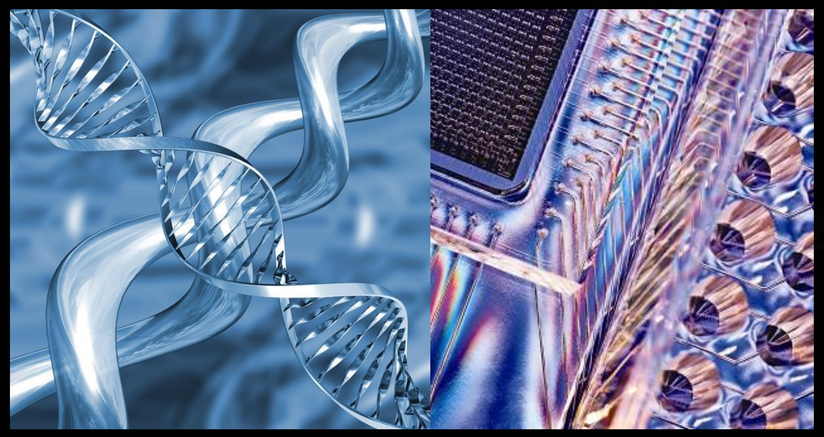
Spotted bat (*Euderma maculatum*) microsatellite marker discovery using Illumina paired-end genomic sequencing

Faith M. Walker[1,2], Jeffery T. Foster[2], Kevin P. Drees[2], and Carol L. Chambers[1]

[1]School of Forestry and [2]The Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ 86011

Background

High throughput sequencing technologies have recently been extended to non-model species lacking prior genomic information. We employed these technologies to discover microsatellite markers in the spotted bat (*Euderma maculatum*), a widely-distributed species (Figure 1) that is charismatic yet poorly understood. Because spotted bats are cryptic (nocturnal and roost solitarily), much of their biology is unknown, making them excellent candidates for elucidation of aspects of population biology and natural history via genetic tools. To this end, we extracted total DNA from a wing punch of a spotted bat mist-netted in northern Arizona in 2010, and subjected the sample to paired-end genomic sequencing using an Illumina MiSeq.



Methods

- Genomic DNA extraction: Qiagen DNEasy Blood & Tissue Kit on spotted bat wing punch
- WGS library prep:
  - DNA fragmented to 300 bp with Matrical SonicMan microplate sonicator
  - sequencing adapters and primers: Kozarewa and Turner (2011)
  - adapter ligation and library amplification: KAPA Library Preparation Kit for Illumina Platforms
  - library quantification: ABI Prism qPCR with KAPA Library Quantification Kit
- Sequencing: Illumina MiSeq 2 x 250 bp
  - 13 million paired 250 bp reads
- *de novo* sequence assembly: ABySS 1.3.2 (Simpson et al. 2009)
  - k=95
  - assembly filtered for 7410 contigs which exceeded 1500 bp
- Microsatellite discovery: msatcommander (Faircloth 2008)
  - 4-6mers with at least 6 repeats
  - primers for 60-220bp PCR products designed with integral Primer 3 (Rozen and Skaletsky 2000)
- PCR annealing temp optimization: 47-63°C temp gradient PCRs (master mix details and cycling conditions available on request)
- Microsatellite testing: polymorphism assessed using DNA from 5 museum-archived heart samples and from 15 tissue and buccal samples that we collected
- Electrophoresis: ABI 3130 Genetic Analyzer
- Data analysis: GeneMapper

Figure 1. Approximate distribution of spotted bats in the U.S., Mexico, and Canada.

Results

We identified 126 potentially amplifiable loci containing repeat regions, and designed primers for 56 of them. Of these, 22 PCR-amplified at either 54 or 60°C, and were polymorphic and easily scored (Figure 2).

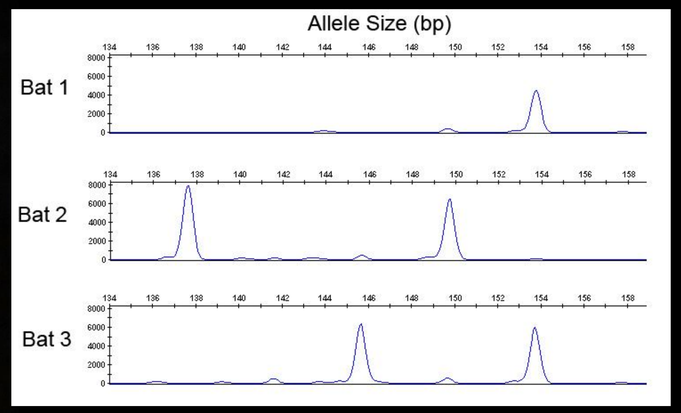


Figure 2. Locus EUMA6 electorphoresis plots from three individuals, showing four allele sizes.

Conclusions

This work demonstrates the utility of genomic sequencing as a means to microsatellite identification, in a non-model species that is otherwise scientifically intractable. The new loci will enable examination of genetic population structure, genetic diversity, and population size, in a rarely-encountered species (e.g., 79 individuals in museum collections). A panel of ten loci that perform well with museum and non-invasive genetic samples will be selected for upcoming microsatellite and mitochondrial genotyping of spotted bats. We have samples from 52 live captures from northern Arizona, and have access to mummies and to tissue from museum specimens spanning the species' distribution, providing depth in time and space. We will assess broad-scale questions such as bat movements between regions, as well as fine-scale questions involving population size estimation in northern Arizona. The latter is relevant to the problem of bat fatalities associated with wind turbines, as our genetic-based population size estimates for this species will assist land managers in setting more accurate mortality thresholds, which are currently based on perception.

References

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Author emails: Faith.Walker@nau.edu; Jeff.Foster@nau.edu; Kevin.Drees@nau.edu; Carol.Chambers@nau.edu