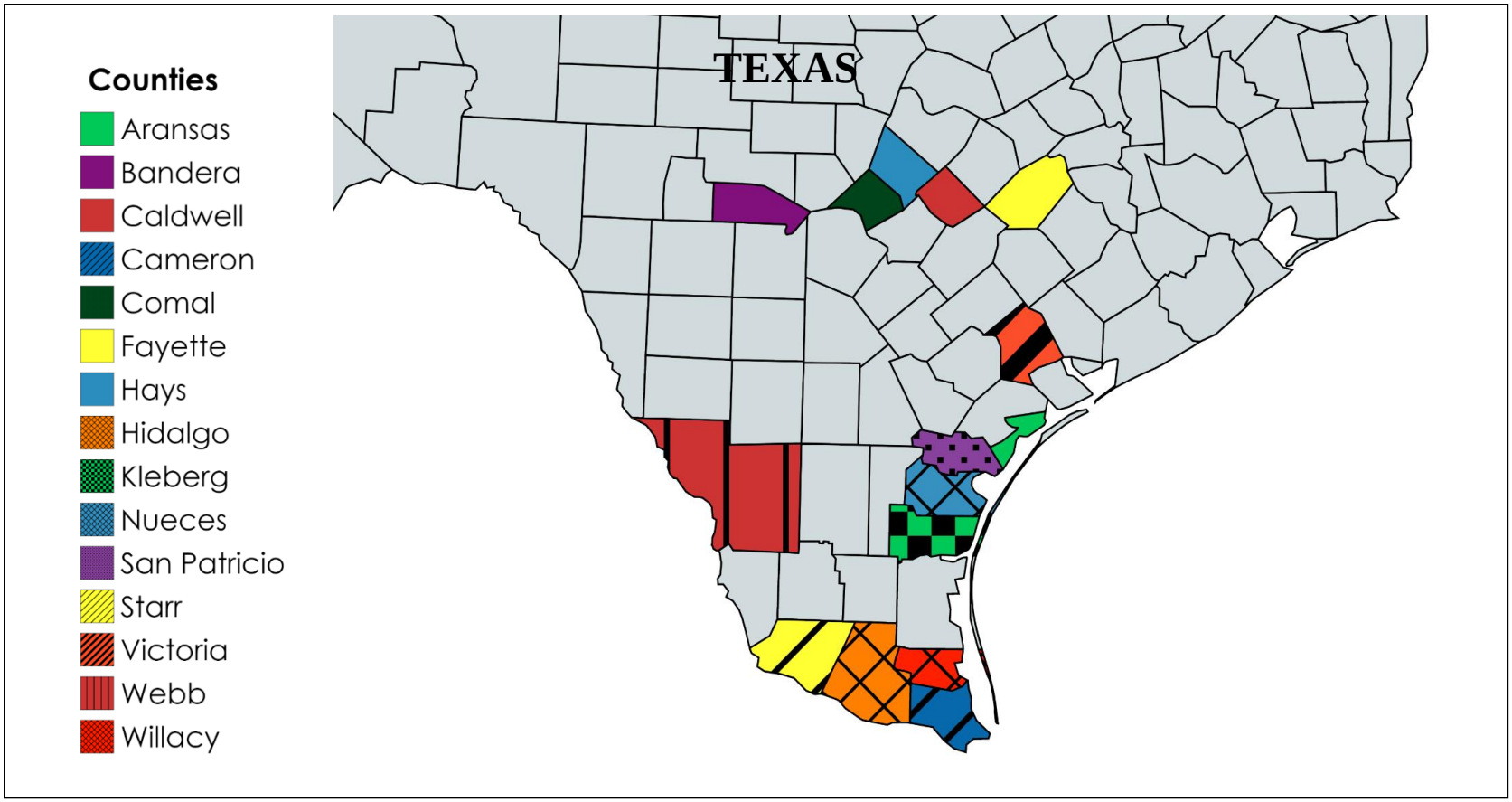

**Table 2.**-Microsatellite loci optimized for the analysis of *Dasypterus ega*. Included are locus name (* indicates loci that were dropped because I was unable to optimize them), forward and reverse primer sequence, allele size range (bp), PCR annealing temperature ($T_A$, °C), Fluorescent WellRed Dye-Label (Black=D2 dye, Green=D3 dye, Blue=D4 dye), and the publication of each primer reported.

| Locus | Primer Sequence - 5'-3' | DNA size range | $T_A$ | Dye Label | Reference |
|---|---|---|---|---|---|
| CoraF11 | F: AAGCTCAGAGACTGCTCCTTC<br>R: ATCCATTATGTTTGCTGATGTTC | 159-193 | 60 | Green | Piaggio et al. (2009) |
| CotoG12 | F: TGCAAGTCTTAACTCACCTCATT<br>R: CCACTCCCCTAGTTTTCATCTAC | 218-250 | 60 | Black | Piaggio et al. (2008) |
| LbG* | F: CTGGGATCACATGGGGAACT<br>R: ATGTGGACTCAGCTCACACA | 196-258 | 60 | Blue | Korstian et al. (2014) |
| LbK* | F: TCTCTCTCCTACCCCTTCCT<br>R: ACTGGCCTCTGGAATTGTGA | 215-259 | 60 | Blue | Korstian et al. (2014) |
| LbT | F: TCCTCTGTCTGGGCACATAC<br>R: TCGCAGATTCCCAAGGATCC | 201-243 | 60 | Blue | Korstian et al. (2014) |
| LcO* | F: GAGGTCCTGTTTGTGCCAAG<br>R: CAGGTCCGCGGTTAATTACG | 195-231 | 61 | Black | Korstian et al. (2014) |
| PrLb02* | F: AGATGAAAAGCGCGTGTTGT<br>R: GGCCCATGCTCATCATCTA | 79-121 | 61 | Green | Korstian et al. (2014) |
| D226 | F: ATCCCAGTTCAAGCAGAGTATG<br>R: ATTCAGGGCTCTGCATTTTAG | 124-184 | 54 | Green | Vonhof and Russell (2015) |
| D200 | F: TGCACAGATGTTTTAAGGTTTG<br>R: TCATGAAATTTGGTTGTCCAC | 142-236 | 54 | Black | Vonhof and Russell (2015) |
| Paur03 | F: CTGGAGTGTTGTTTTGCCCTTC<br>R: GCTGATGGTGGAGTCTCCTTTTTC | 224-264 | 55 | Blue | Burland et al. (1998) |

contained 6.25 µL of master mix, 0.625 µL of both forward and reverse primers at 10µM each, 20-100 ng of DNA, and the remainder of RNase free water. The PCR amplifications and thermal profiles were modified during the project, by either changing annealing temperature or adjusting DNA concentration in order to obtain the best PCR product (Table 3). To verify the PCR products, subsamples of DNA underwent PCR and were analyzed via electrophoresis gel. Each gel was 1.5% sodium borate and was run for 30 min at 120V. The optimized conditions were run with all individuals with WellRed Dye-labeled forward (Sigma-Aldrich Corp., St. Louis, MO) and unlabeled reverse (Alpha DNA, Montreal, Quebec) primers (Table 2).

*Genotyping procedures* – Dye-labeled PCR products were genotyped on a capillary electrophoresis genetic analysis system (CEQ™8000, Beckman-Coulter Inc., Brea, CA) using a 400 bp GenomeLab DNA Size Standard Kit (AB Sciex, Concord, Ontario) as the size standard. Genotypes were scored by eye following sequencing.

*Analysis methods* – Micro-Checker v 2.2.3 was used to check for large-allele dropout, null alleles, and errors due to stutter (van Oosterhout et al., 2004). GenAlEx v 6.503 (Peakall and Smouse, 2006, 2012) was used to check levels of genetic diversity including alleles per locus ($N_a$), expected heterozygosity ($H_e$), and observed heterozygosity ($H_o$). Heterozygosity was calculated separately for four counties that had more than one bat from that county in this study. Cameron, Hidalgo, Nueces, and Webb Counties were calculated for heterozygosity, excluding Paur03, to determine if there was a difference in heterozygosity within each county.

**Table 3.-**Polymerase chain reaction thermal profiles utilized at each microsatellite locus for *Dasypterus ega*.

| Thermal profile | Loci |
|---|---|
| 95˚C for 5 min, followed by 35 cycles of 94˚C for 30s, $T_A$ for 1.5min, and 72˚C for 1.5min, with a final extension at 60˚C for 30min | CoraF11, CotoG12, LbT, D226, D200, LbG, LbK, LcO, PrLb02 |
| 95˚C for 5 min, followed by 35 cycles of 94˚C for 30s, 55˚C for 1.5min, and 72˚C for 1.5min, with a final extension at 72˚C for 10min | Paur03 |

## RESULTS

*Optimization* – Loci LbG, LbK, LcO, and PrLb02 were excluded from this study because I was unable to optimize these loci for *D. ega*. LcO and PrLb02 were multiplexed in the same PCR and were tried at annealing temperatures (˚C) of 56, 59, 60, 61, and 62. These two loci never amplified and never exhibited any banding at any of the different annealing temperatures. LbG was run with annealing temperatures (˚C) of 59, 60, 61, 62, and 63. Annealing temperatures 59-61˚C yielded smeared and stuttering bands, while annealing temperatures 62 and 63˚C did not exhibit any banding. LbK was run with an annealing temperature of 60˚C that yielded stuttering, while annealing temperature 61˚C exhibited no banding. I was successful in the optimization of CoraF11, CotoG12, LbT, D226, D200, and Paur03. The loci that I was able to optimize were amplified for all 75 individuals; excluding five missing data points located at locus D226 for individuals: TK171041, TK173083, ASK10857, and TK171206 and at locus CotoG12 for the individual ASK12713. The percentage of missing genotype data at locus D226 was 5.3% and the missing data at locus CotoG12 was 1.3%, calculated by the number of individuals missing data at each locus divided by the total number of individuals. The overall percentage of missing data for all 6 loci was 1.1%.

*Null alleles* - Loci CotoG12, LbT, D226, D200, and Paur03 registered as having null alleles from Micro-Checker, while locus CoraF11 was the only locus that did not register as having null alleles. Hardy-Weinberg equilibrium (HWE) was calculated and loci CotoG12, LbT, D226, D200, and Paur03 were not in HWE while locus CoraF11 was the only locus in HWE.

*Genetic variation* – Heterozygosity values were calculated for *D. ega* at each locus as well as overall heterozygosity values (Table 4). The values were compared to other bats including *Lasiurus borealis, Aeorestes cinereus, Pipistrellus pipistrellus,* and *P. pygmaeus* (Table 5). The heterozygosity value for *D. ega* was 0.321 with a standard error of 0.083. The individual observed heterozygosity values per locus ranged from 0 - 0.493. CoraF11 and D226 each had the highest heterozygosity levels at 0.493 while Paur03 had the lowest at 0. Because Paur03 is a locus suspected of being X-linked (Burland et al., 1998) and would be expected to lower the heterozygosity calculation, it was removed. The calculation of average observed heterozygosity without this locus was 0.385 with a standard error of 0.064. Cameron, Hidalgo, Nueces, and Webb counties had heterozygosity calculated for each county. Cameron county (n=26) had a heterozygosity of 0.382, Hidalgo (n=12) had a heterozygosity of 0.400, Nueces (n=19) had a heterozygosity of 0.337, and Webb (n=7) had a heterozygosity of 0.429.

*Dasypterus ega* had a lower heterozygosity than any of the other bats being compared, with the closest heterozygosity value of 0.586, found in *C. townsendii ingens* (Lee et al., 2015). In addition, the number of alleles per locus ranged from 3 - 11 for *D. ega*, with an average of 8.4 alleles per locus (Table 4). *Lasiurus borealis* had a heterozygosity of 0.787 and *Aeorestes cinereus* had the highest heterozygosity of 0.825 (Korstian et al., 2015). *Eidolon helvum* had a heterozygosity of 0.643 which was not that different from the 0.665 value of *P. pygmaeus* (Peel et al., 2010; Sztencel-Jabłonka and Bogdanowicz, 2012). *Pipistrellus pipistrellus* had a heterozygosity value 0.709 which was intermediate compared to the values of the least concerned species (Sztencel-Jabłonka and Bogdanowicz, 2012).

**Table 4.-**Genetic diversity values of *Dasypterus ega* across 6 microsatellite loci. $N_{gen}$ is number of genotyped individuals, $N_a$ is number of alleles at each locus, $H_o$ is observed heterozygosity, and $H_e$ is expected heterozygosity.

| Locus | $N_{gen}$ | $N_a$ | $H_o$ | $H_e$ |
|---|---|---|---|---|
| CoraF11 | 75 | 3 | 0.493 | 0.502 |
| CotoG12 | 74 | 11 | 0.473 | 0.807 |
| LbT | 75 | 10 | 0.187 | 0.610 |
| D226 | 71 | 9 | 0.493 | 0.663 |
| D200 | 75 | 9 | 0.280 | 0.585 |
| Paur03 | 75 | 3 | 0.000 | 0.052 |
| Overall w/ Paur03 | 74 | 7.5 ± 1.455 | 0.321 ± 0.083 | 0.537 ± 0.105 |
| Overall w/o Paur03 | 74 | 8.4 ± 1.400 | 0.385 ± 0.064 | 0.633 ± 0.051 |

**Table 5.-**Genetic diversity values of various bat species. $N_{gen}$ is number of genotyped individuals, $L_{ana}$ is the number of loci analyzed, $N_a$ is number of alleles at each locus, $H_o$ is observed heterozygosity, and $H_e$ is expected heterozygosity. This study calculated heterozygosity without the Paur03 locus. IUCN Status indicates the conservation status of each species (T=Threatened, LC=Least Concern, NT=Near Threatened, E=Endangered).

| Species | $N_{gen}$ | $L_{ana}$ | $N_a$ | $H_o$ | $H_e$ | IUCN Status | Citation |
|---|---|---|---|---|---|---|---|
| *Dasypterus ega* | 74 | 5 | 3-11 | 0.385 | 0.633 | T | This study |
| *Lasiurus borealis* | 400 | 6 | 6-23 | 0.787 | 0.796 | LC | Korstian et al. (2015) |
| *Aeorestes cinereus* | 212 | 6 | 7-46 | 0.825 | 0.844 | LC | Korstian et al. (2015) |
| *Pipistrellus pipistrellus* | 183 | 8 | - | 0.709 | 0.860 | LC | Sztencel-Jabłonka and Bogdanowicz (2012) |
| *Pipistrellus pygmaeus* | 170 | 8 | - | 0.665 | 0.847 | LC | Sztencel-Jabłonka and Bogdanowicz (2012) |
| *Eidolon helvum* | 135 | 20 | 2-46 | 0.643 | 0.769 | NT | Peel et al. (2010) |
| *Corynorhinus townsendii ingens* | 18 | 15 | - | 0.586 | 0.577 | E | Lee et al. (2015) |

# DISCUSSION

The objective of this study was to evaluate the genetic diversity of *D. ega,* a species designated as "threatened" in the state of Texas, through the use of microsatellites. Low heterozygosity values in *D. ega* indicated low genetic diversity within the region of southern Texas where samples were obtained. It has been shown that threatened populations have reduced heterozygosity and allelic richness, and this reduced genetic diversity can affect the adaptability and long-term sustainability of a species (Willoughby et al., 2015). A low heterozygosity can be caused by many factors, whether it be a genetic bottleneck, genetic drift, or being a leading-edge population.

When the genetic variation values of *D. ega* are compared to other non-threatened bat species, it is evident that *D. ega* has a low genetic diversity in the Texas population. *Dasypterus ega* has a much lower heterozygosity value than *L. borealis* or *A. cinereus*. A study performed by Sztencel-Jabłonka and Bogdanowicz (2012) reported heterozygosity values of two nonthreatened *Pipistrellus* bats from central Europe. These *Pipistrellus* bats had a higher heterozygosity value than *D. ega.* Heterozygosity has been reported for a near threatened bat, *Eidolon helvum*; this provides data for a species known to have lost genetic diversity (Peel et al., 2010). Even though this bat is near threatened, it has a much higher heterozygosity value than *D. ega.* It is not unexpected that the endangered *Corynorhinus townsendii ingens* has a lower heterozygosity (Lee et al., 2015) than the species designated as "least concern". The heterozygosity differences between the non-endangered bats and *D. ega* is staggering. The heterozygosity value of *D. ega* is 0.385 while the next lowest heterozygosity value was the endangered *C. townsendii ingens* with a heterozygosity value of

0.586 (Table 5). The highest heterozygosity value was 0.825 for *A. cinereus* (Table 5). These comparisons highlight the low genetic diversity within the Texas populations of *D. ega*.

It is of course, possible that this comparison is not valid. The sample size for this study includes 75 *D. ega* which is substantially lower than the sample size of 400 *L. borealis* (Table 5). I also had a lower number of tested loci than I was hoping for because of the loci that I could not optimize. This left me with five loci that were tested, which is less than the number of loci tested in the other studies used for comparison (Table 5). Additionally, without primers designed for *D. ega*, it is uncertain whether the primers worked to their maximum potential. Furthermore, this study did not include individuals from Central and South America and therefore might not be representative of the entire range of the species.

Most of the *D. ega* samples used in this study were collected near the Gulf of Mexico (Corpus Christi, Nueces County) and the Rio Grande River (Brownsville, Cameron County) around urbanized areas. Some bats also were collected in the San Antonio and Austin areas. Most of the bats were submitted to the DSHS for rabies testing by the general public, so it is expected that the majority of bats were from populated urban areas. The bats are likely distributed in other counties throughout southern Texas, however they might not have been encountered as often in the rural areas. My samples were from the northernmost edge of the *D. ega* range which could cause the low genetic diversity values. The bat species *Plecotus austriacus* showed reduced genetic diversity on the edge of their range (Razgour et al., 2013). Similarly, a study on pearly heath butterflies (*Coenonympha arcania*) by Besold et al. (2008) showed a reduction in genetic diversity in peripheral populations, while the central populations showed no genetic differentiation as well as high genetic diversity. These studies

suggest that the majority of the genetic diversity for *D. ega* might be in Central and South America (Kurta and Lehr, 1995).

Leading-edge populations are important for species in order to increase the range that a species inhabits, especially with the changing climate. Climate change is exceeding the rate that species are able to adapt or shift their ranges (Razgour et al., 2013) making leading-edge populations all the more important. With this rapid climate change, three population-level responses are possible: range migration, adaptation, or death (Gibson et al., 2009). The Texas population of *D. ega* is important for the gradual range shift that occurs with species. Without this important population, much of the entire species could be at risk down the line due to the changing climate as the other *D. ega* populations may be unable to adapt. The importance of conservation strategies for leading-edge populations with a low genetic diversity are demonstrated by the data collected for *D. ega* in this study.

This study included primers for microsatellite loci that were developed for red bats or other closely related bat species belonging to the family Vespertilionidae (Burland et al., 1998; Ammerman et al., 2012). Six of the primer sets worked reliably in *D. ega* once optimal conditions were identified. However, several primer sets were excluded from this study including LbG, LbK, LcO, and PrLb02. These primers were developed specifically for *Lasiurus borealis* (Korstian et al., 2014, 2015). I chose the primers for this project because they were shown to work with several red bat studies, but they did not amplify in *D. ega*. It was surprising that I was unable to optimize LbG, LbK, LcO, and PrLb02 because they were designed for *L. borealis*. I expected these primers to amplify more clearly because of the close relationship between *D. ega* and *L. borealis*. It is possible that these primers did not

amplify because *D. ega* has a different genetic sequence at that specific locus. A different genetic sequence would not allow the primer to bind properly to the DNA, which would not allow for the amplification at that locus. The three major steps in PCR are the denaturing, annealing, and extension phases (Delidow et al., 1993). If the primer binding site has a different genetic sequence, the annealing phase is disrupted, and no DNA amplification will occur. It is likely that *D. ega* has a similar genetic sequence as *L. borealis*, indicated by the successful amplification at multiple loci, but that there are minor differences that separate the species at different loci. It is interesting that the CoraF11 and CotoG12 loci were able to be amplified so clearly. These 2 primers were developed for a genus, *Corynorhinus*, that is not as closely related to *D. ega* as the red bats (Roehrs et al., 2010). The sequence of the regions flanking these microsatellite loci must be highly conserved to work in both genera of bats. I would have expected the *L. borealis* primers to work before the primers optimized for a different genus, simply because of the relatedness of species. For those loci that did amplify, I did, however, find that five of the six loci potentially were affected by null alleles.

A null allele is any allele at a microsatellite locus that consistently fails to amplify to detectable levels via the polymerase chain reaction. Changes in the nucleotide sequences in the flanking regions may prevent primer annealing, causing a null allele (Chapuis and Estoup, 2006). The large number of null alleles that I found could be indicative of the primers not working with specific alleles of *D. ega*. While there were clear microsatellite peaks, it is possible that for some alleles there were base substitutions or insertions/deletions that prevented either the forward or reverse primer from binding. If this was the case, then any individuals homozygous for the affected allele would have failed to amplify and no

peaks would be detected. I observed at least one peak at every locus in this study, except five missing data points located at locus D226 for individuals: TK171041, TK173083, ASK10857, and TK171206 and at locus CotoG12 for the individual ASK12713. It is possible that these individuals were homozygous with an allele that failed to amplify. It is also possible for other individuals that one allele had a mutation not allowing for amplification, which could have only showed one allele at the locus (appearing homozygous). When null alleles are present, it is possible that the individual is truly a heterozygote at that locus, but the individual appears to be homozygous at that locus (Rico et al., 2017), causing excess homozygotes. There was an excess of homozygote genotypes present in this study, however that is not necessarily indicative of null alleles. It is possible that the genotypes of *D. ega* are largely homozygous due to founder effects, bottlenecks, and/or genetic drift. The threatened status of *D. ega,* coupled with the fact that they are an edge population could indicate a true excess of homozygotes due to the tendency toward lower diversity at the edges of species distributions (Razgour et al., 2013). A true excess of homozygotes could give the impression of null alleles and it is difficult to distinguish between these two possibilities without more data. Both scenarios are possible; the loci that I analyzed were truly affected by null alleles or the excess of homozygote genotypes is real and *D. ega* has low genetic diversity in this region of Texas. Including more loci or sampling individuals from the center of the distribution could provide information to determine which scenario is correct.

It is also possible that Southern yellow bats exhibit low genetic diversity across their entire range. Genetic conservation is important in order to maintain healthy populations that are capable of adapting to their environment. Without species conservation efforts, many

species that we know today would not be present in captivity or in the wild. When population sizes decrease, heterozygosity typically declines as well (Nei et al., 1975). When a population is reduced and then the population increases, it is considered a bottleneck. The bottleneck effect can have severe implications for populations. When a bottleneck occurs, many low frequency alleles are eliminated (Nei et al., 1975) which lowers the genetic diversity. A classic example of a genetic bottleneck is the African cheetah (*Acinonyx jubatus jubatus*).

The African cheetah was once widely distributed across Africa. Through a mixture of loss of habitat, human hunting, and the changing ecosystem the cheetah population became extremely low (Menotti-Raymond and O'Brien, 1993). In the early 1980s a genetic analysis was performed on cheetahs because of the difficulty of breeding them in captivity. The analysis showed that the genetic diversity levels of cheetahs was comparable to deliberately inbred lab mice and livestock (Menotti-Raymond and O'Brien, 1993). This showed a 90-99% allele variation reduction which led to a variety of impairments including high infant mortality, increased sensitivity to disease agents, and decreased fecundity. This reduction of genetic diversity was caused by a genetic bottleneck in recent evolutionary history (O'Brien et al., 1985). Population conservation continues today for this species and it remains listed as vulnerable on the IUCN Red List (Dalton et al., 2013).

Protecting species before reaching such small population sizes is ideal. Species conservation along with zoo conservation efforts can help to rebuild a population. Many large mammals have already become extinct in the wild and only survive under managed breeding programs in wildlife preserves and zoological parks (O'Brien et al., 1985). An

example of successful species conservation efforts to increase genetic diversity within an endangered population is showcased by the Florida Panther (*Puma concolor coryi*). The Florida Panther is an endangered species and is the only puma species in the eastern United States (Johnson et al., 2010). In the 1990s the population was down to 20-25 adults due to habitat loss. These individuals had a clear loss of genetic variation as well as several morphological and biomedical abnormalities (Hostetler et al., 2012). In 1995, 8 female pumas were moved from a Texas population to increase genetic diversity and improve population numbers (Johnson et al., 2010). The population of Florida Panthers has increased since the introduction of the Texas individuals. It has been shown that the genetic restoration of the population contributed to the increase of the population size (Hostetler et al., 2012).

These examples of extreme genetic loss show the importance of genetic conservation. It is much healthier for a population to not experience the genetic loss in the first place. With strict conservation efforts, as seen with the Florida Panther, a population can grow to a healthy size with increased genetic diversity. However, alleles will likely be lost with a reduced population as seen with bottlenecks. A preventative method of conservation is more effective and healthier for populations. Protecting these populations before they get to drastically low population sizes is necessary to ensure genetic diversity.

This project on genetic diversity in Southern yellow bats was possible primarily through the samples obtained from the DSHS. They received bats that have been encountered by the general public (or their pets) and tested them for the rabies virus. *Dasypterus ega* seems to be fairly commonly encountered by the public when palms used in landscaping are being trimmed of dead fronds (Ammerman et al., 2012; Jimenez, 2016). Once the rabies test

is completed, the bats (primarily the rabies-negative bats) can be prepared as vouchers and be deposited in natural history collections for further research. Bats that have been received by the DSHS and placed into the Angelo State Natural History Collection have been important not only in genetic analyses, but also for refining our understanding of species distributions (Demere et al., 2012).

Rabies is caused by a virus and is found in mammals. It is an acute viral disease that affects the central nervous system of mammals and is transmitted through the bite of a rabies infected animal (Mayes et al., 2013). Between 2001-2010, 94% of laboratory confirmed rabies cases in Texas were from wildlife species, most commonly from skunks and bats (Mayes et al., 2013). There are about two to three human rabies cases in the U.S. per year (Mayes et al., 2013). Bats are examined by public health agencies frequently due to the association with rabies, and about 4-10% of bats submitted to laboratories test positive for rabies (Yancey et al., 1997). This number can be misleading due to the way these bats are submitted for testing. Most of these bats are brought into the health agencies by the general public. Typically, for the bat to be brought in, it is sick or dead near human activity (Yancey et al., 1997). This leads to a bias because healthy bats are usually not found in close proximity to humans and are not brought in for testing. Yancey et al. (1997) caught 171 bats from Big Bend Ranch State Park and all 171 bats tested negative for rabies. This indicates that the majority of wild bats do not have rabies. Rabies has a notable impact on healthcare but is more manageable now than it has been in the past. There are preexposure and postexposure vaccinations available; however, vaccines can be costly and can exceed $3,000 (Rabies, 2015).

Despite public health concerns, insectivorous bats have a large ecological role and a significant economic impact in agricultural settings. While it is not known specifically what *D. ega* eats, they most likely consume small, flying insects (Ammerman et al., 2012). Bats are among the most economically important non-domesticated mammals due to their crop pest suppression, as well as their role in pollination and seed dispersal (Boyles et al., 2013). It is estimated that bats in south-central Texas provide pest suppression services of about $74/acre, based on the cotton-dominated landscape (Boyles et al., 2011). It is shown that bats in the southern part of Texas play a tremendous role in the consumption of crop pests. As a result, less insecticides are needed because of bats acting as natural pest control.

There are several items of interest that could increase the knowledge of *D. ega*. More ecological information is needed to truly assess their role in the ecosystems they inhabit. The insects that these bats consume are currently unknown. It is nearly impossible to estimate what impacts *D. ega* has on the ecosystem without knowing which food webs these bats fit in. It is possible that *D. ega* has an impact on crop pests, and therefore helps to control these insect populations. Another aspect that would further benefit this molecular study is the development of primers specifically for microsatellite regions in *D. ega*. With the development of these primers, I could collect data to better understand heterozygosity levels of this bat, specifically pertaining to null alleles. I am currently unsure whether the excess homozygotes are due to the selection of microsatellite markers and use of cross-amplification of primers or whether there is a true homozygote excess within the population. Primers specifically for *D. ega* would help to validate the results of this study, as well as including *D.*

*ega* individuals from throughout their range instead of only sampling those from the northern edge.

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# VITA

Samuel James Harrison was born in Rapid City, South Dakota, on August 16, 1996. He attended Angelo State University for all four years of his undergraduate career. He chose ASU because of his interests in the science fields. He will be graduating with a Bachelor of Sciences in Biology with Highest University Honors with a minor in Chemistry. He presented research posters at various regional and national Honors and Biology conferences. While at ASU Sam was active in the Honors Student Association and served as a mentor for the Honors Program. He will attend UT Health San Antonio in fall 2018 to begin a Ph.D. program in the Integrated Biomedical Sciences.

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