Genetic Diet Analysis of Coyote Scat from Populations in Long Beach

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Abstract

Interactions between humans and local wildlife are inherent to urbanization and have created a demand for management solutions. Coyotes (*Canis latrans*) are prominent in urban ecosystems and can potentially cause a variety of residential threats, as seen at Long Beach, CA. Currently, a project is underway assessing the coyote population, in order to better understand how the animals function in the city and how best to manage them. The main components of the study include monitoring coyote activity and dispersal patterns, how the urban environment affects coyote living strategies, and a dietary analysis. The dietary analysis has two components: a solid analysis of the bones found in the coyote scat and a parallel study on the genetic material of prey items found in the scat. This study will focus on the genetic analysis of the coyote scat using species-specific primers for PCR.

Introduction

- Understanding Long Beach coyote diet is important for developing an informed and effective wildlife management plan.
- Previous research in Southern California showed that urban coyotes acquire up to 25% of their food from anthropomorphic sources, such as garbage, pet food and pets (Riley *et al*. 2003).
- It is difficult to determine coyote diet through observation (Klare *et al*. 2011), so scat analysis is the preferred method (Marucco *et a*l. 2008; van Dijk *et al*. 2007).
- A novel approach to analyzing coyote diet is by utilizing genetics to identify DNA of prey items deposited in their scat.
- Species-specific primers were developed for PCR using the mitochondrial cytochrome b gene, which is the preferred gene for mammal species identification (Bradley and Baker 2001).
- PCR products of different base pair lengths create a unique banding pattern on the agarose gel, which can be used to identify prey item DNA in the scat sample.

Question: What prey items are most present in Long Beach urban coyote diet? Do domesticated pets such as cats and dogs make up a significant proportion of the diet?

Hypothesis: Domesticated pets do not make up a large portion of urban coyote diet, which is instead significantly comprised of naturally occurring mammals.

Methods

- Scat samples were collected near fire station 19 in Long Beach, CA; Latitude: 33-49'22'' N, Longitude: 118-08'03'' W
- DNA was extracted using either chelax
- Ten species of interest were chosen for this experiment ranging from common prey items to domesticated animals
- The list consisted of : *Canis Latrans* , *Canis lupus familiaris*, *Felis Catus*, *Sciurus niger*, *Otospermophilus beecheyi* , *Neotoma fuscipes, Sylvilagus audubonii*, *Thomomys bottae*, *Rattus rattus*, and *Rattus norvegicus*.
- Genetic sequences for cytochrome b were retrieved from NCBI Gen bank
- Forward primers were designed by the alignment of the the sequences, using GenStudio software, in order to find a parsimonious universal primer
- Reverse primers unique to each species were designed using Primer 3 software using the cyt b gene and inputting the chosen forward primer
- The minimum base amplification pair length chosen was ~100bps long, with increasing increments of ~50bps, creating a unique base pair size for each species of a multiplex.
- The reference tissues were used for positive control tests to confirm that the primers do not amplify for any other species
- The PCR products were visualized on gel using Gel Electrophoresis

Results

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                 210        220        230        240        250
Sylvilag   CAGTAACTCA TATTTGTCGA GACGTAAACT ACGGCTGACT AATCCGATAT
Thomomys   CAGTAACACA TACCTGCCGA GATGTAAATT ATGGATGACT AATCCGCTAC
Neotoma_   CTGTAACTCA CATCTGTCGA GATGTTAATT ACGGATGACT AATCCGATAT
Rattus_r   CAGTTACTCA CATCTGCCGA GACGTAAACT ACGGCTGACT AATCCGATAC
Rattus_n   CAGTCACCCA CATCTGCCGA GACGTAAACT ACGGCTGACT AATCCGATAC

                 260        270        280        290        300
Sylvilag   CTTCATGCTA ACGGAGCATC AATATTCTTC ATCTGCCTTT ACATACACGT
Thomomys   ATACATGCAA ACGGAGCCTC CCTATTCTTC ATCTGCCTAT ACATCCATAT
Neotoma_   ATGCACGCAA ACGGAGCCTC TATATTCTTT ATCTGCCTAT TTCTCCACGT
Rattus_r   TTACATGCCA ACGGAGCCTC AATATTCTTT ATCTGCTTAT TCCTCCATGT
Rattus_n   CTACACGCCA ACGGCGCCTC AATATTTTTC ATCTGCCTAT TCCTCCATGT

                 310        320        330        340        350
Sylvilag   CGGCCGCGGT ATTTACTACG GCTCCTACAC TTACCTAGAA ACCTGAAATA
Thomomys   CGGACGTGGC ATCTACTACG GTTCCTATCT TTATAAAGAA ACATGAAATG
Neotoma_   AGGACGAGGA ATCTACTATG GCTCATATAC ATTCATAGAA ACCTGAAACA
Rattus_r   AGGCCGAGGG ATATACTACG GATCCTACAC CTTCCTAGAA ACATGAAACA
Rattus_n   GGGACGAGGA CTATACTATG GATCCTACAC TTTCCTAGAA ACCTGAAACA

                 360        370        380        390        400
Sylvilag   TTGGCATTAT CCTGCTATTC GCAGTAATAG CCACAGCTTT CATAGGCTAT
Thomomys   TTGGCATCCT ACTTCTATTT CTAACAATAG CAACTGCATT CGTCGGATAT
Neotoma_   TCGGCATTGC CTTATTATTT GCCGTAATAG CGACAGCATT CATGGGATAT
Rattus_r   TTGGAATTAT CCTACTATTT GCAGTCATAG CAACCGCATT CATAGGTTAT
Rattus_n   TTGGAATCAT TCTACTATTT GCAGTCATAG CAACTGCATT CATGGGCTAT

                 410        420        430        440        450
Sylvilag   GTACTCCCAT GAGGCCAAAT ATCCTTCTGA GGCGCAACCG TAATCACTAA
Thomomys   GTCCTACCAT GAGGACAAAT ATCATTCTGA GGGGCCACCG TAATTACAAA
Neotoma_   GTACTACCAT GAGGACAAAT ATCTTTCTGA GGAGCTACAG TAATTACAAA
Rattus_r   GTACTCCCAT GAGGACAAAT ATCATTCTGA GGAGCCACAG TAATCACAAA
Rattus_n   GTACTCCCAT GAGGACAAAT ATCATTCTGA GGAGCTACAG TAATTACAAA

                 460        470        480        490        500
Sylvilag   TCTTTTATCA GCCATCCCCT ATATTGGAAC AGCCCTAGTC GAATGGATCT
Thomomys   CCTATTATCA GCAATCCCTT ATATTGGCCA AGATCTAGTA GAATGAATCT
Neotoma_   CCTTCTATCA GCTATTCCTT ATATTGGAAC TACCCTAGTA GAATGAATCT
Rattus_r   CCTATTATCA GCCATTCCCT ACATTGGCAC CACTCTAGTC GAATGAATCT
Rattus_n   CCTATTATCA GCTATCCCTT ACATTGGGAC TACCCTAGTC GAATGAATCT

                 510        520        530        540        550
Sylvilag   GAGGGGGCTT CTCAGTTGAT AAAGCCACAC TCACTCGCTT CTTCGCATTT
Thomomys   GAGGCGGATT CTCAGTGGAT AAAGCTACTC TTACACGATT CTTCGCGTTT
Neotoma_   GAGGAGGATT CTCAGTAGAC AAAGCCACAC TAACACGTTT TTTTGCTTTC
Rattus_r   GAGGAGGCTT CTCAGTAGAC AAAGCAACCC TAACACGTTT TTTCGCATTC
Rattus_n   GAGGAGGCTT CTCAGTAGAC AAAGCAACCC TAACACGCTT CTTCGCATTC

                 560        570        580        590        600
Sylvilag   CATTTTATTC TACCATTCAT TATTGCAGCC CTAGTTATAG TTCACTTACT
Thomomys   CACTTTATTC TCCCATTTAT CATTGCAGCT ATAGCTATAG TTCACCTTCT
Neotoma_   CACTTTATCC TTCCATTCAT TATCACAGCC CTAGTTGTAG TTCACCTACT
Rattus_r   CACTTCATCC TCCCATTCAT CATCGCCGCC CTTGCAATTG TACATCTCCT
Rattus_n   CACTTCATCC TCCCATTCAT TATCGCCGCC CTTGCAATTG TACATCTTCT
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Figure 1. Alignment of mitochondrial cytochrome b gene of five target prey species in multiplex 2 with highlighted species-specific primer attachment sites. *Sylvilagus audubonii* (red), *Thomomys bottae* (orange), *Neotoma fuscipes* (teal), *Rattus rattus* (green) *and Rattus norvegicus* (pink).

Table 1. Depiction of the uniquely designed forward and reverse primers for multiplex 1, including the species name and expected product length in base pairs.

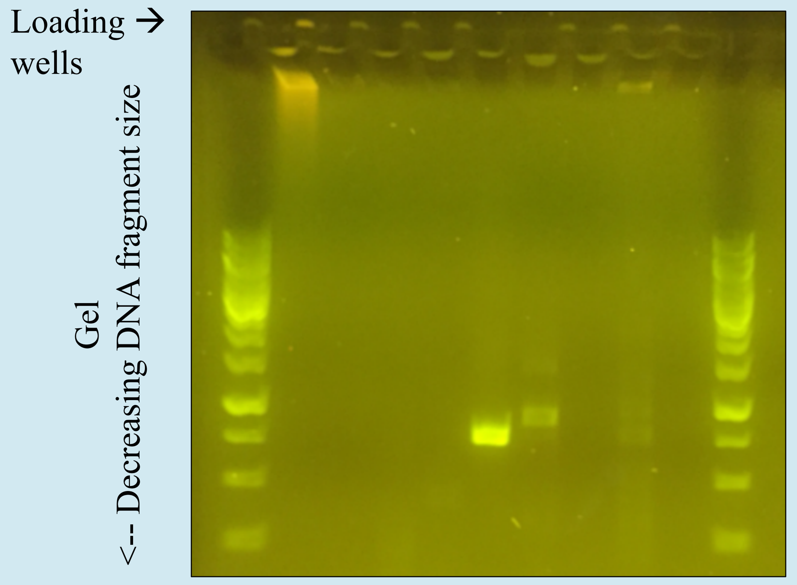
Species name	Primer direction	Primer sequence 5'-3'	Product size
Spermophilus beecheyi	Forward	AAAGCTACCCTAACACGATT	75
	Reverse	GTGAACTATGACTAGGGCTGTAA	
Sciurus niger	Forward	AAAGCAACTCTTACACGATT	136
	Reverse	TATCAGAATCGGAGATCAGG	
Canis lupus familiaris	Forward	AAAGCAACCCTAACACGATT	232
	Reverse	GGTCAGGTGAAAATAAAACTAGTGA	
Canis latrans	Forward	AAAGCAACCCTAACACGATT	304
	Reverse	ATCATTCGGGTTTGATATGTG	
Felis catus	Forward	AAAGCCACCCTAACACGATT	389
	Reverse	AGTACTAGGATGGAGAGTACTAGGG	

Table 2. Depiction of uniquely designed forward and reverse primers for multiplex 2, including the species name and expected product length in base pairs.

Species name	Primer direction	Primer sequence 5'-3'	Product size
Sylvilagus audubonii	Forward	TACGGCTGACTAATCCGATA	106
	Reverse	GGTAAGTGTAGGAGCCGTAGTAAA	
Thomomys bottae	Forward	TATGGATGACTAATCCGCTA	163
	Reverse	CGAATGCAGTTGCTATTGTTAG	
Neotoma fuscipes	Forward	TACGGATGACTAATCCGATA	226
	Reverse	GAAGGTTTGTAATTACTGTAGCTCCT	
Rattus rattus	Forward	TACGGCTGACTAATCCGATA	273
	Reverse	TCAGATTCATTCGACTAGAGTGGT	
Rattus norvegicus	Forward	TACGGCTGACTAATCCGATA	328
	Reverse	TGAAGTGGAATGCGAAGAAG	

Results

Loading → wells



Lane	Species	Results
L	Reference ladder	(each band = 100 bases)
1	Pocket gopher	Did not amplify (dilute)
2	CA ground squirrel	Did not amplify
3	Northern raccoon	Did not amplify
4	Virginia opossum	Did not amplify
5	Coyote	Amplified, ~300bp
6	Fox squirrel	Amplified, ~325bp
7	Striped skunk	Did not amplify
8	Desert cottontail	Amplified, multiple bands
9	Negative control	No amplification
L	Reference ladder	

Figure 2. Coyote species-specific primers successfully amplified coyote DNA at the correct band size. Cross amplification occurred with fox squirrel and desert cottontail. Cross amplification needs to be removed by optimizing primers/PCR reaction. Coyote primer successfully did not amplify other mammal DNA.

Discussion

The results from the preliminary PCR indicate that our method is effective in characterizing DNA on the species level.

Further testing will be conducted with the species-specific primers individually and in multiplex against known tissue samples to determine their effectiveness in amplifying the correct DNA segments. These primers will then be used in PCR to test coyote scat samples from the Long Beach research site to determine the prey items present in the coyote diet.

Characterizing the diet of urban coyotes is essential for developing proper management techniques. Importantly, residents are concerned that coyotes prey on household pets. This concern leads to anxiety amongst residents and to potentially dangerous urban "solutions", such as shooting, trapping or poisoning coyotes (Weckel, 2010). Accurately determining the composition of Long Beach coyotes' diets can help the city of Long Beach to educate its residents, mitigate some of their anxiety from urban predators and reduce dangerous population control techniques.

In a relevant study, a similar methodology looking at the diet of Fin whales and Adelie penguins through prey DNA amplification in the feaces was shown to be effective, adding to the validity of this coyote study (Jarman et al., 2004)

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