

SUPPORTING INFORMATION

Deep barriers, shallow divergences: reduced phylogeographical structure in the collared pika (Mammalia: Lagomorpha: *Ochotona collaris*)

Hayley C. Lanier and Link E. Olson

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Appendix S1 Specimen information and degraded DNA amplification

Table S1 Voucher specimens and geography

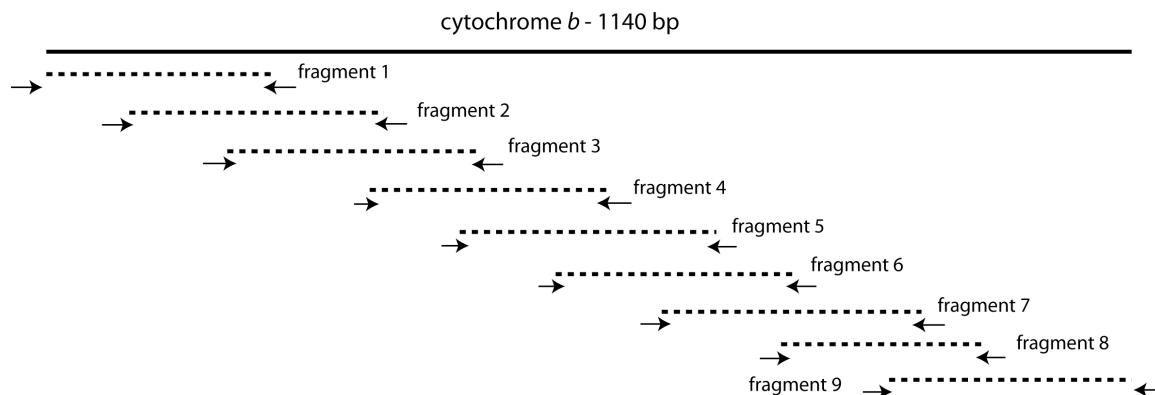
Mountain range, locality ID, population, and museum catalogue number for *Ochotona collaris* samples used in this study. Starred samples (*) from antique sources. Unless otherwise indicated, specimen numbers are catalogue numbers for voucher specimens housed in the University of Alaska Museum's Mammal Collection. AF = Alaska Field number for specimens deposited but not yet catalogued at UAM, CMN = Canadian Museum of Nature, Hik = samples from David Hik (University of Alberta Edmonton), KU = University of Kansas Natural History Museum, USNM = US National Museum of Natural History (Smithsonian).

Mountain Range	ID	Population	Institution & catalogue numbers
Alaska Range	a	Southwest Alaska Range	100773; 100775; 100776; 100795; 100796; 100833; 100834; 100839; 100840; 100848; 100849; 100867; 100870; 100890; 100943; 93191; USNM 13651*
	b	Denali	98296; 102573; 102574; AF64794; 102561
	c	Central Alaska Range	102478; 102481; 102482; 102483; 102486; 102487; 102488; 102490; 102492; 102493; 102494; 102495; 102496; 102497; 102498; 102500; 102501; 102502; 102503; 102504; 102505; 102506; 102507
Chugach Mountains	d	Anchorage	31645; 31646; 31647; 51299; 64363; 102564; 102565; 102566; 102567; 102568
	e	Thompson Pass	100896
	f	Southern Wrangell-St Elias	57694; 102419; 102420; 102422; 102423; 102424; 102429; 102430; 102431; 102432; 102434; 102435; 102438
Yukon-Tanana Uplands	g	White Mountains	63931; 63932; 63935; 63936; 63938; 67030; 102554; 102556; 102557; 102558; 102559; 102560
	h	Yukon-Charley Rivers NPP ¹	58204; 58205; 58206; 58207; 58208; 58211; 58212; 58213; 58214; 58219; 58242; 58243; 58244; 58245; 58248; 58249; 58250; 58251; 58257; 58258; 58316; 58338; 58399; 58400; 58407; 58422; 58444; 58445; 58446; 58448; 58546; 58547; 58548; 58549; 58550; 58551; 58552; 58553

Mountain Range	ID	Population	Institution & catalogue numbers
	i	Ogilvie Mountains	CMN 29410*; CMN 29411*; CMN 30647*; CMN 44998*; CMN 45001*; CMN 45002*; CMN 45006*
Wrangell-St Elias	j	Wrangell Mountains	KU 147383*; 102366; 102416; 56066; 56067; 56093; 56094; 56099; 56814; 57920
	k	Kluane	Hik 1205; Hik 1207; Hik 1326; Hik 1329; Hik 1355; Hik 1385; Hik 1555; Hik 1628; Hik 1649; Hik 431; Hik 441; Hik 446; Hik 492; Hik 499
Coast Mountains	l	Whitehorse	CMN 31161*; 71652
	m	British Columbia	KU 29099*; KU 29101*; 35126; 51896; USNM 127142*; USNM 128581*; USNM 128582*
	n	Canol Road	CMN 30648*
Mackenzie Mountains	o	Northwest Territories	CMN 18106*; CMN 42788*; 88532; 88534
	p	Bonnet Plume Lake	CMN 35314
	q	Keele Lake	CMN 35320*; CMN 35328*
	r	Ross River	CMN 17803*
	s	Little Hyland River	CMN 31167*
Richardson Mountains	t	Horn Lake	CMN 30301*; CMN 30302*; CMN 30304*; CMN 46541*

¹Shown as Yukon-Charley NPP in figures and tables

Figure S1 Degraded DNA amplification strategy



Samples were amplified in nine separate PCR reactions and unidirectionally sequenced using the primers in Table S2 (in order, with first primer pair in Table S2 targeting fragment 1) to yield a high degree of overlap (see Table S2) between all fragments (dotted line) for complete coverage of the cytochrome *b* gene.

Sequence verification

Concatenation of multiple overlapping short fragments to reconstruct longer sequences is fraught with risk, especially in the case of degraded DNA (Olson & Hassanin, 2003). We used resequencing and analytical methods to rule out the possibility of investigator error and inadvertent chimerism. Approximately one-third of the fresh samples were amplified and sequenced twice for verification. All historical samples were amplified and sequenced in separate reactions, with regions of overlap between contiguous products spanning polymorphic sites wherever possible (Olson & Hassanin, 2003). In addition, a 250-bp sliding window approach indicated no relationship between gene region and reticulation (as would be expected if the reticulation was the result of chimerism, a common concern in studies using degraded DNA). This approach involved building a network from sequence data for a 250 bp region in SPLITS TREE (Huson & Bryant, 2006), counting the number of reticulations resulting from that window, shifting the sampling window and re-estimating the network and reticulation count, to yield estimates of the degree of reticulation that can be traced to a particular region of the gene. The reticulation appears to be the result of true homoplasy in our data set, although it is not clear whether it results from parallel mutations or reversals.

REFERENCES

- Huson, D.H. & Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, **23**, 254–267.
- Olson, L.E. & Hassanin, A. (2003) Contamination and chimerism are perpetuating the legend of the snake-eating cow with twisted horns (*Pseudonovibos spiralis*). A case study of the pitfalls of ancient DNA. *Molecular Phylogenetics and Evolution*, **27**, 545–548.

Table S2 Primers used in the amplification sequencing of fresh and antique tissues

Fragment length¹	Overlap²	Primer	Sequence (5' to 3')
238 bp	115	CB-HLF1*	CCA CCG TTG TAG TTC AAC TA
		CB-HLaR1	TGC GCC GTT AGC GTG CAG GTA G
271 bp	166	CB-HLaF2	GAC CTC CCA ACC CCC TCA AAC ATC TC
		CB-HLaR2	CGG TAG CTA TGA CTG CGA ATA ATA
269 bp	116	CB-HLaF3	ACA TCA GAC ACA CTA ACA GCA TT
		CB-HLaR3	CAA GGT CGG TGC CGA TGT ACG GGA
248 bp	153	CB-HLaF4	ACA CCT ACT CAG AAA CAT GGA A
		CB-HLiR2*	AGC CTG TTT CGT GGA GGA AGA GTA
269 bp	170	CB-HLaF5	CCT TCT GAG GGG CAA CCG TAA
		CB-HLaR5	CTA GGG TGA GGA GGA GGG AGAT
253 bp	140	CB-HLiF2*	AGC CAC CCT AAC TCG ATT CT
		CB-HLaR6	GGT TTG ATA TGT GGA GGT GTG
272 bp	148	CB-HLaF7	CAG GAA TCA TCC CAG ACG CAG AC
		CB-HLaR7	TGC TTC GTT GTT TTG ATG TGT G
215 bp	103	CB-HLaF8	GAG ACC CAG ACA ACT ACA CCC
		CB-HLaR8	CAT GTG AGT GTG AGT AGA TCG GC
256 bp	NA	CB-HLaF9	ACT TGG CGG CGT ATT AGC CCT TAT
		CB-HLR1*	GGT TTA CAA GAC CAG GGT A

¹Not including primers

²Overlap (in base pairs) with next fragment (at 3' end)

* Previously published in Lanier & Olson (2009)