Department of Defense
Legacy Resource Management Program

PROJECT 12-612
(AGREEMENT N0. W9132T-12-2-0050)

Monitoring Species of Concern Using Noninvasive Genetic Sampling and Capture-Recapture Methods: Pilot Demonstration With Coyote, a Predator to Species at Risk and Game Species on DoD Lands

LISETTE WAITS

Oral Presentation for Wildlife Society Annual Meeting, Milwaukee, WI

Presented October 2013

Balancing Scat Deposition Rates and Fecal DNA Degradation Rates to Optimize Noninvasive Genetic Sampling of Carnivores

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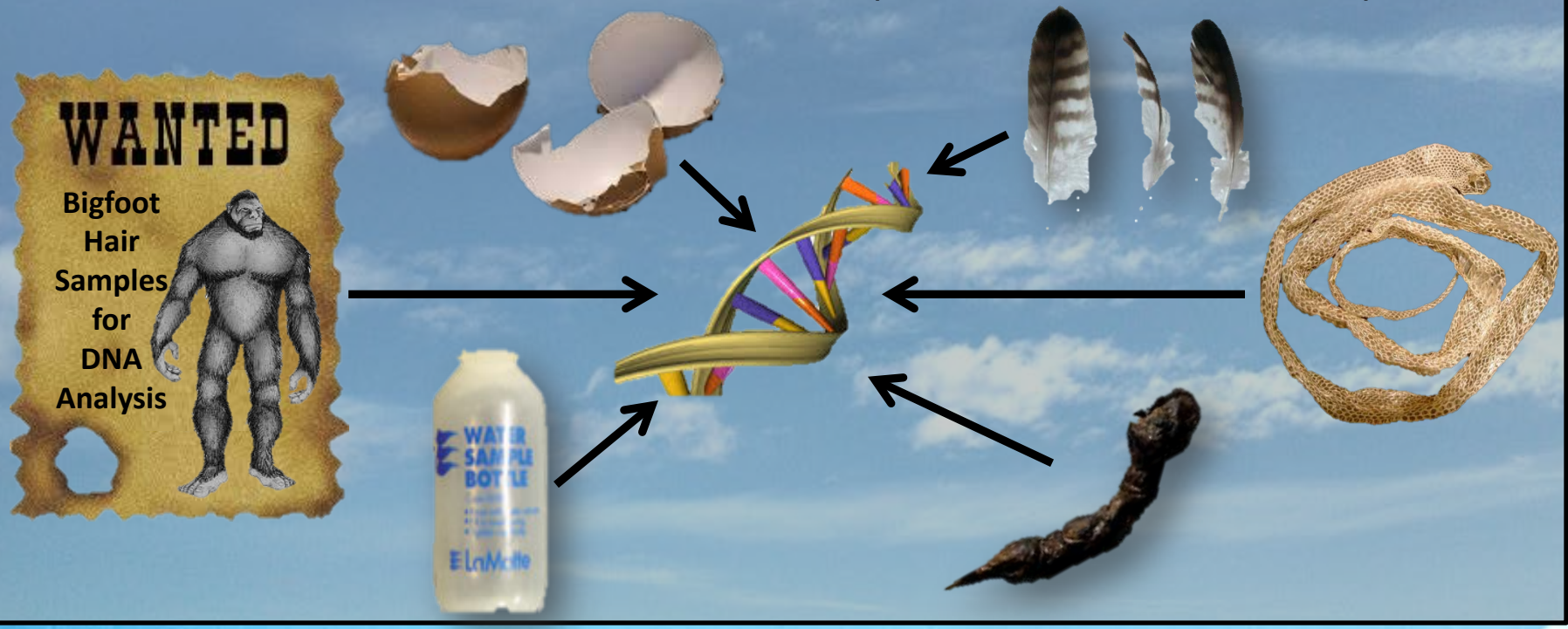
Noninvasive Genetic Sampling (NGS)

Inventory and monitor species by collecting feces, hair, feathers, saliva, etc. (Taberlet et al. 1999)

Eliminates need to disturb individuals (↓stress, trauma, risk)



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NGS Challenges:

- DNA degradation
  - Time since deposition (Piggott 2004, Murphy et al. 2007, Santini et al. 2007)
  - Environmental conditions (Murphy et al. 2007, Piggott 2004, Santini et al. 2007)
  - Diet (Murphy et al. 2003)
  - Storage medium (Panasci et al. 2011)
  - Locus length (DeMay et al. 2013)
  - Species (Piggott 2004)

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NGS Challenges:

- DNA degradation
- Contamination
- Genotyping error (Broquet et al. 2004)
  - Allelic dropout (ADO)
  - False allele (FA)

Target Species

Kit fox *(Vulpes macrotis)*

- Species of Concern
- In decline across range
- High proportion of mortalities attributable to predation by coyotes

Coyote *(Canis latrans)*

- Populations have increased in abundance and distribution
- Intraguild predator of kit foxes
- NGS can be accomplished concurrently

Study Site

U.S. Army's Dugway Proving Ground, UT

- Great Basin cold desert
- Elevation: 1228m to 2154m
- Winters: Cold-dry
- Summers: Moderate-dry

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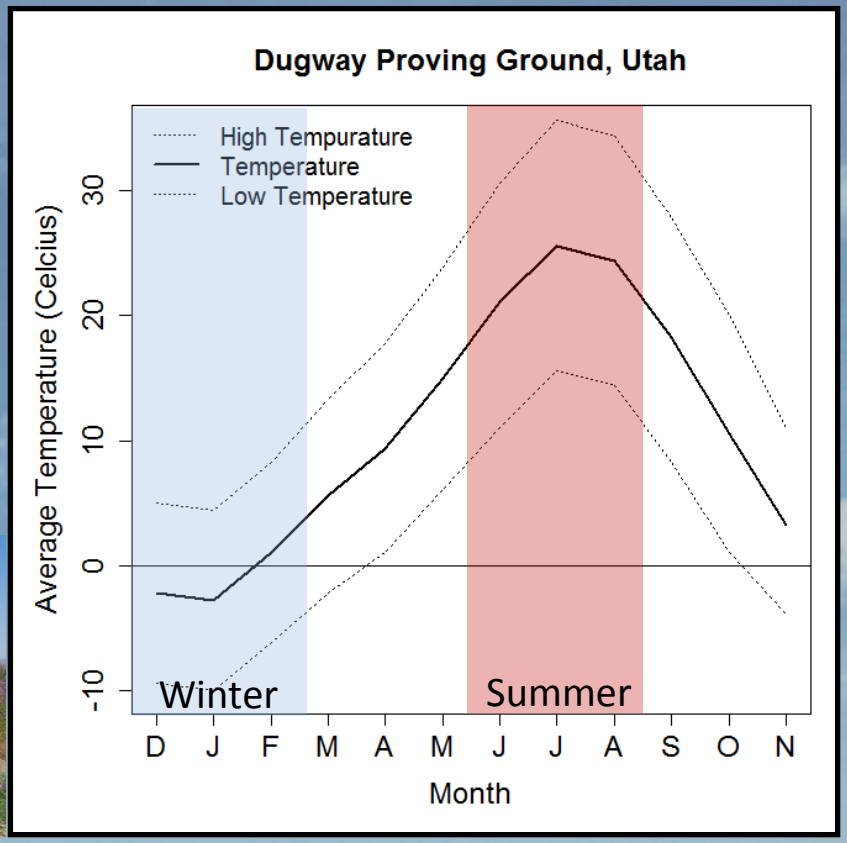

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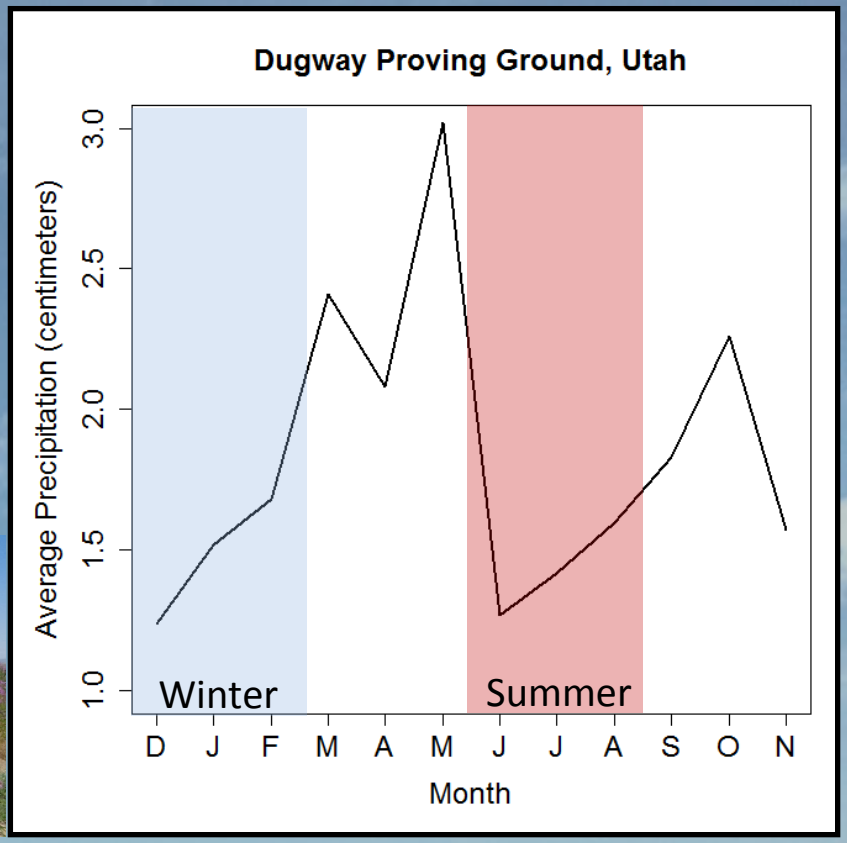


Objectives

Overall goal: Improve NGS efficiency for long-term monitoring

For both species, evaluate:

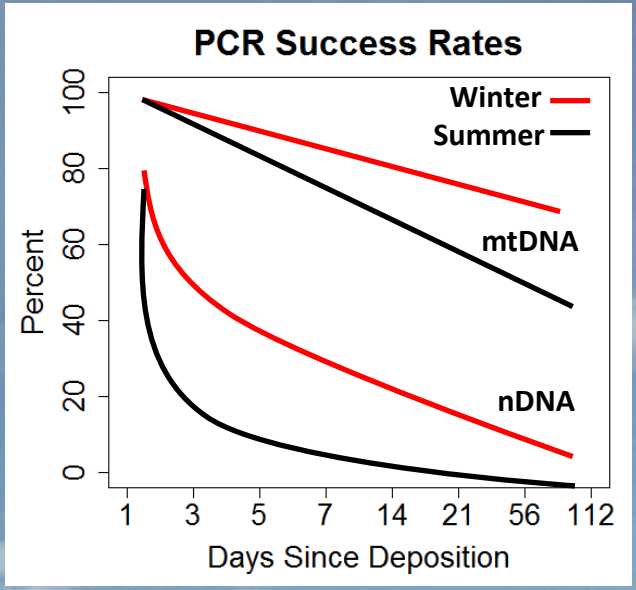
1. Scat deposition rates:
   - Winter vs. Summer
2. DNA degradation over time (i.e., PCR success, genotyping errors):
   - Winter vs. summer
   - Mitochondrial DNA (mtDNA) vs. nuclear DNA (nDNA)
   - nDNA locus lengths
3. Optimal sampling interval (duration between sampling events)

Hypotheses

For kit foxes and coyotes, evaluate:

1. Scat deposition rates:
   - Coyote > Kit fox
   - Summer > Winter

2. DNA degradation (PCR success and genotyping errors rates):
   - Coyote mtDNA > kit fox mtDNA
   - No difference in nDNA
   - PCR Success: shorter loci > longer loci



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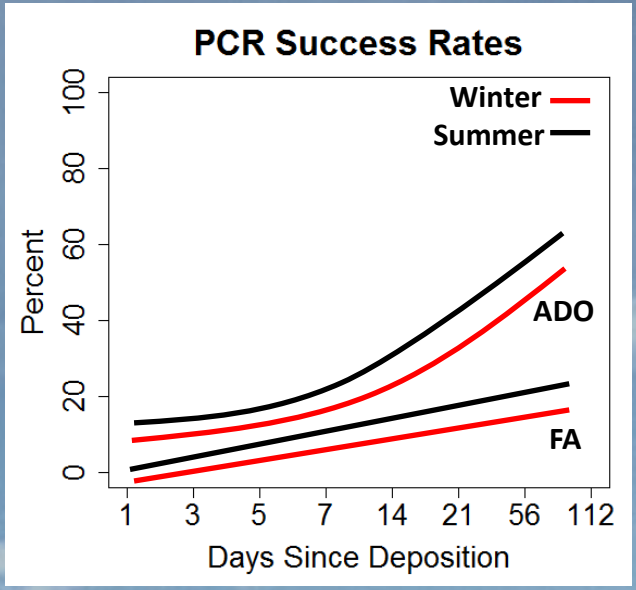
3. Optimal sampling interval:
   - Intermediate length



Scat Deposition Methods

Many carnivores utilize linear features for regular movements (Kohn et al. 1999)

Transect surveys:

- Cleared transects
- Subsequently surveyed for carnivore scats
- Data provided by Bryan Kluever and Steven Dempsey (Utah State University)
- Survey intensity:

  Winter = 75km
  Summer = 170.5km
  Spring = 150km

DNA Degradation Methods

Sample acquisition (20 scats/species in each season):

- Kit fox: Live-trapped free-ranging individuals (Bryan Kluever)
- Coyote: USDA Predator Research Facility, Logan, UT (Eric Gese)

Set up:

- Natural environment
- Protected from removal/disturbance

Sampling time points:

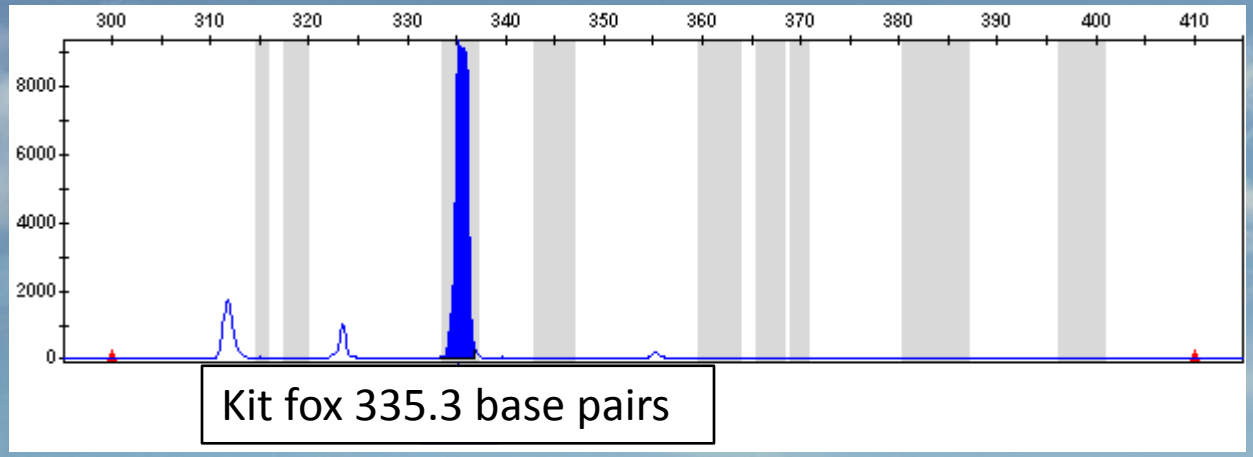
- Winter: <1, 3, 7, 14, 21
- Summer: <1, 3, 5, 7, 14, 21, 56, 112

Laboratory Methods

DNA extraction: Qiagen QIAamp DNA Stool Mini Kits

Species identification test: mtDNA (Onorato et al. 2006)

- Kit fox: 335.4 - 337.1 base pairs



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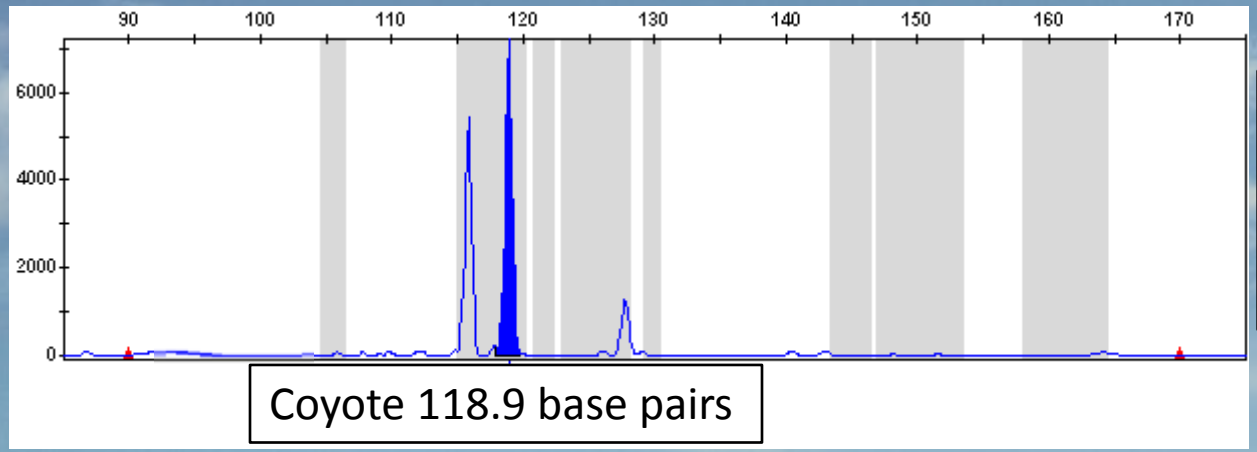
- Coyote: 115.0 - 120.1; 359.6 - 363.9 base pairs



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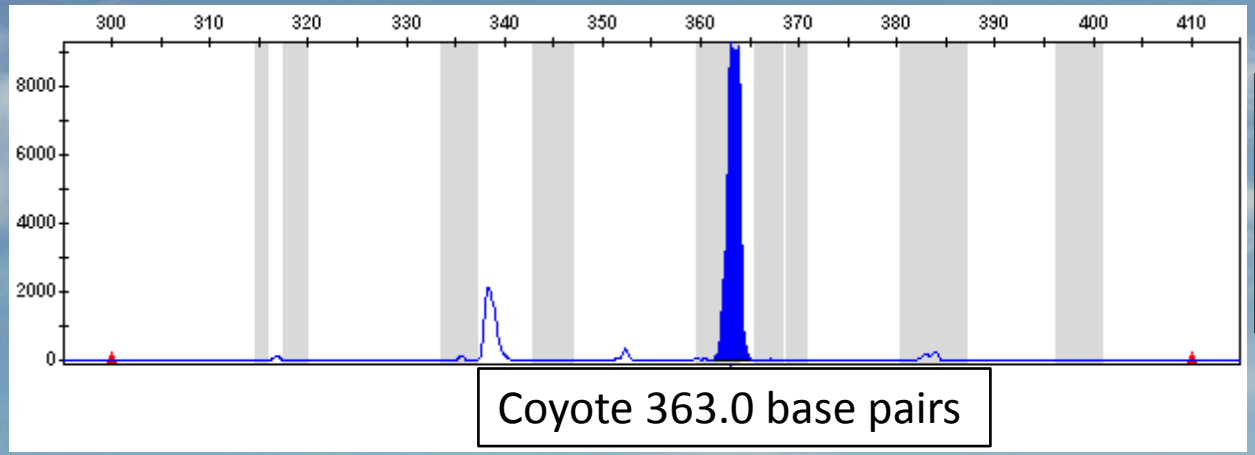

Coyote 363.0 base pairs

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Laboratory Methods

DNA extraction: Qiagen QIAamp DNA Stool Mini Kits

Species identification test: mtDNA (Onorato et al. 2006)

- Kit fox: 335.4 - 337.1 base pairs
- Coyote: 115.0 - 120.1; 359.6 - 363.9 base pairs
- Failed samples repeated a second time

Individual identification test: nDNA

- Kit fox: 7 microsatellite loci (Schwalm 2012)
  - Locus size range = 119 – 281 base pairs
- Coyote: 9 microsatellite loci (Stenglein et al. 2010b)
  - Locus size range = 79 – 226 base pairs

Data Analysis

Scat Deposition:

- Generalized linear model: quasi-Poisson distribution (log link)
- Likelihood ratio test and contrast analysis

Main effects:	Species
	Season

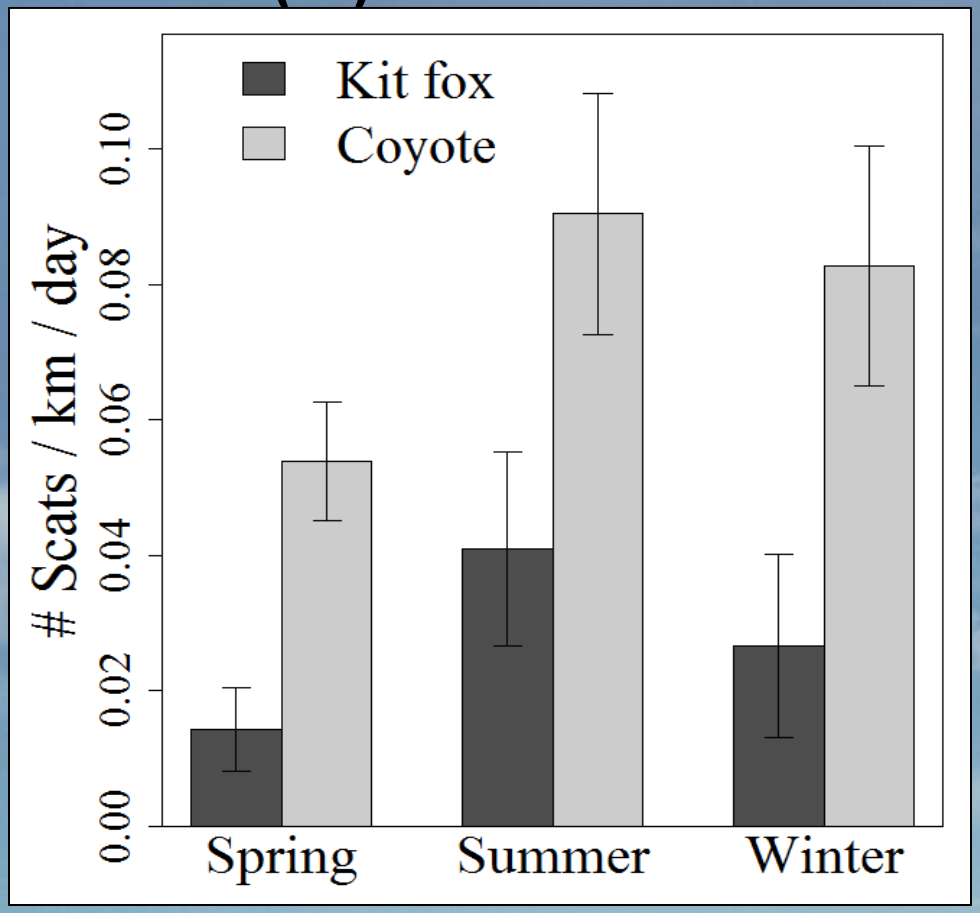
DNA Degradation:

- Mixed effects logistic regression: PCR Success, ADO, FA

Fixed effects:	Time	Locus length
	DNA type	Season
	Species	
Random effects:	Sample	

Scat Deposition Results

Mean (SE) Scat Accumulation

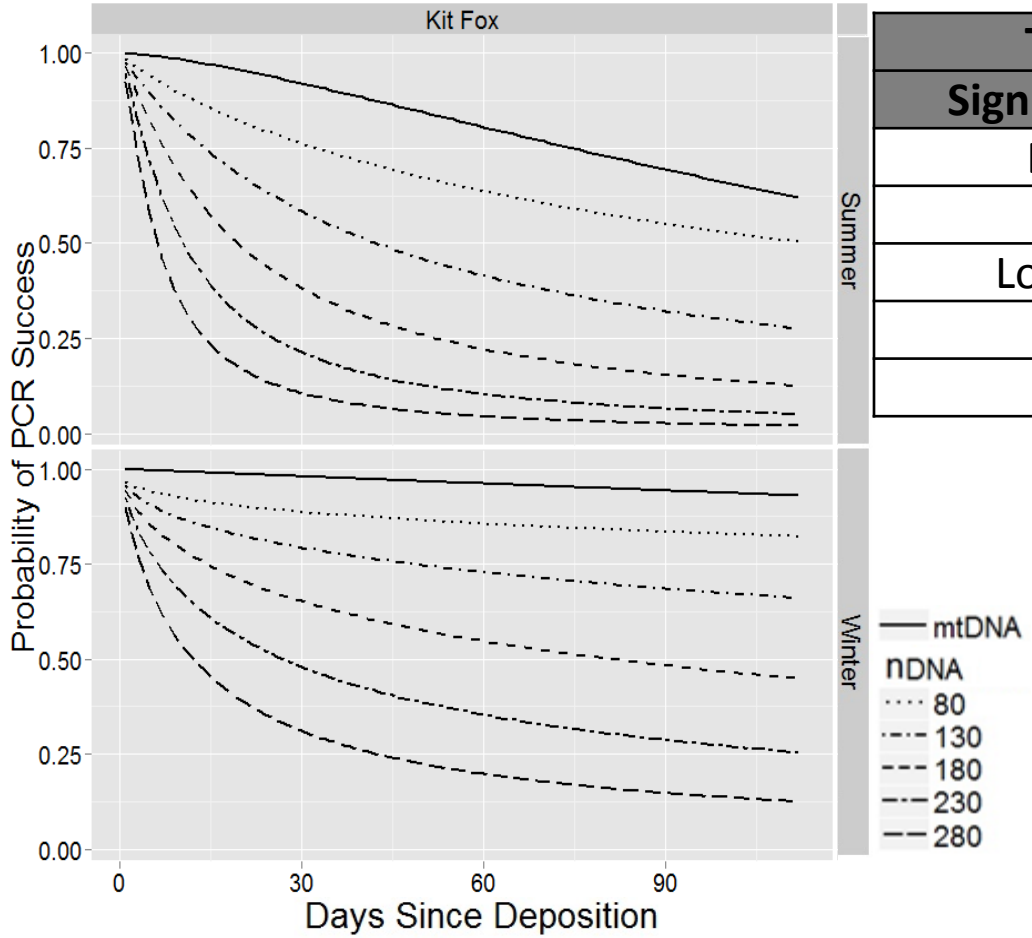


LRT (*p*-value = 0.673)

Model Parameters	z value	*p*-value
Intercept	-12.37	**<0.001**
Summer	2.38	**0.019**
Winter	1.36	0.177
Kit fox	-3.83	**<0.001**

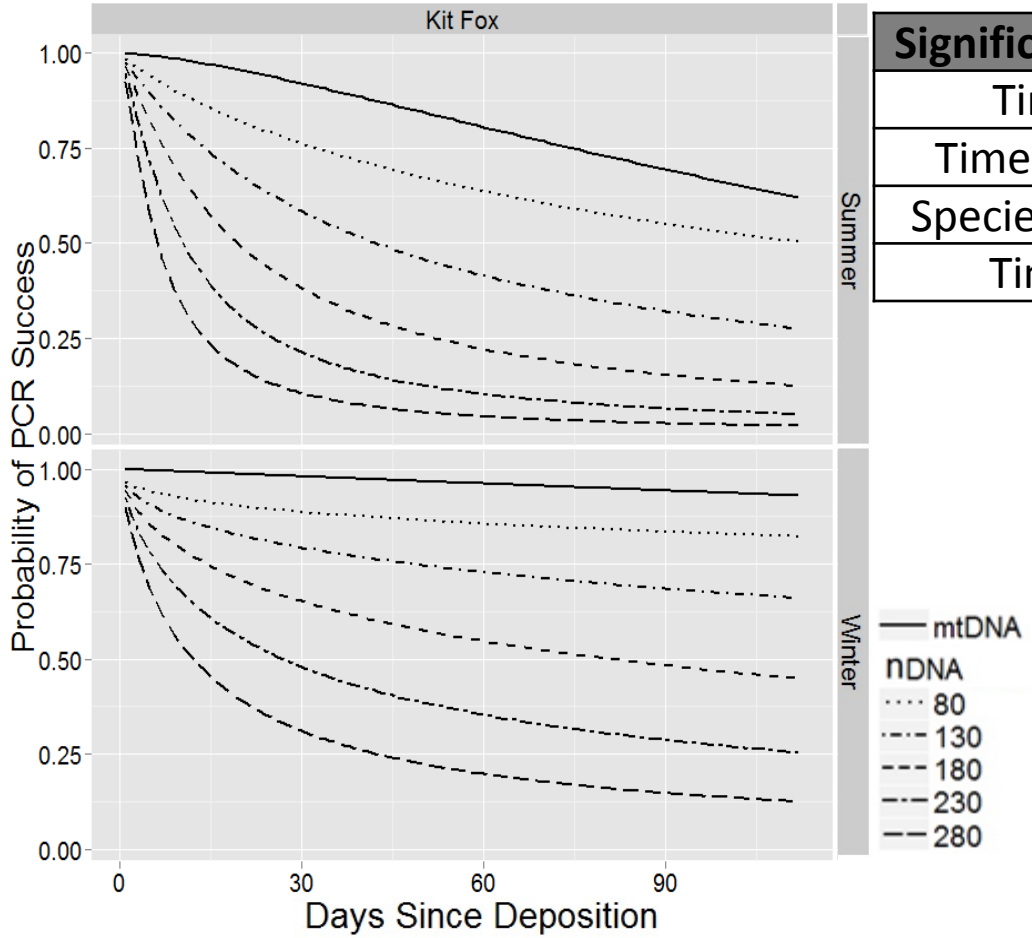
Contrasts	z value	*p*-value
Coyote vs. Kit fox	-9.09	**<0.001**
Spring vs. Summer	5.99	**<0.001**
Spring vs. Winter	-3.16	**0.002**
Summer vs. Winter	1.89	**0.059**

PCR Success Model Results



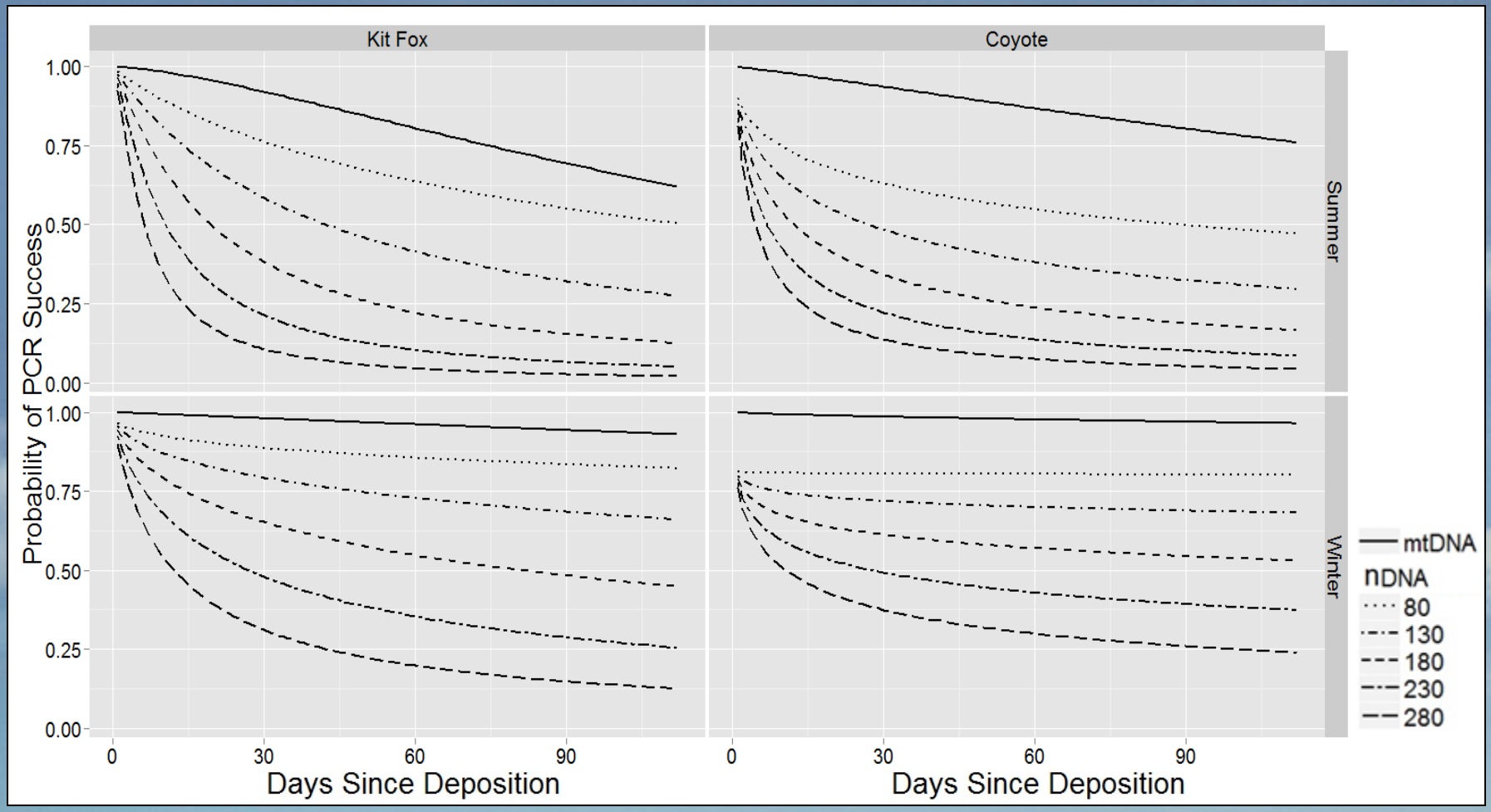
Type III Tests for Fixed Effects		
Significant Effects	***F* value**	***p*-value**
DNA Type	224.06	**<0.0001**
Time	4.93	**0.0263**
Locus Length	8.73	**0.0031**
Season	4.02	**0.0449**
Species	25.90	**<0.0001**

PCR Success Model Results

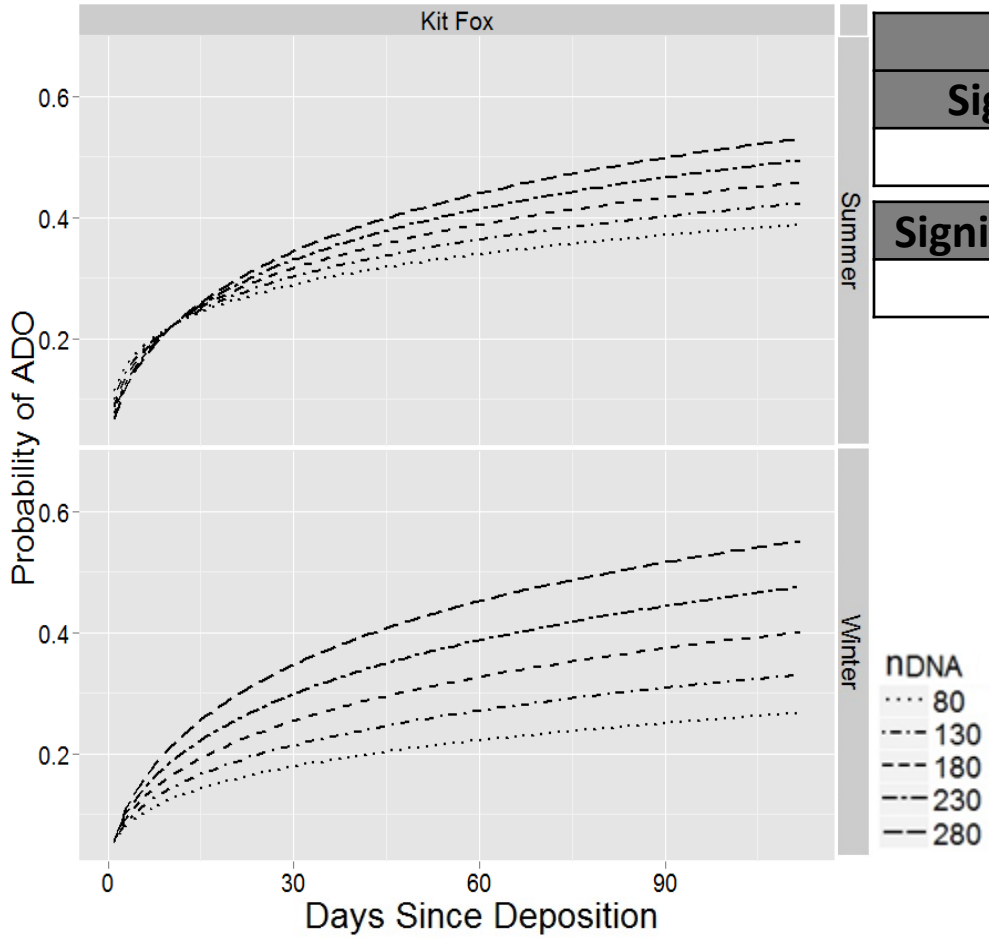


Significant Interactions	*F* value	*p*-value
Time*Season	42.02	**<0.0001**
Time*Locus Length	13.38	**0.0003**
Species*Locus Length	8.36	**0.0038**
Time*Species	24.15	**<0.0001**

PCR Success Model Results

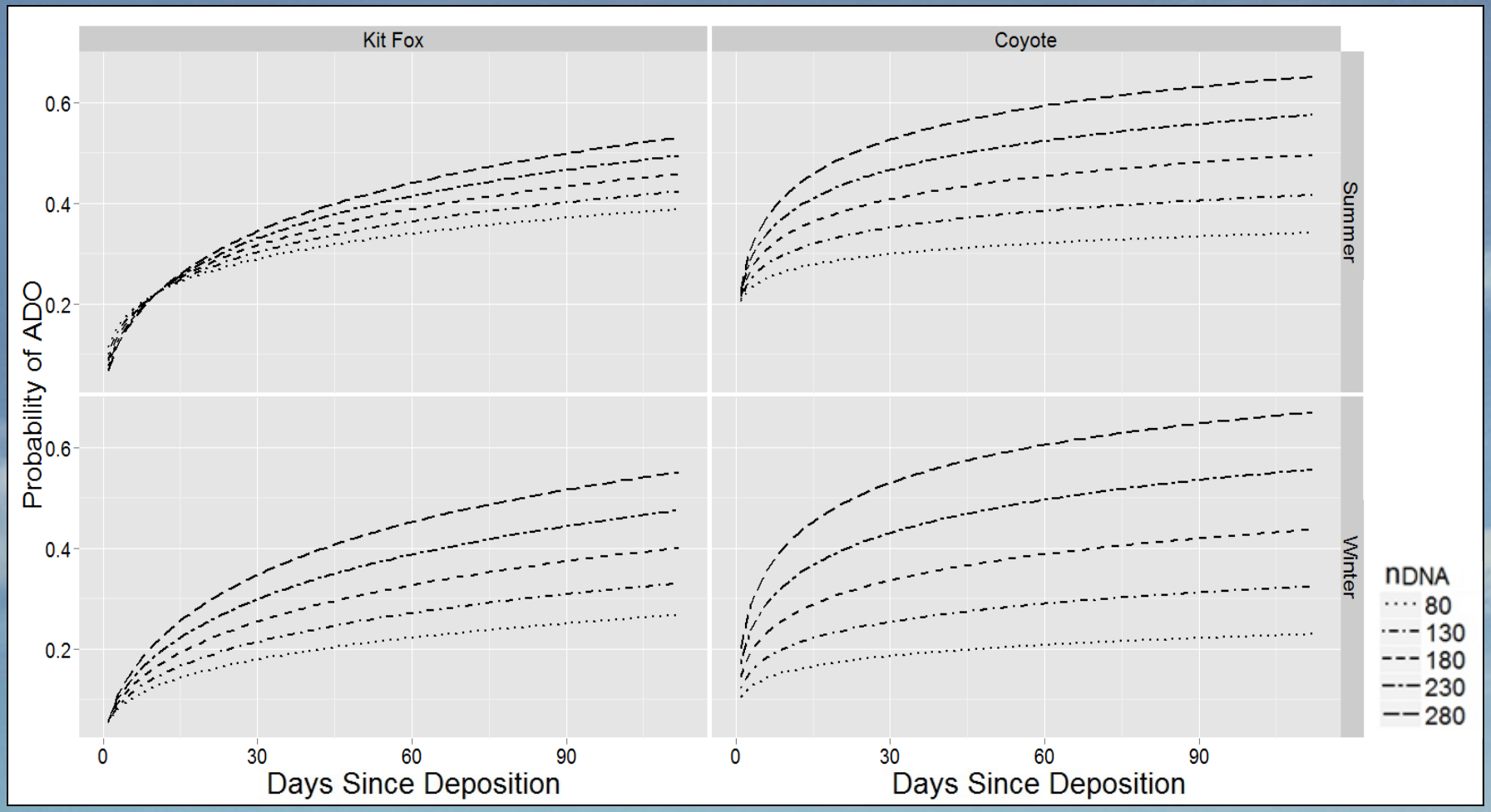


Allelic Dropout Model Results

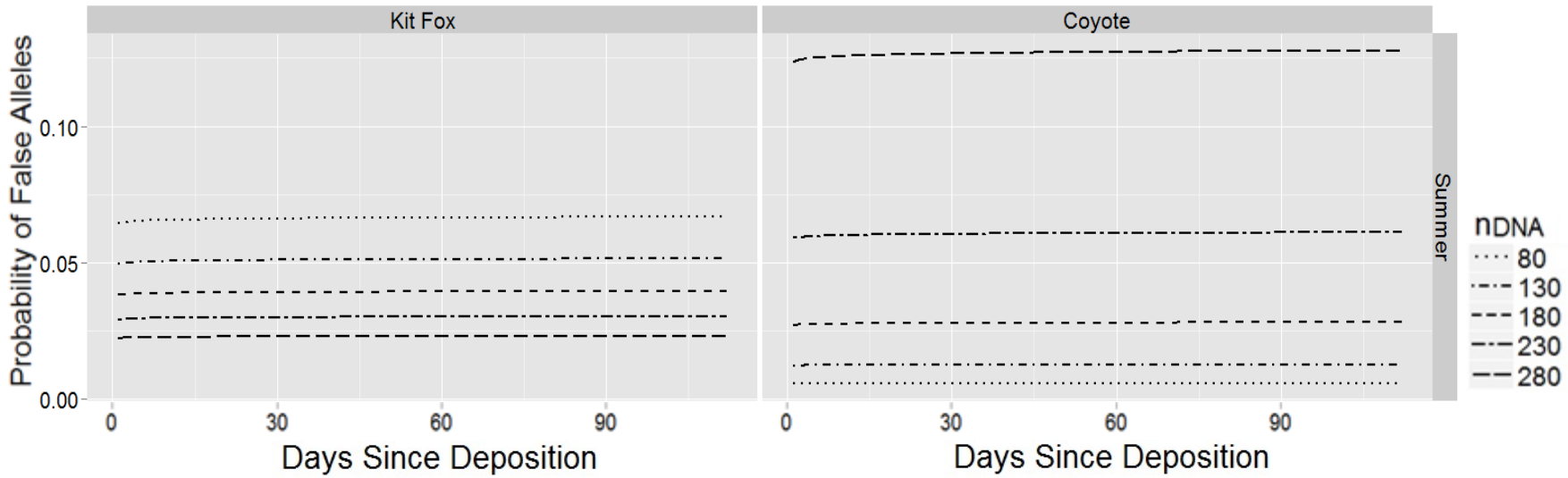


Type III Tests for Fixed Effects		
Significant Effects	***F* value**	***p*-value**
Season	4.11	**0.0428**
Significant Interactions	***F* value**	***p*-value**
Time*Species	42.02	**<0.0001**

Allelic Dropout Model Results

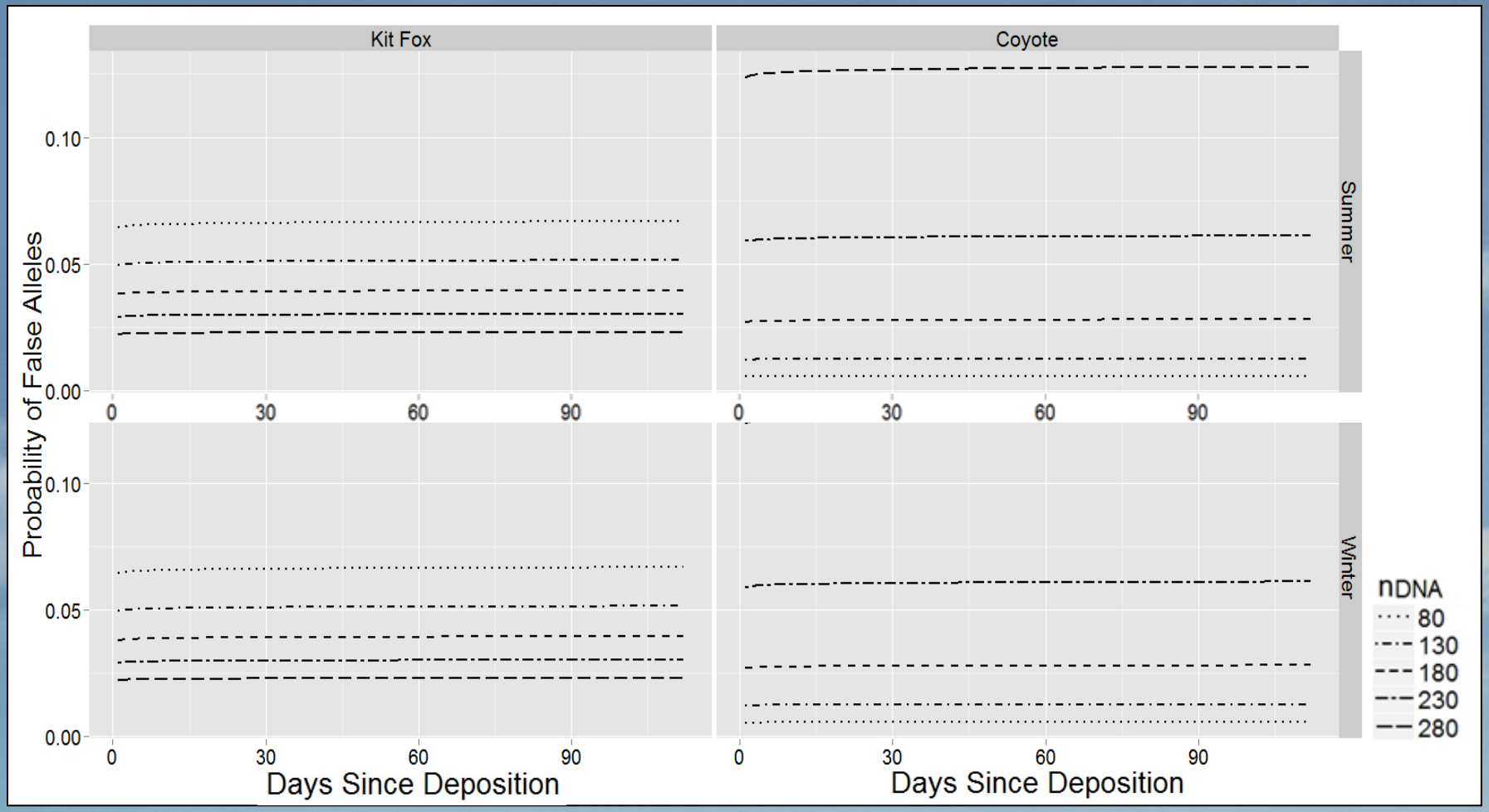


False Allele Model Results



Type III Tests for Fixed Effects		
Significant Effects	***F* value**	***p*-value**
Species	13.02	**0.0003**
Significant Interactions	***F* value**	***p*-value**
Locus Length*Species	9.23	**0.0024**

False Allele Model Results



Cost Per Sample & Sampling Interval

Cost Per Sample & Sampling Interval

Laboratory Costs

Samples Collected

PCR Success Rates

Genotyping Error Rates

Total Cost Per Successful Sample

Field Costs

Scat Deposition

Survey Effort

Samples Desired

Sampling Events

Cost Per Sample & Sampling Interval

Laboratory Costs

Samples Collected

PCR Success Rates

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Total Cost Per Successful Sample

Model Predicted

Field Costs

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Samples Desired

Sampling Events

Cost Per Sample & Sampling Interval

Laboratory Costs

Samples Collected

PCR Success Rates

Genotyping Error Rates

Total Cost Per Successful Sample

150km

Kit Fox (n=200)
Coyote (n=400)

Field Costs

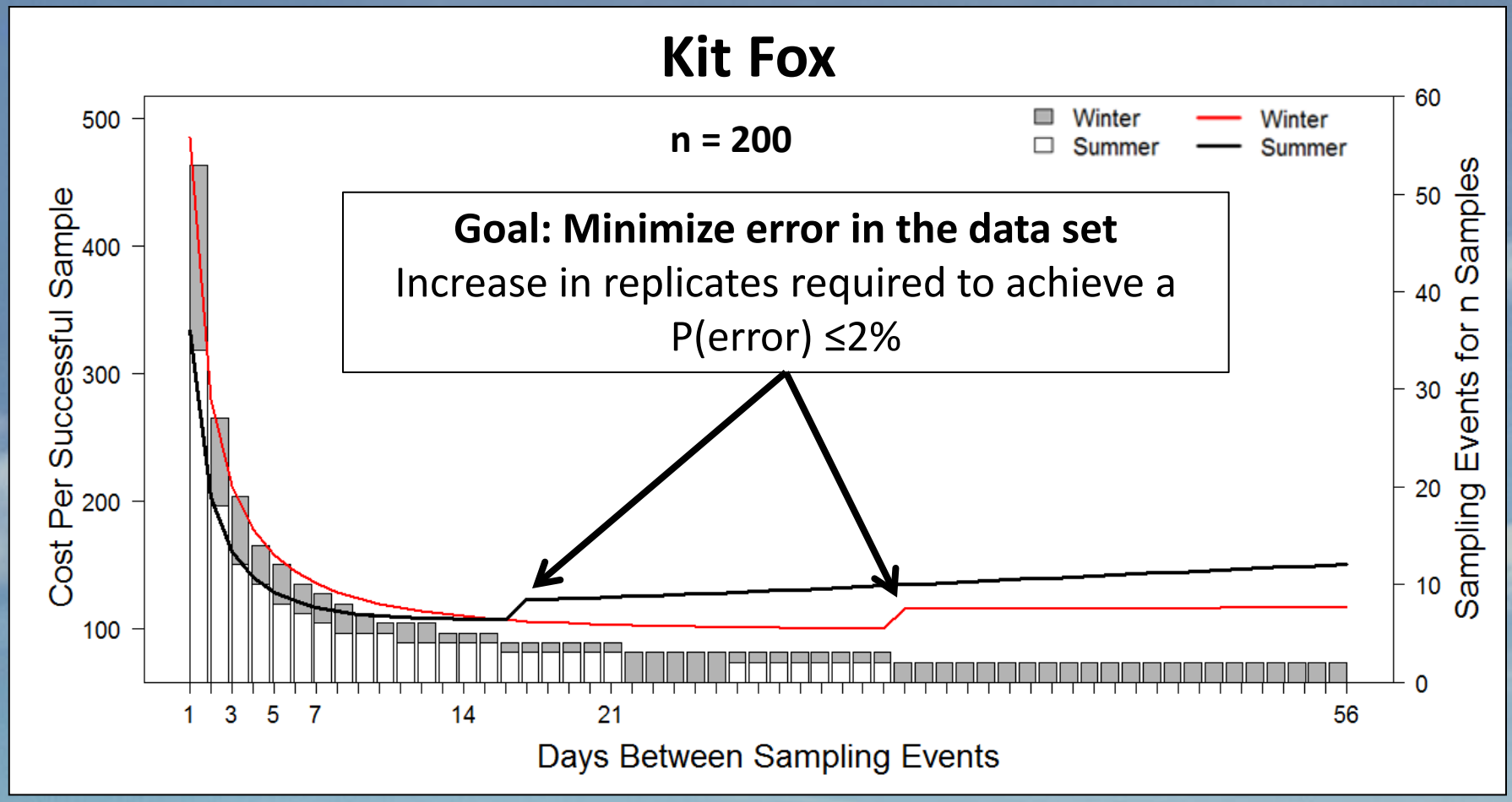
Scat Deposition

Survey Effort

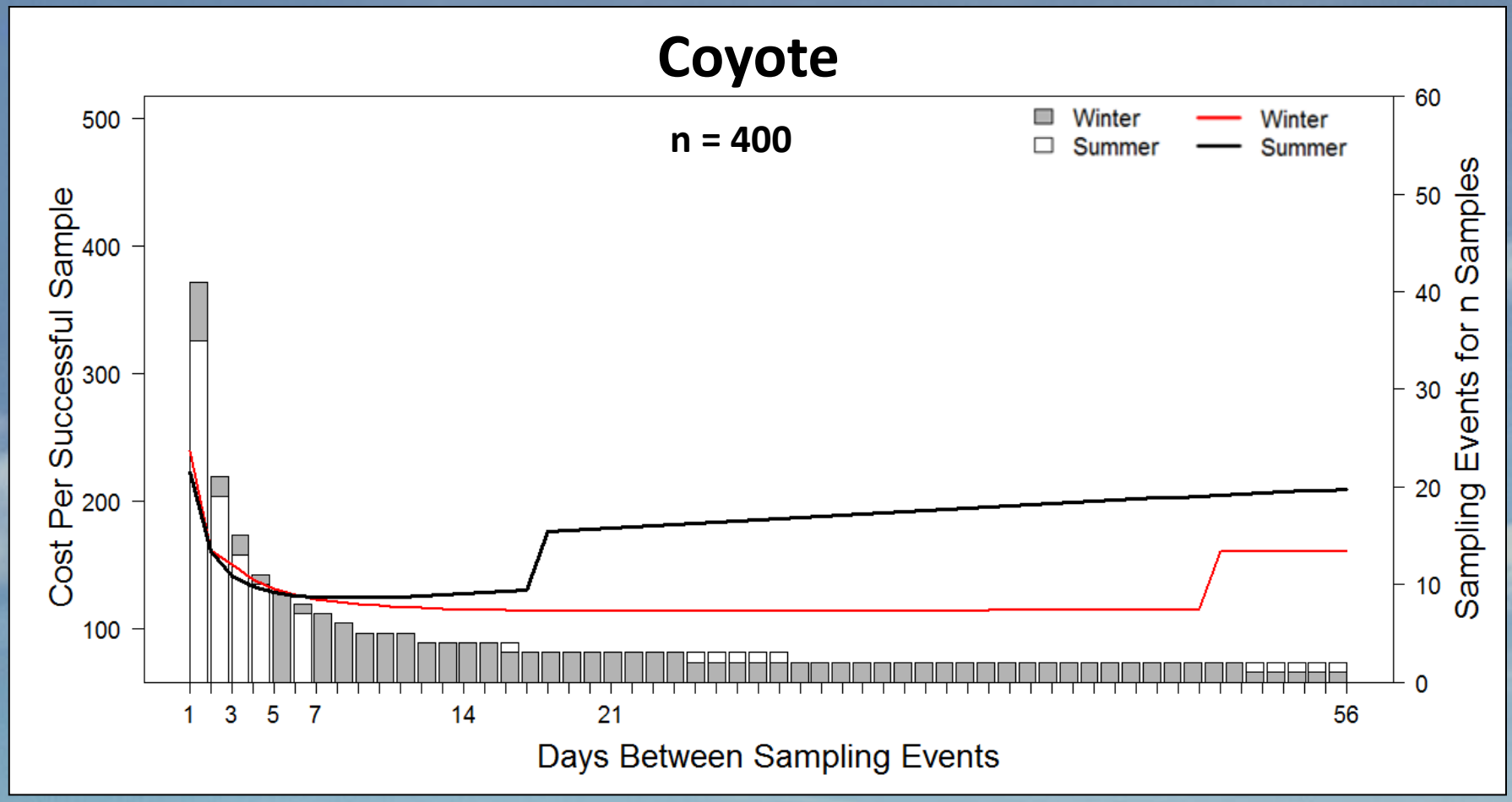
Samples Desired

Sampling Events

Cost Per Sample & Sampling Interval



Cost Per Sample & Sampling Interval



Discussion

Effects	Scat Deposition	PCR Success	ADO	FA
DNA Type	**N/A**			
Time Since Deposition	**N/A**			
Locus Length	**N/A**			
Season	✓			
Species	✓			
Interactions				

Deposition: coyote > kit fox

summer > winter > spring

Discussion

Effects	Scat Deposition	PCR Success	ADO	FA
DNA Type	**N/A**	✓		
Time Since Deposition	**N/A**	✓		
Locus Length	**N/A**	✓		
Season	✓	✓		
Species	✓	✓		
Interactions		✓		

PCR Success: mtDNA > nDNA
decreases with time
decreases with increasing locus length (nDNA)
faster rate of decline in summer than winter
rate of decline increases w/ locus length (nDNA)

Discussion

Effects	Scat Deposition	PCR Success	ADO	FA
DNA Type	**N/A**	✓		
Time Since Deposition	**N/A**	✓		
Locus Length	**N/A**	✓		
Season	✓	✓	✓	
Species	✓	✓		✓
Interactions		✓	✓	✓

Genotyping errors:

ADO > FA across both species
ADO appears to be higher for longer loci
ADO appears to increases over time
seasonal differences in ADO
species differences in FA

Discussion

Combining accumulation and degradation:

- One of the first studies
- Improves overall efficiency of NGS

Optimization of NGS:

- Feasible monitoring strategy when funding is limited
- Increases spatial and temporal extents
- Extends sampling to more species

Considerations for optimization:

- Cost
- Violations of assumptions

Management Implications

- Pilot studies
- High mtDNA success: Opportunities to survey for kit fox populations (evaluate distribution and/or occupany)
- Field costs: Can be reduced by monitoring sympatric species concurrently
- Laboratory costs: Can be reduced by selecting shorter loci and optimal sampling seasons
- Shorter intervals: May be cost prohibitive
- Extended intervals: Use may be limited due to genotyping error and model assumptions

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