Leptin Gene Polymorphisms in Wild and Captive American Mink

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EAAP 2010, August 23-27

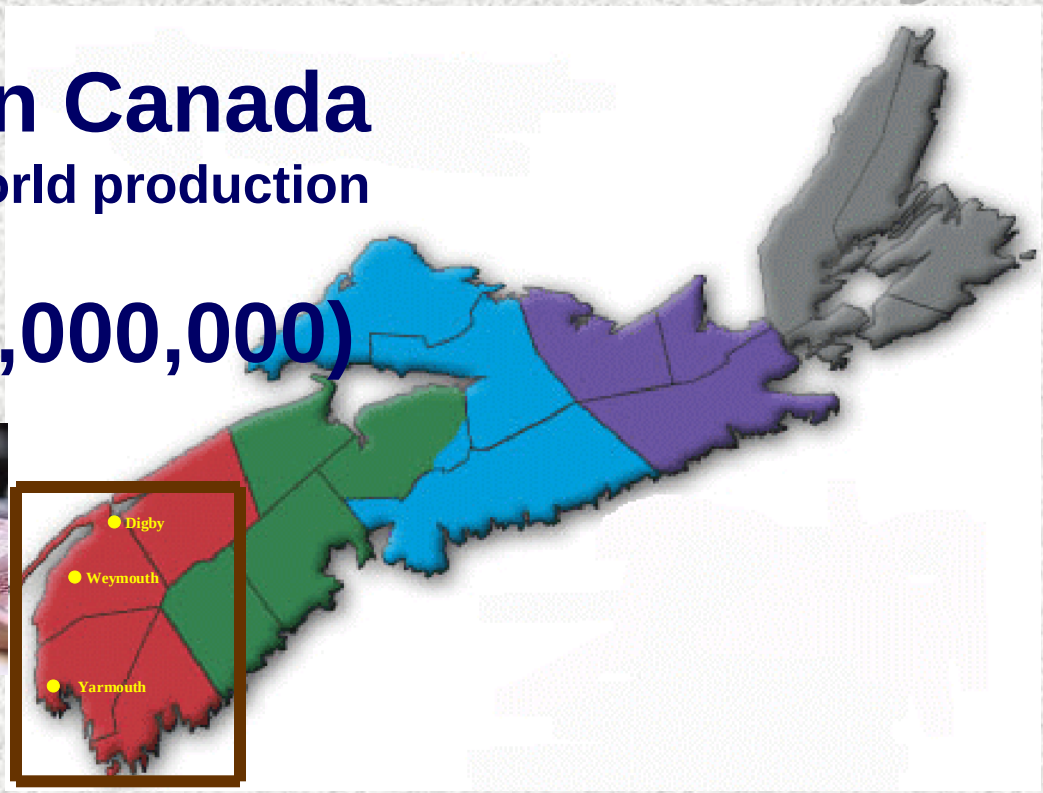
The Canadian Mink Industry

2,300,000 pelts in Canada

~ 4.2% of the world production

~ 50% in NS ($75,000,000)

Export

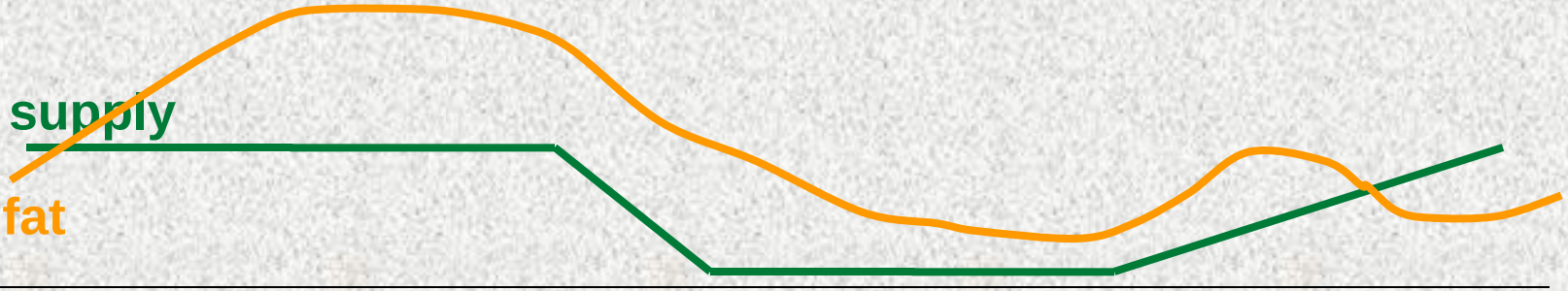


> 80%

Wild

Feed supply

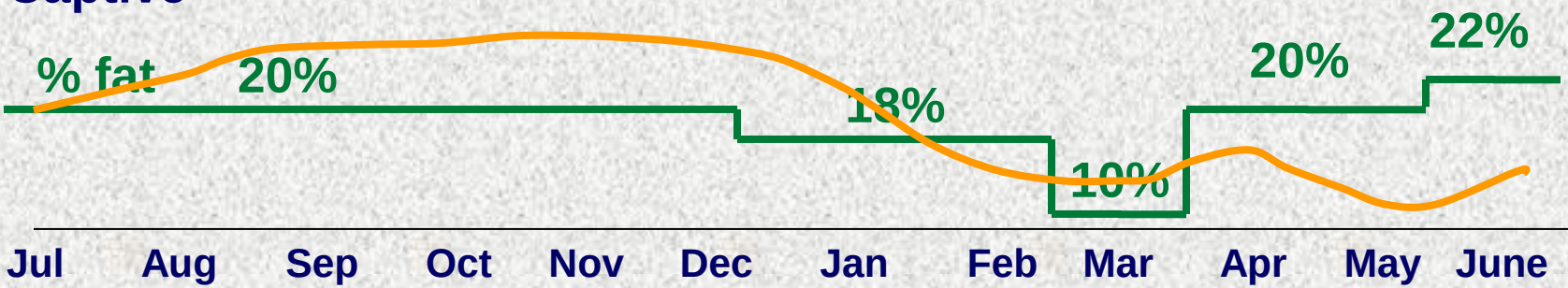
Body fat

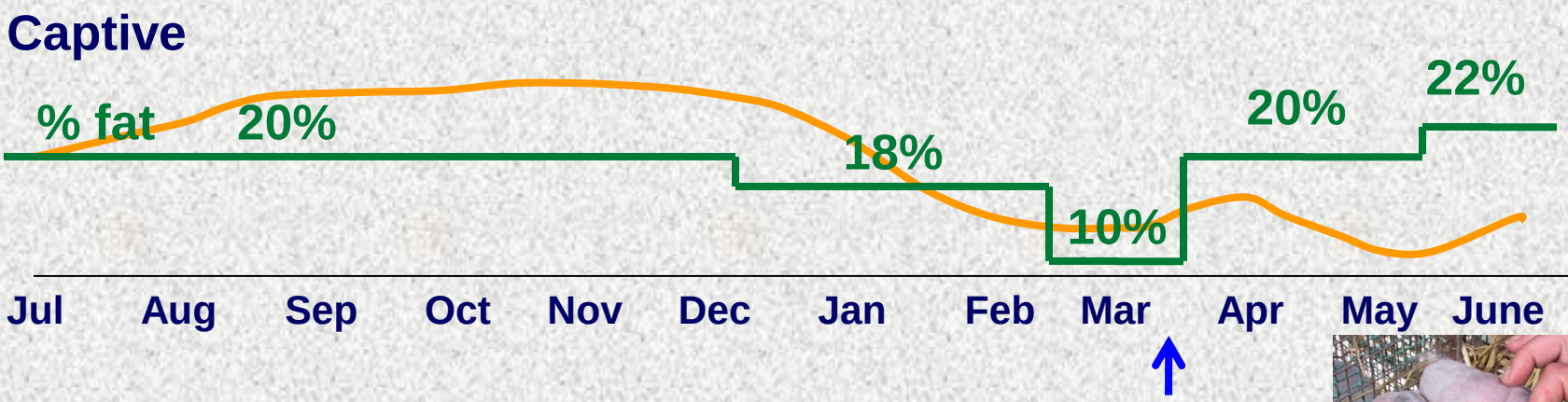


Summer **Fall** **Winter** **Spring**

Breeding (day length, feed) Apr May
Whelping (~ 50 d) May June

Captive





Flushing

Amount of feed

2 weeks, 80% maintenance

1 week 150 % maintenance

→ fertility, litter size

Nutritional signals → Reproduction

Leptin hormone

Objectives

1. To sequence the leptin gene in the mink

2. To identify single nucleotide polymorphisms

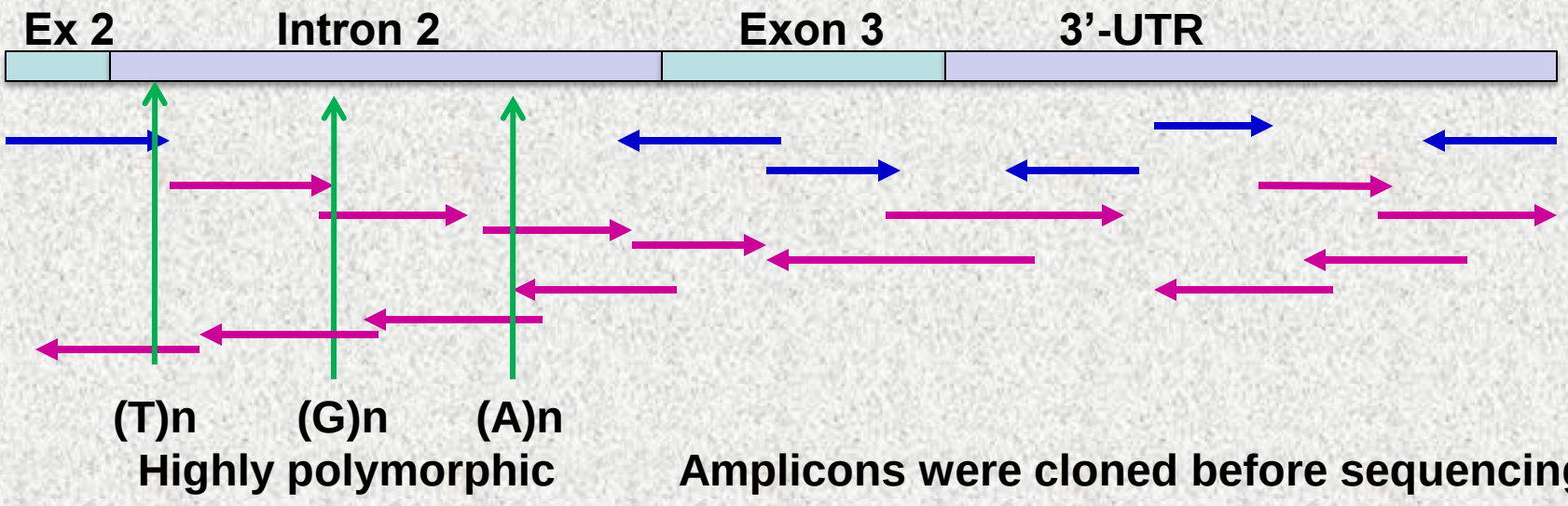
3. To investigate changes that have taken place in the leptin gene as a result of domestication and adaptation to captivity

Materials and Methods

1. Sequencing of the leptin gene

Primers were designed based on the dog leptin gene sequence

PCR amplification & bi-directional sequencing



2. SNP detection

Unrelated mink

Black

Nova Scotia

(20 ranches)

Brown

Nova Scotia

Manitoba

Sapphire

Ontario

Pastel

Nova Scotia

PEI

Wild

New Brunswick

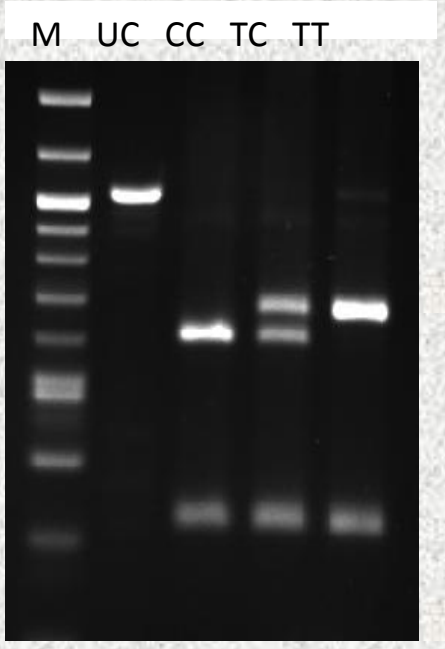
40^2 km

Sequencing: 4 unrelated mink/5 color types

3. Genetic variability

Genotyping: 16 additional mink / color types (20 mink/type)

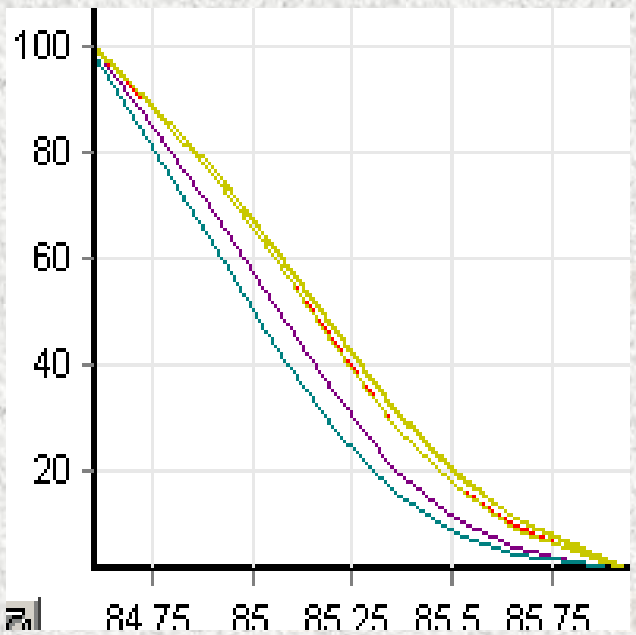
RFLP-PCR



High Resolution Melt

Real time PCR and

SYBR Green I



4. Statistical analysis

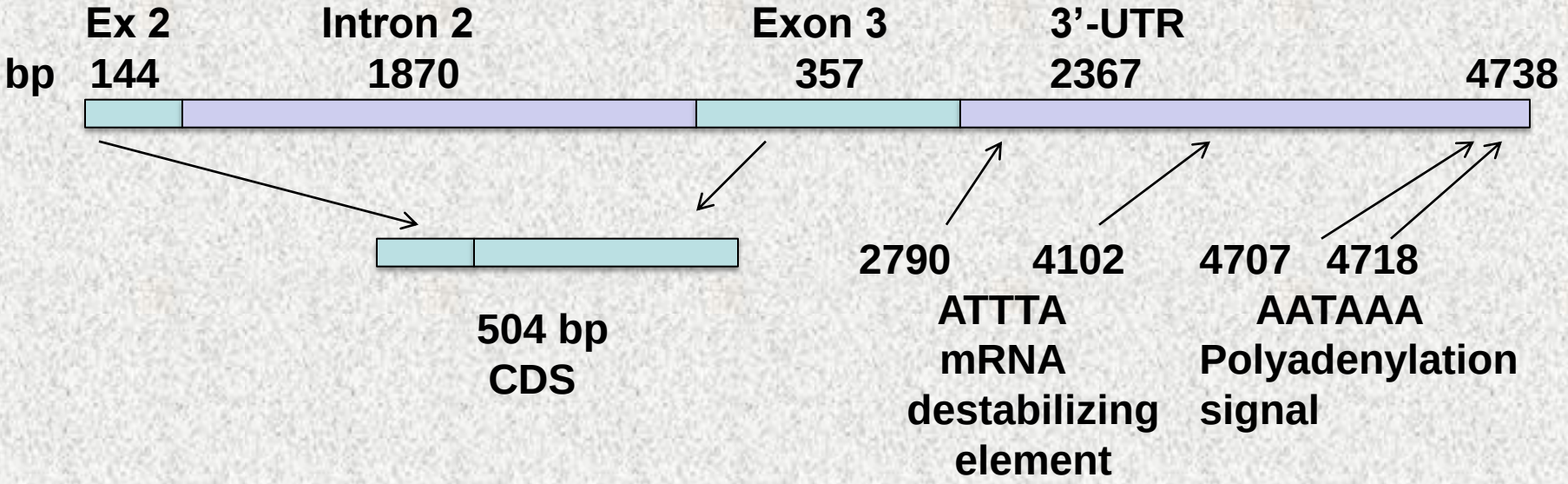
Genotype & allele frequency distributions

Direct count

Pairwise tests for homogeneity of allele frequency distributions

GENEPOP software

Results and Discussion

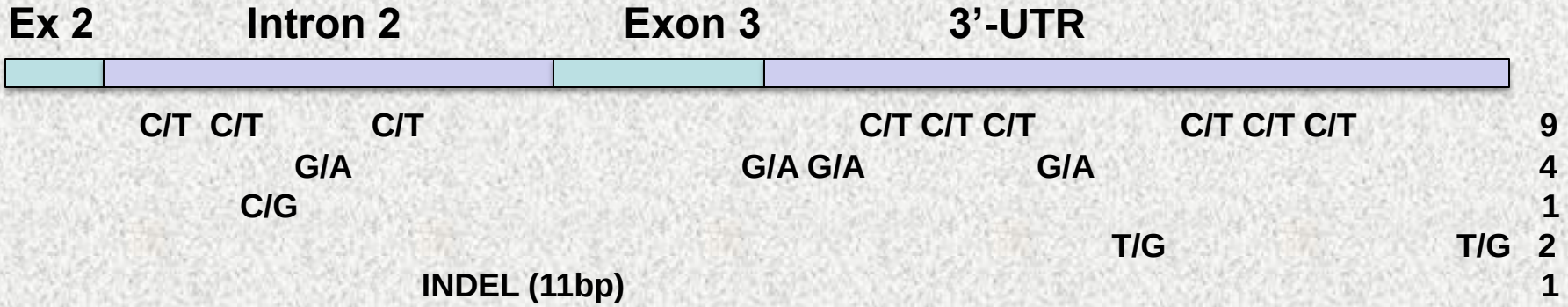


1st ATTTA

~ the same position relative to the stop codon in 10 species

SNP

Inton 2: 6 3'UTR: 11 CDS: 0



Transitional mutations (C ↔ T, G ↔ A): 13

10 at CpG dinucleotides

Methylation-induced deamination of 5-methylcytocine to thymidine

Transversional mutations (C ↔ G, T↔G): 3

3'UTR SNPs mRNA stability

miRNA target sites→expression regulation

Allele frequency distributions

- 32 of 101 comparisons among color types were significant
- 24 involved wild mink

Allele frequency distribution of wild and captive mink

SNP	Allele	Wild	Captive	Dif (Prob.)
$T^{306}T$	T	0.58	0.72	0.14 (0.125)
$G^{1540}A$	G	1.00	0.72	0.28 (0.000)
$C^{1568}T$	C	0.75	0.49	0.26 (0.004)
Del^{1607}	+	0.87	1.00	0.13 (0.000)
$A^{1971}G$	G	0.98	0.64	0.34 (0.000)
$A^{2541}G$	A	0.95	0.98	0.03 (0.346)
$C^{2551}T$	C	1.00	0.97	0.03 (0.587)
$C^{2616}T$	T	0.88	0.23	0.65 (0.000)
$C^{3901}T$	T	0.60	0.96	0.36 (0.000)
$C^{4020}T$	T	0.65	0.83	0.18 (0.028)
$C^{4215}T$	T	0.60	0.96	0.36 (0.000)

Significantly different at 8 of the 11 polymorphic sites

Conclusion

The leptin locus has been under selection pressure in captive mink

Acknowledgements

Financial support received from Nova Scotia Fur Institute and Canada Mink Breeders Association.


Thank You