Ducks, DNA, and Hemoglobin Proteins of the Andes

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I·A·B

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Fundamental question:

How do organisms adapt to novel, changing, or extreme environments, and if subjected to the same new environment, will different organisms adapt in the same ways?

How do CONVERGENT and PARALLEL EVOLUTION really work?

Answers to this question are hugely important to all of biology

- **Systematics:**

  Convergent and parallel evolution homoplasy
  Biodiversity/speciation

- **Ecology:**

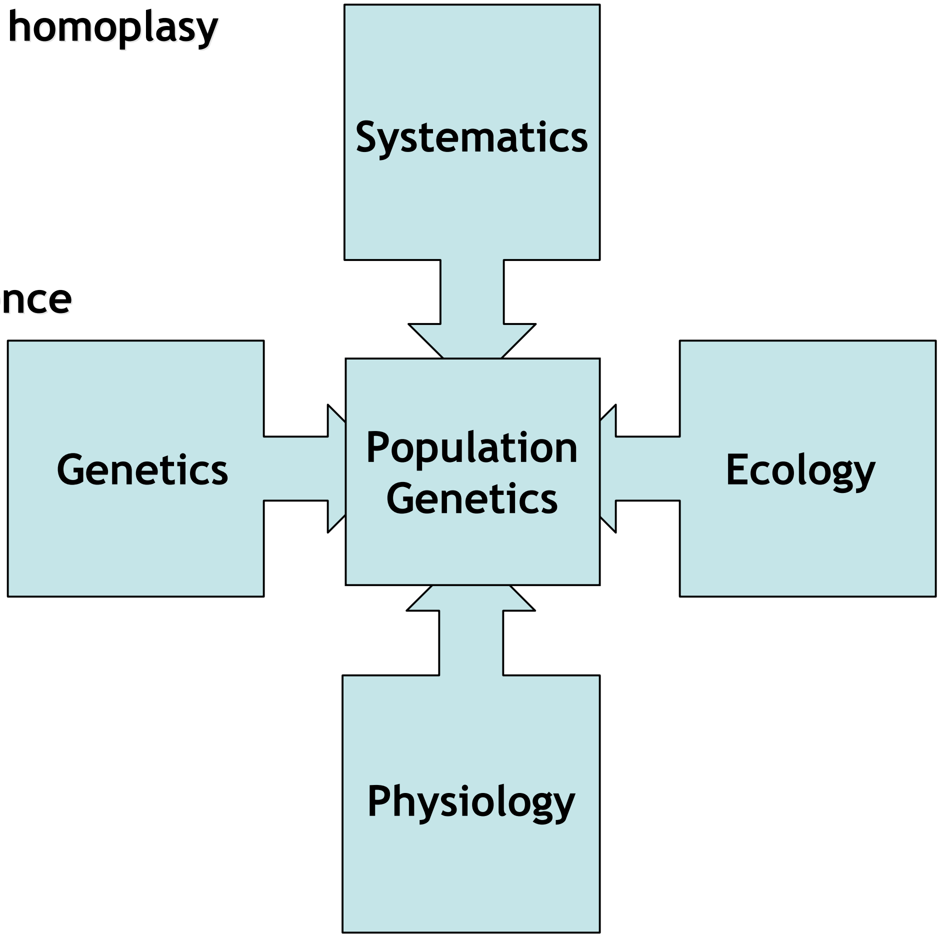
  Adaptive radiation, extinction/resilience

- **Physiology:**

  Mechanisms of adaptation

- **Genetics:**

  Molecular basis of adaptation


Systematics
Genetics
Population Genetics
Ecology
Physiology

South American Ducks in the Andes:

Eight clades of ducks (22 species/37 subspecies) independently colonized and are co-distributed in the same extreme high-altitude environment in the Andes.

Ruddy Duck
(3 subspecies)

Sheldgeese
(6 species)

Torrent Duck
(7 subspecies)

Crested Duck
(2 subspecies)

South American Ducks in the Andes:

Eight clades of ducks (22 species/37 subspecies) independently colonized and are co-distributed in the same extreme high-altitude environment in the Andes.

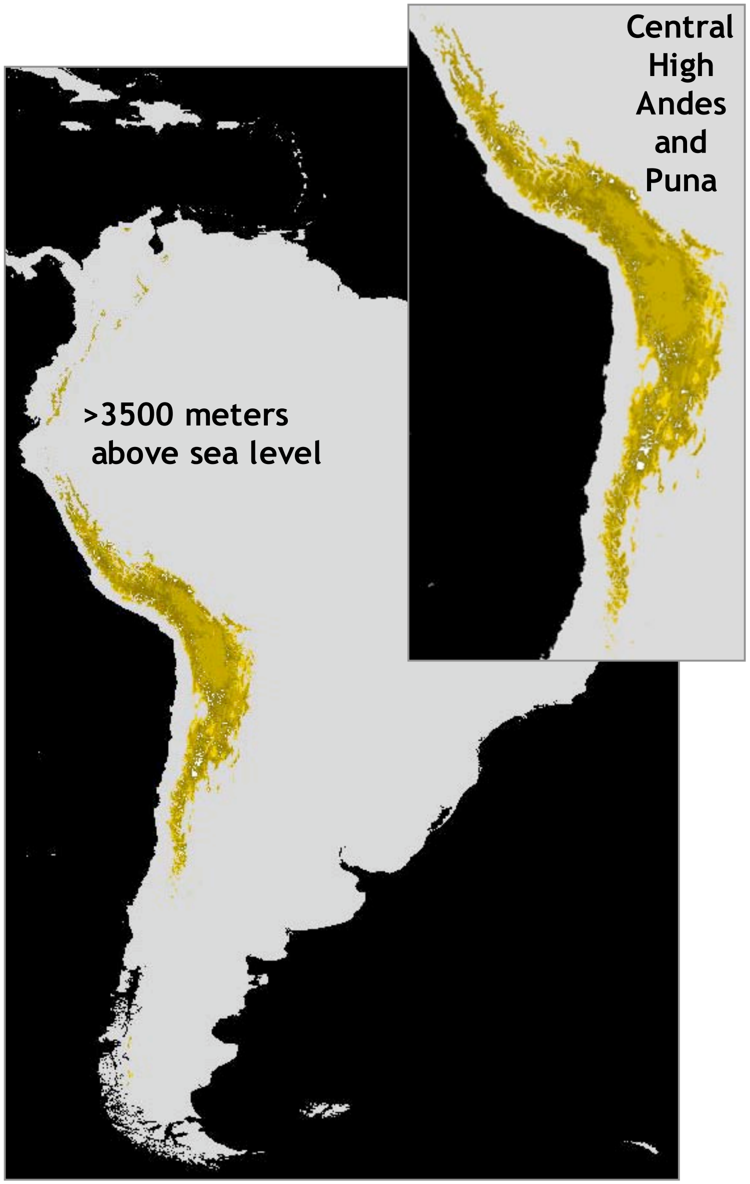
Puna Teal
Silver Teal
(2 subspecies)

Cinnamon Teal
(5 subspecies)
Blue-winged Teal
(2 subspecies)

Yellow-billed Pintail
(3 subspecies)
White-cheeked Pintail
(3 subspecies)

Speckled Teal
(4 subspecies)

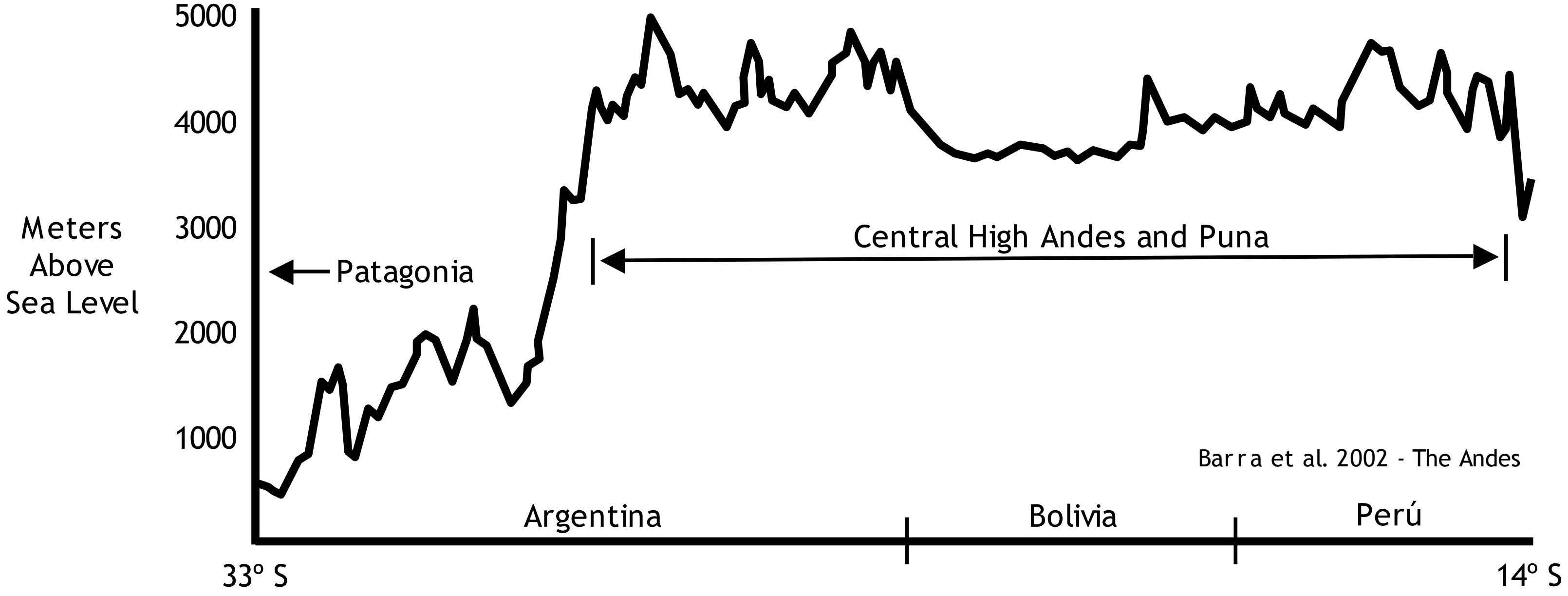
Dpto. Arequipa, Peru



GTOPO30 - Global 30 Arc Second Elevation Data
U.S. Geological Survey

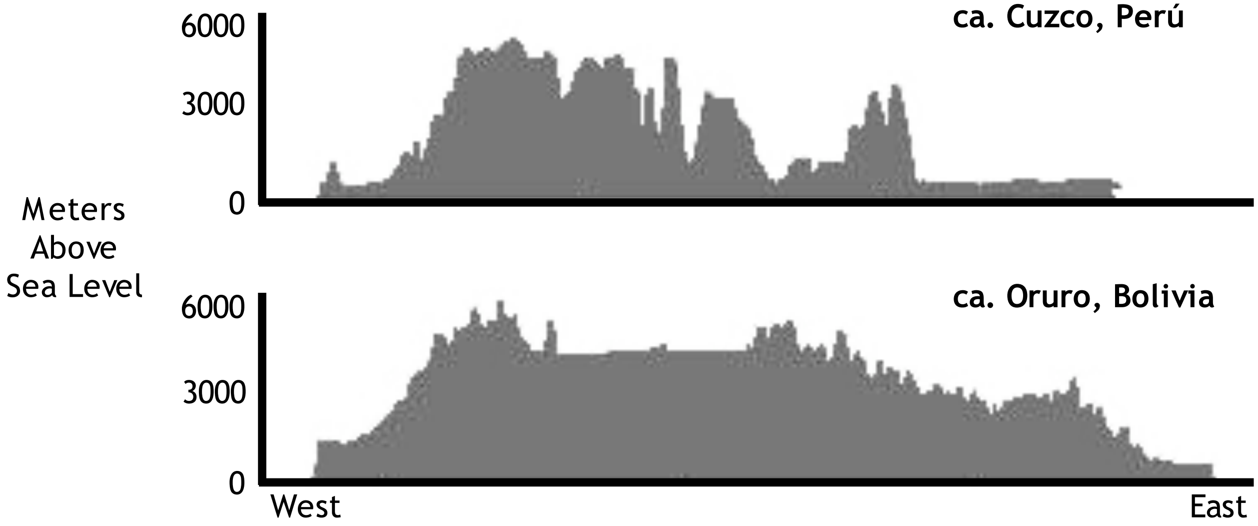
Crested Ducks (*Lophonetta s. alticola*) and Speckled Teal (*Anas f. oxyptera*) - Salta, Argentina

North-South Transect

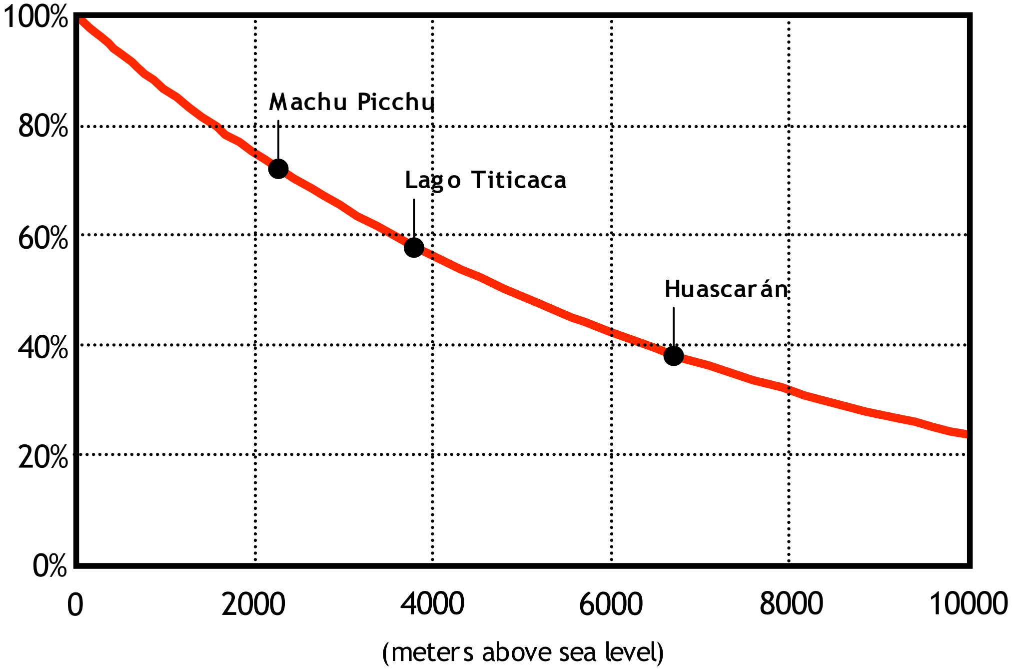


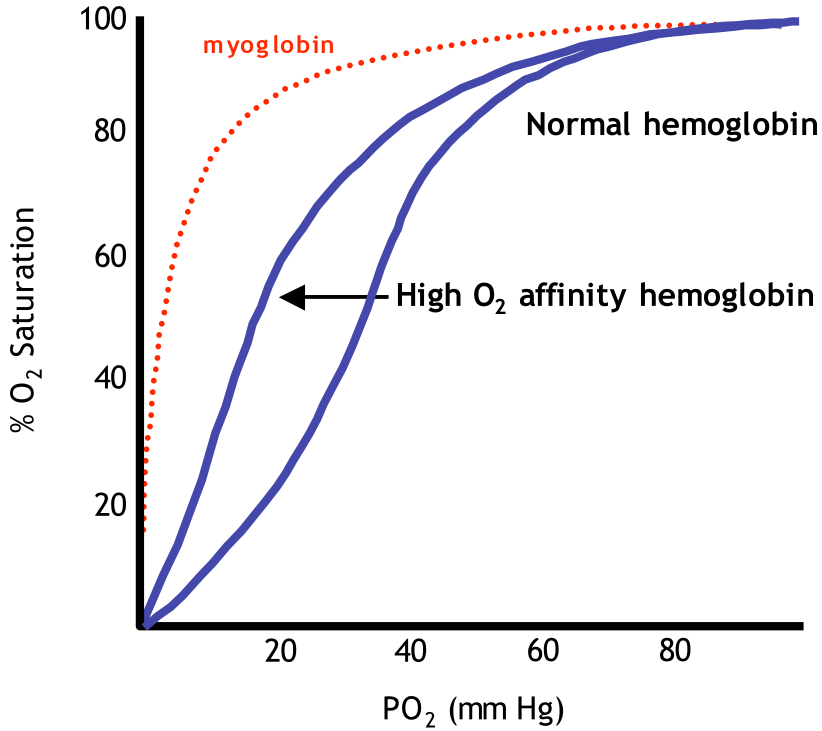
Barra et al. 2002 - The Andes

East-West Transects



Partial Pressure of Oxygen




100
80
60
40
20
% O_2 Saturation
myoglobin
Normal hemoglobin
High O_2 affinity hemoglobin
20
40
60
80
PO_2 (mm Hg)

Hemoglobins---background:

a. Vertebrate hemoglobins are iron porphyrin molecules composed of two □-chain subunits and two □-chain protein subunits (these occur on different chromosomes).

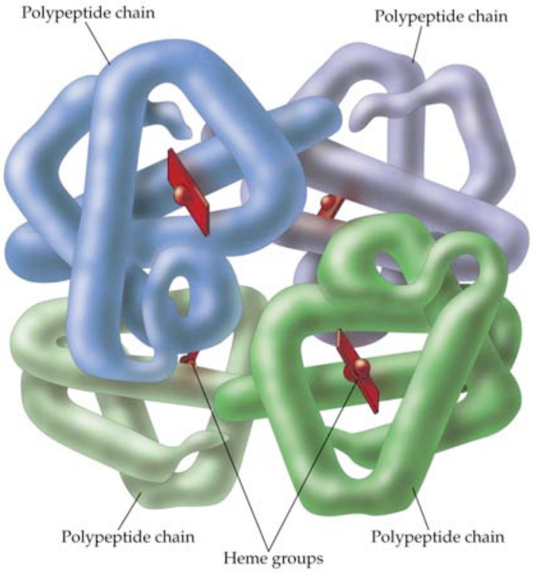
b. Bind and deliver oxygen (e.g., Bohr/root effects). High-altitude species typically show higher oxygen affinity (<P_{50}) with increased oxygen loading at the lungs.

c. Seven hemoglobin genes are expressed in birds:

3 adult subunits:
□-A, □-D, □-A

4 embryonic subunits:
□-□, □-□, □-□, □-H

*linked in the order expressed



How have humans adapted to alpine hypoxia?

a. Humans have independently colonized three high-altitude regions of the world: Himalayas and Tibetan Plateau, East African Plateau, and the South American Andes.

b. No left-shifted O_2 saturation; suggesting that amino acid substitutions of large effect (leading to ↑O_2 affinity) have not occurred in the human adult hemoglobin.

c. Highlanders have responded to chronic hypoxia using other physiological mechanisms:

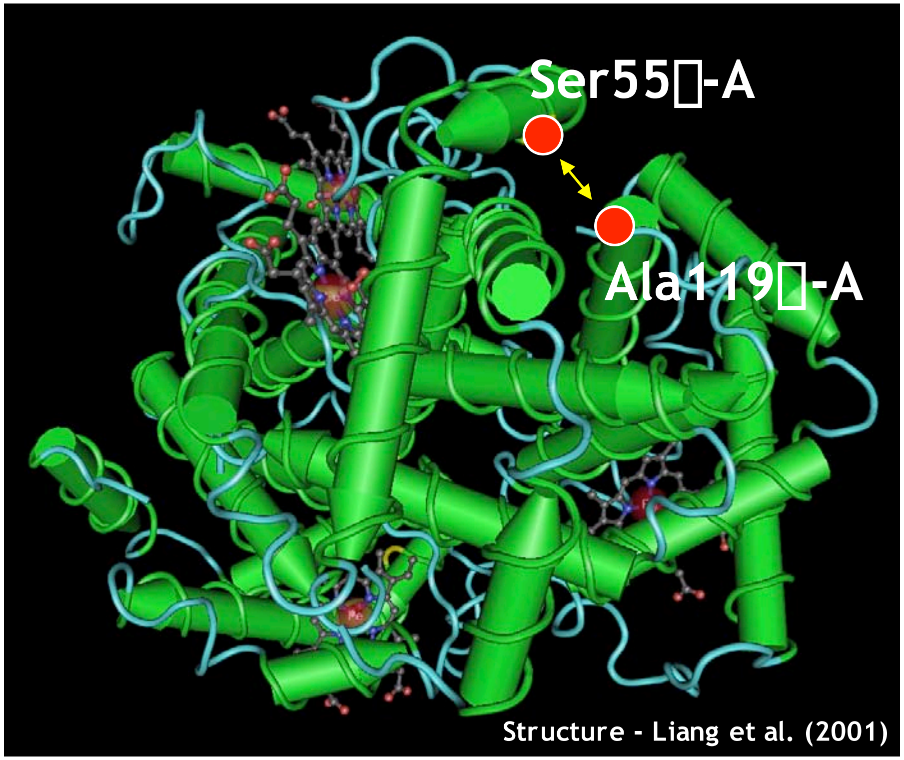
- Reduced HVR to counteract alkalosis.
- Reduced HPVR to minimize hypertension.
- Increased circulatory/blood volume
- Increased erythropoiesis/RBC mass
- Modified fuel preferences (e.g., ↑ATP yield)

Hochachka and Somero (2002) - *Biochemical Adaptation*

How have birds adapted to alpine hypoxia?

a. <u>Pro119□-A ---> Ala</u> increases O_2 affinity in Bar-headed Goose, which inhabits the steps of Asia (Perutz 1983).

b. <u>Leu55□-A ---> Ser</u> results in the same □/□ inter-subunit conformation change in Andean Goose in the Central High Andes (Heibel et al. 1987).



Structure - Liang et al. (2001)

How have birds adapted to alpine hypoxia?

a. Site-directed mutagenesis of the human hemoglobin demonstrated that Ala119□-A and Ser55□-A confer equivalent effects with no detectable changes in other hemoglobin properties (Jessen et al. 1991).

Andean Goose (*Chloephaga melanoptera*)

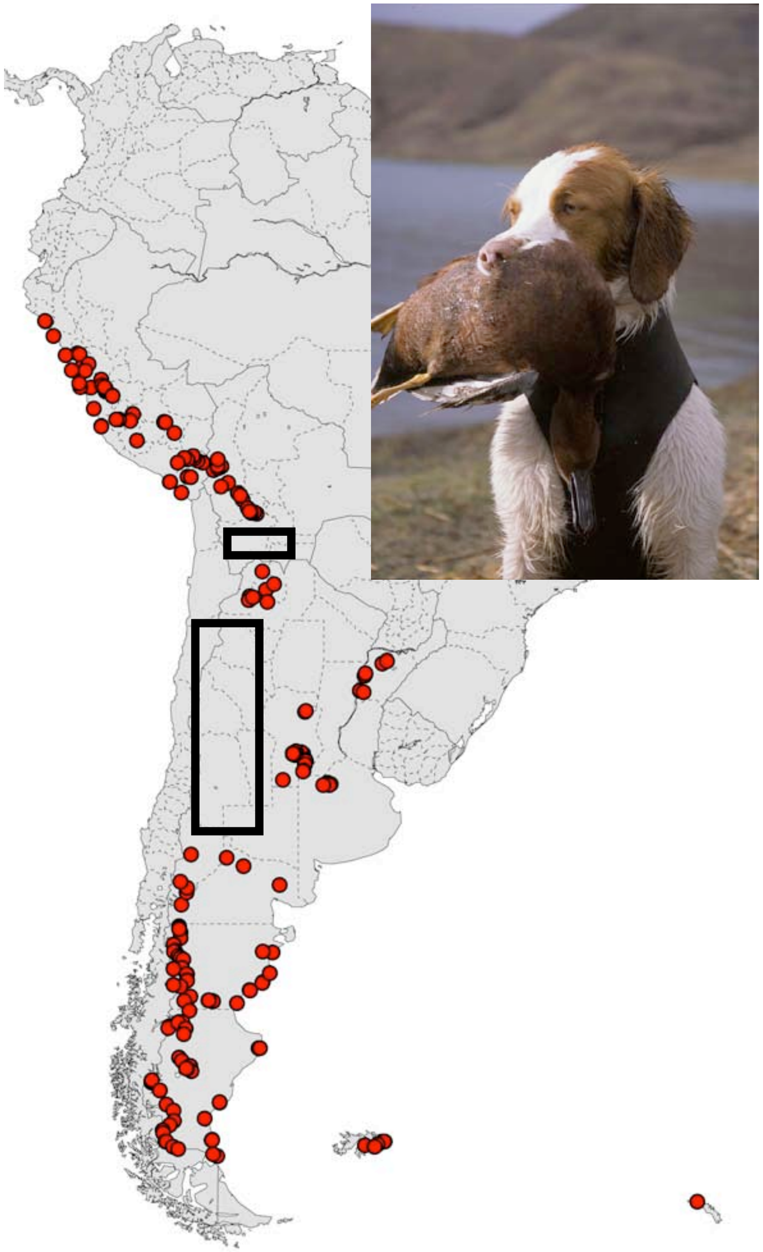
Bar-headed Goose (*Anser indicus*)

So what are we doing collecting ducks in South America?

- Six collecting expeditions to lowland and highland regions of South America since 2001---the "Megatransect".

- Largest series of vouchered waterfowl specimens (>750) collected in >50 years.

- Extensive series of louse ectoparasites, blood parasites, and cloacal swabs.



Initial findings:

- South American duck in Argentina has the "longest bird penis ever" found.
- 42.5 cm (17 inches)! Shaped like a corkscrew, and covered in black spines!

Kevin G. McCracken (Copyright 2000)

Lake Duck (*Oxyura vittata*)

DNA sequencing completed to date:

Locus	Length (bp)	1	2	3	4	5	6	7	8	Other	Total
mtDNA control region	1000	39	-	-	74	222	59	169	109	-	672
▯-A hemoglobin	700	11	15	7	62	172	59	90	109	72	600
▯-A hemoglobin	1600	5	11	6	60	32	59	87	109	57	426
▯-D hemoglobin	900	-	-	-	53	-	-	-	-	13	66
ornithine decarboxylase*	350	-	-	-	-	-	-	169	109	-	278
glyceraldehyde-3-phosphate dehydrogenase*	400	-	-	-	-	-	59	-	-	-	59
lamin A*	300	-	-	-	-	222	59	-	-	-	281

* = introns

Key to clades:

1 = Ruddy Ducks
2 = Sheldgeese
3 = Torrent Duck

Core focus clades:

4 = Crested Duck, Steamer Ducks, Spectacled Duck, Brazilian Teal
5 = Cinnamon Teal, Blue-winged Teal
6 = Puna/Silver Teal
7 = Pintails
8 = Speckled Teal

Questions to be answered:

a. Have the hemoglobins of different Andean ducks evolved convergently or in parallel? How and to what extent has this occurred?

b. Where do these amino acid substitutions occur on the □- and □-subunits (these occur on different chromosomes) and do they lie physically close to each other in the □/□-tetramer; are they complementary?

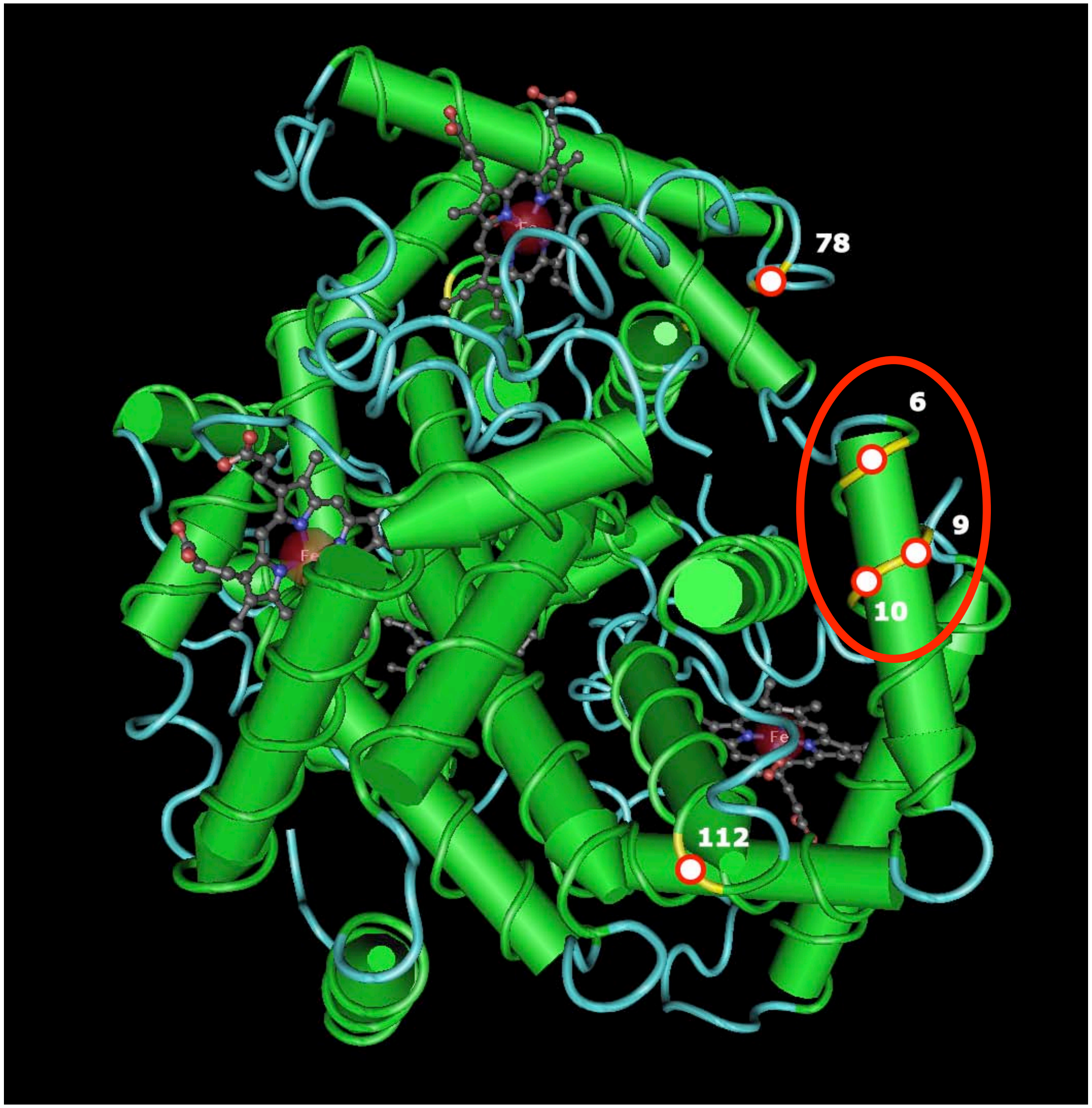
c. What is the magnitude of selection on ‘Andean’ hemoglobin alleles, how are they maintained in populations, and how did they originate (i.e., What can population genetics tell us about the roles that selection, gene flow, and mutation might have played contributing to and reinforcing population subdivision)?

Parallel α-A and β-A-chain amino acid replacements in seven of eight clades of Andean Ducks

Andean Waterfowl and Alleles		**Hemoglobin Amino Acid Position**									
Clade	**Species**	**Alpha-A**					**Beta-A**				
		6	**9**	**10**	**78**	**112**	**14**	**87**	**95**	**117**	**134**
1	*Oxyura j. ferruginea* - Lowland	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Oxyura j. ferruginea - Highland	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
2	Other Sheldgeese	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Chloephaga melanoptera	Ala	Ala	Asn	Thr	Ile	Gly	Ser	Asp	Ala	Met
3	*Merganetta a. garleppi* - 2500 m	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Merganetta a. garleppi - 3200 m	Ala	Thr	Asn	Thr	Ile	Gly	Ala	Asp	Ala	Leu
4	*Lophonetta s. specularioides*	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Lophonetta s. alticola	Thr	Ala	Asn	Ala	Ile	Gly	Ala	Glu	Ala	Met
5	*Anas d. discors*	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Anas c. septentrionalium	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Anas c. orinomus	Ala	Thr	Ser	Ala	Ile	Gly	Ala	Asp	Ala	Leu
6	*Anas versicolor*	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Anas puna	Ala	Thr	Asn	Thr	Thr	Gly	Ala	Glu	Ala	Leu
7	*Anas g. spinicauda* - Lowland	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Anas g. spinicuada - Highland	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ser	Met
8	*Anas f. flavirostris*	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu
	Anas f. oxyptera	Ala	Thr	Asn	Thr	Ile	Ser	Ala	Asp	Ser	Met
	Other Ducks	Ala	Thr	Asn	Ala	Ile	Gly	Ala	Asp	Ala	Leu

High altitude species, subspecies, population, or allele = 🟨

Where do amino acid substitutions occur in the □/□-protein tetramer?



<u>□-A subunit hotspot</u>

<u>Thr6 □-A:</u>
Crested Duck

<u>Ala9 □-A:</u>
Andean Goose
Crested Duck

<u>Ser10 □-A:</u>
Cinnamon Teal

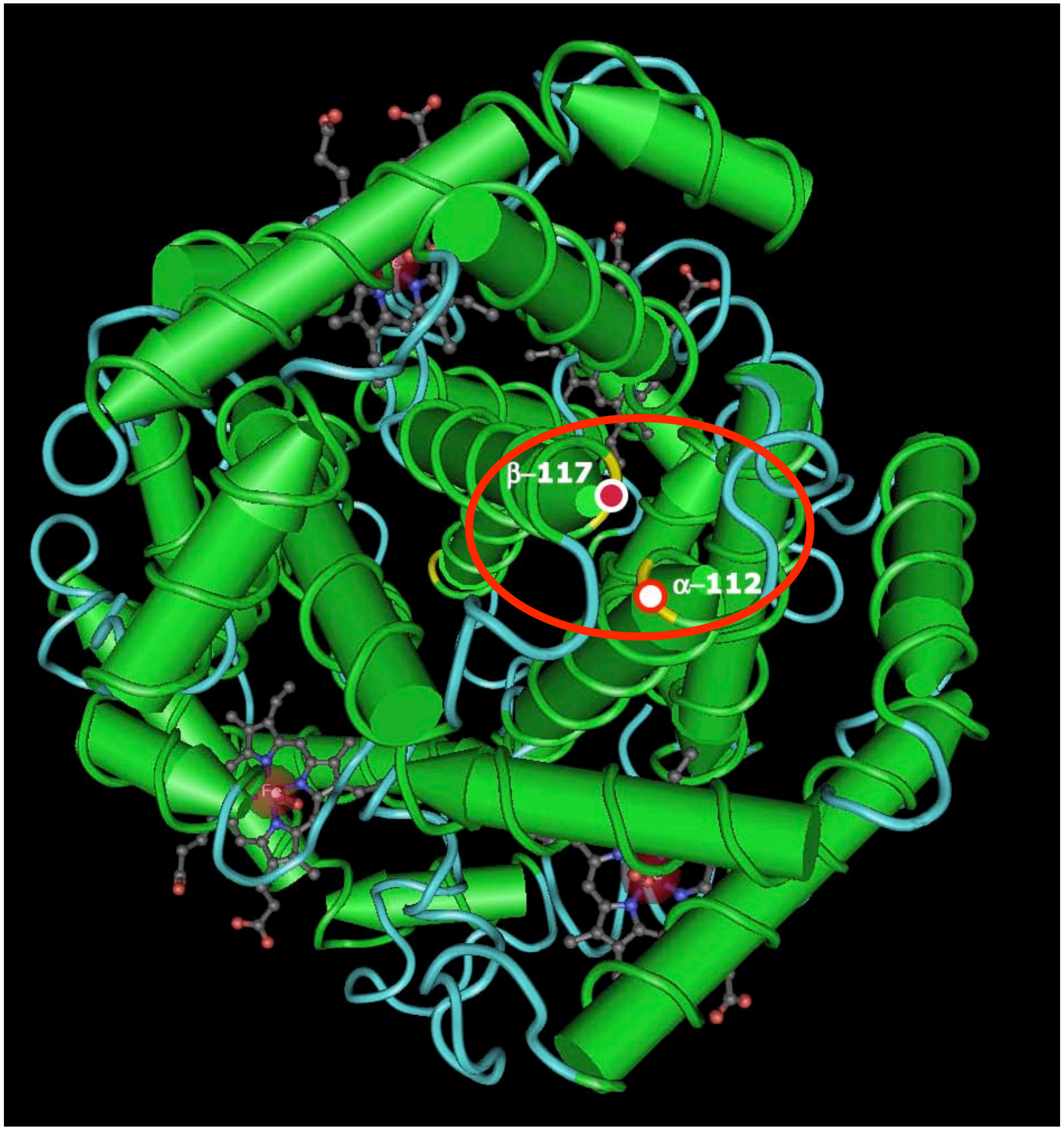
Structure - Liang et al. (2001) The structure of Greylag Goose oxy haemoglobin: The roles of four mutations compared with Bar-headed Goose haemoglobin. Acta Crystallogr D Biol Crystallogr. 57:1850-56.

Where do amino acid substitutions occur in the □/□-protein tetramer?

□-A/□-A subunits interface

Thr112 □-A
Puna Teal

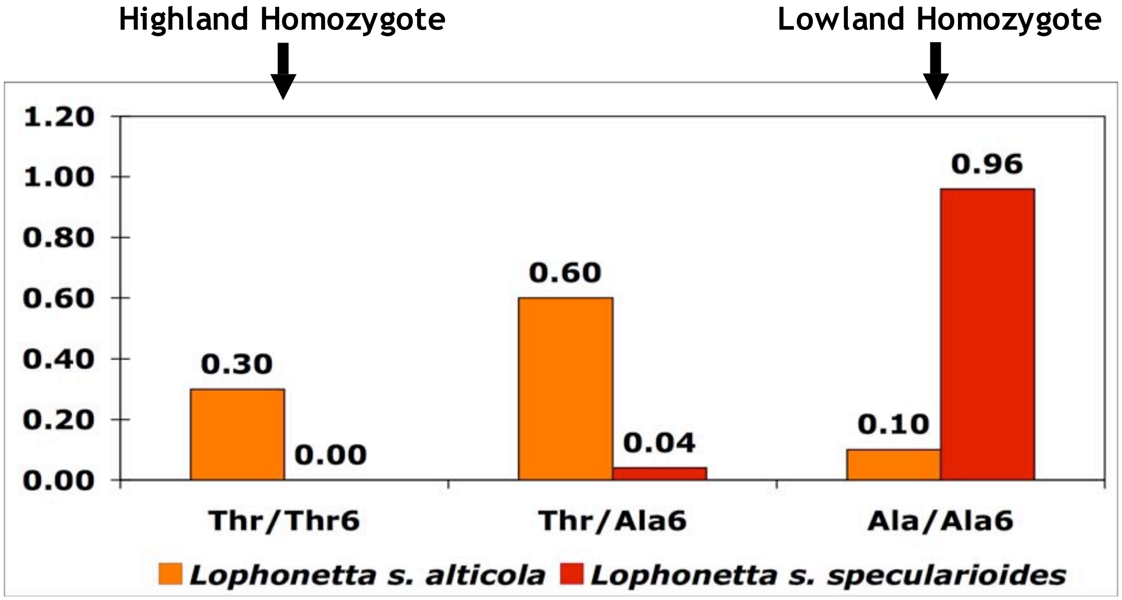
Ser117 □-A
Yellow-billed Pintail
Speckled Teal



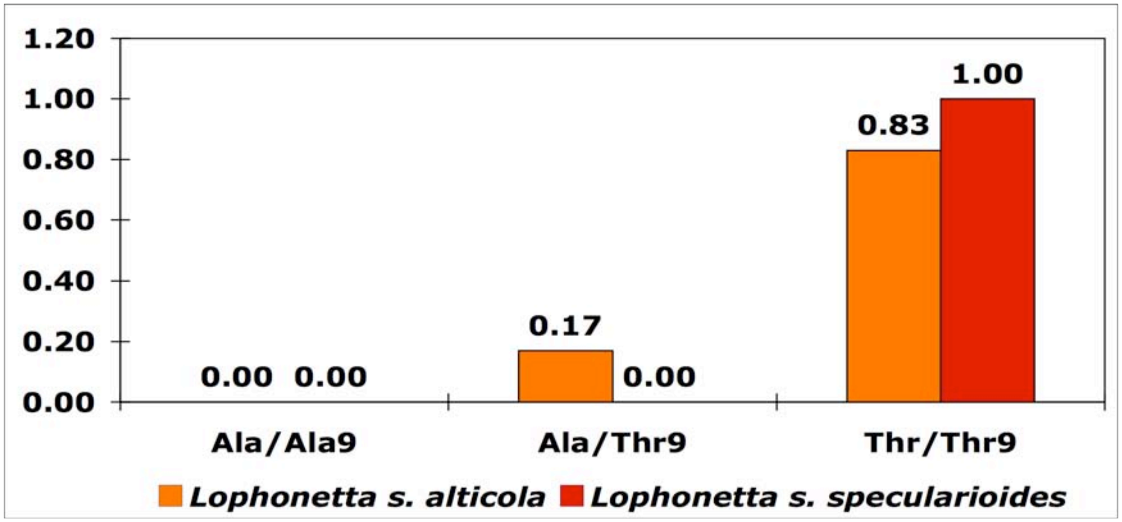
Structure - Liang et al. (2001) The structure of Greylag Goose oxy haemoglobin: The roles of four mutations compared with Bar-headed Goose haemoglobin. Acta Crystallogr D Biol Crystallogr. 57:1850-56.

What is the magnitude of selection on the ‘Andean’ □-A-hemoglobin subunit alleles?

□6
Fst = 0.53

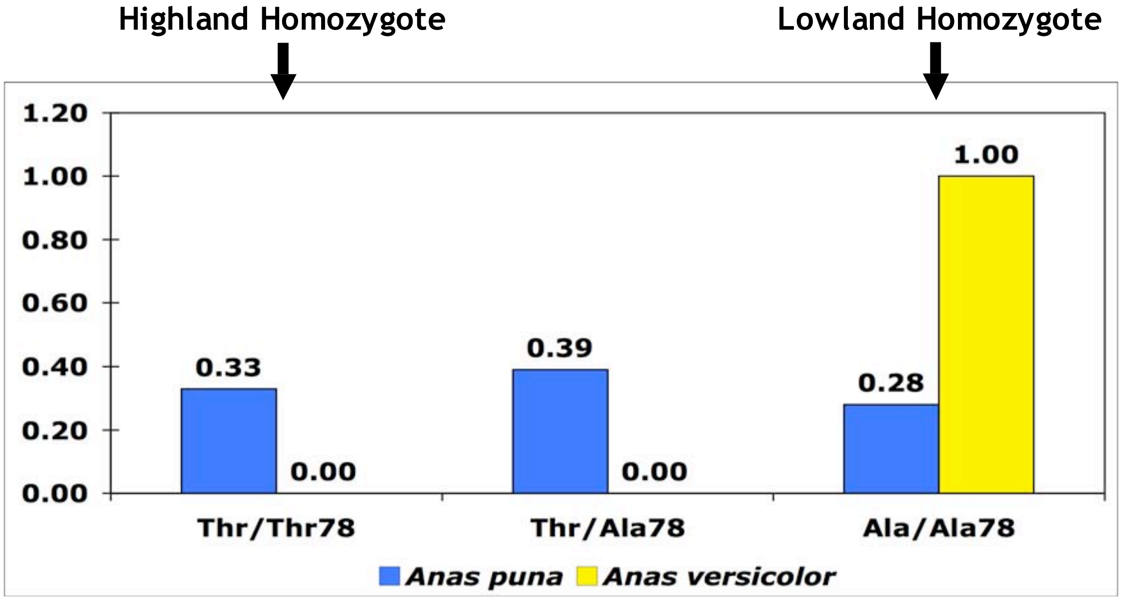


□9
Fst = 0.06

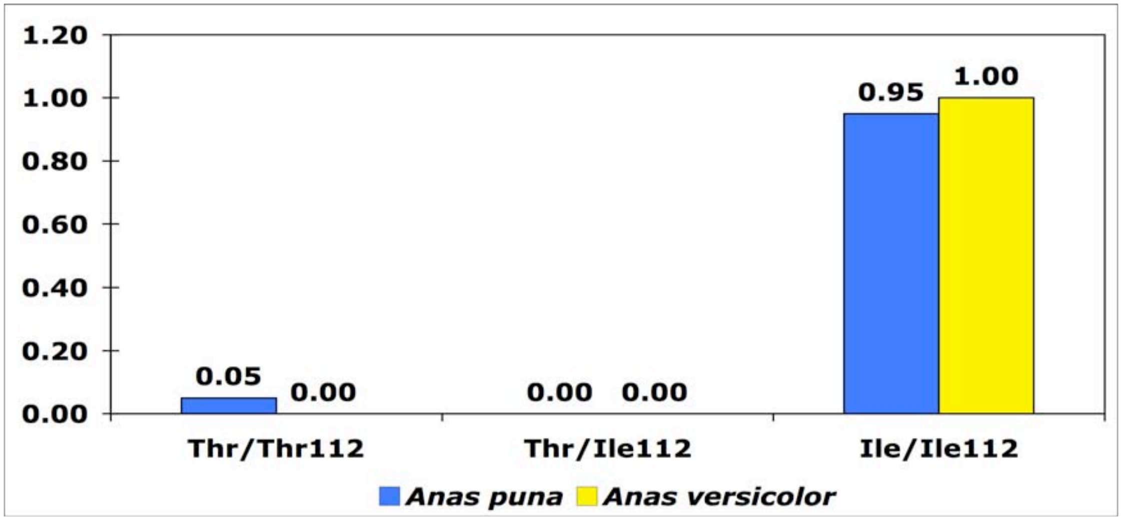


What is the magnitude of selection on the ‘Andean’ □-A-hemoglobin subunit alleles?

□78
Fst = 0.44



□112
Fst = <0.01

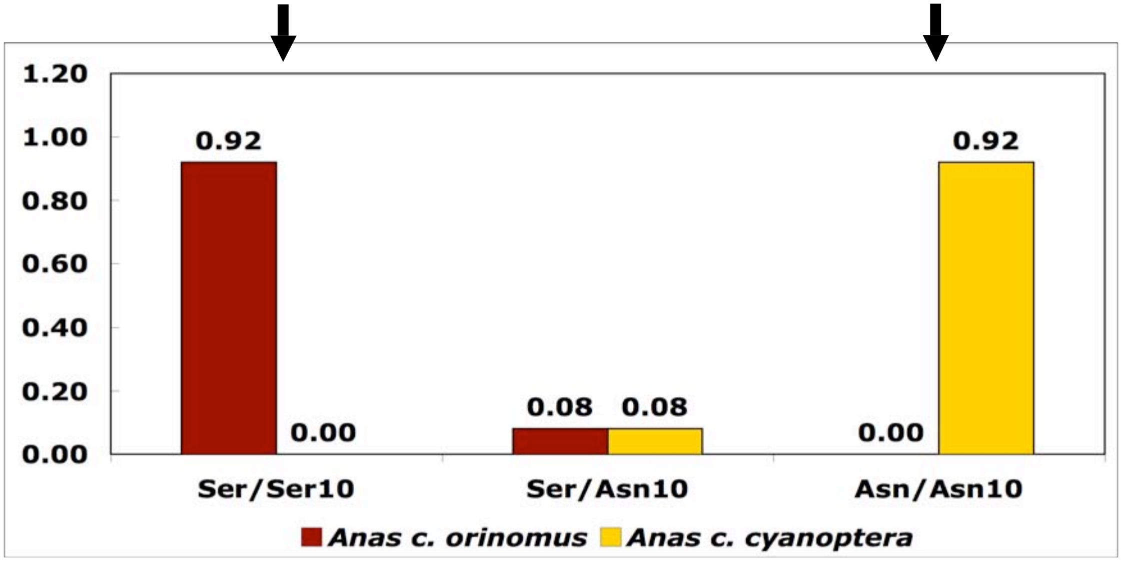


What is the magnitude of selection on the ‘Andean’ □-A-hemoglobin subunit alleles?

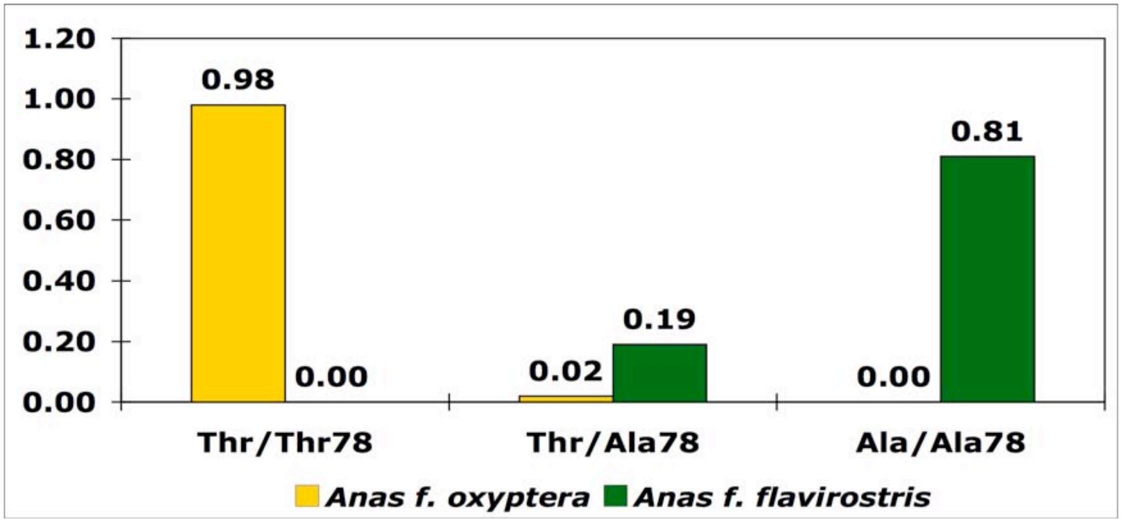
Highland Homozygote

Lowland Homozygote

□10
Fst = 0.91



□78
Fst = 0.89



Duck mtDNA Control Region

Fig. 2A. Speckled Teal

Unweighted most parsimonious tree illustrating 51 mtDNA control region haplotypes (length = 110, CI = 0.77) for three subspecies of Speckled Teal.

🟫 **Central/Northern Andes (Highland)**

🟩 **Patagonia (Lowland)**

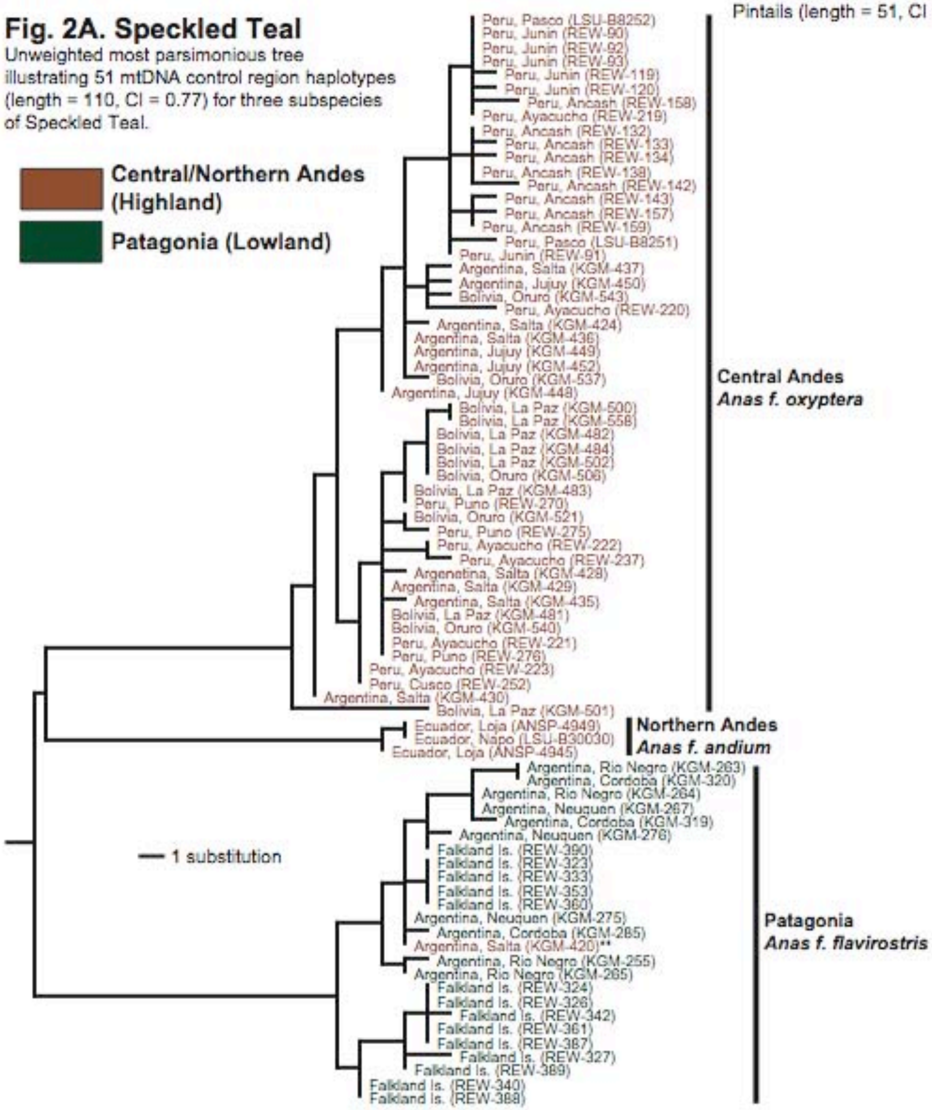
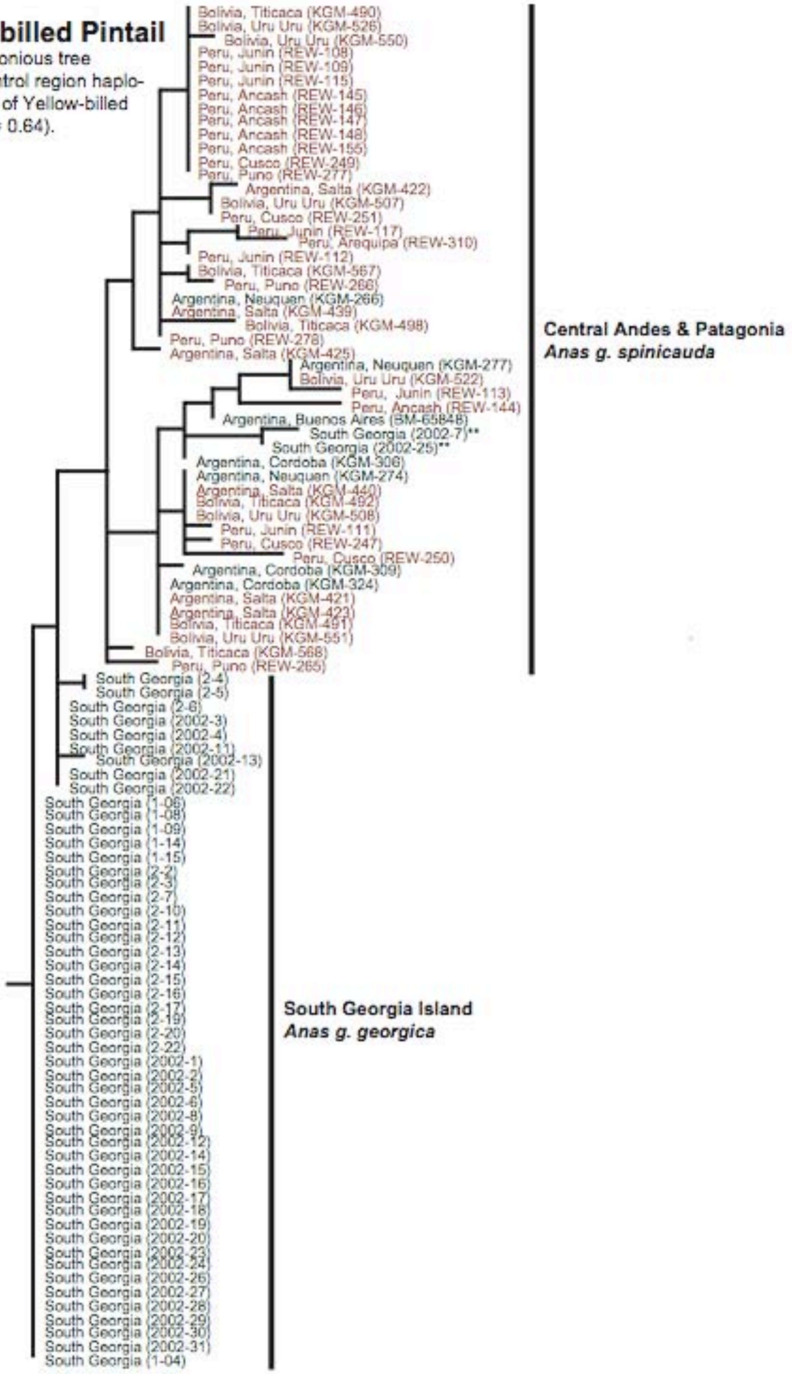
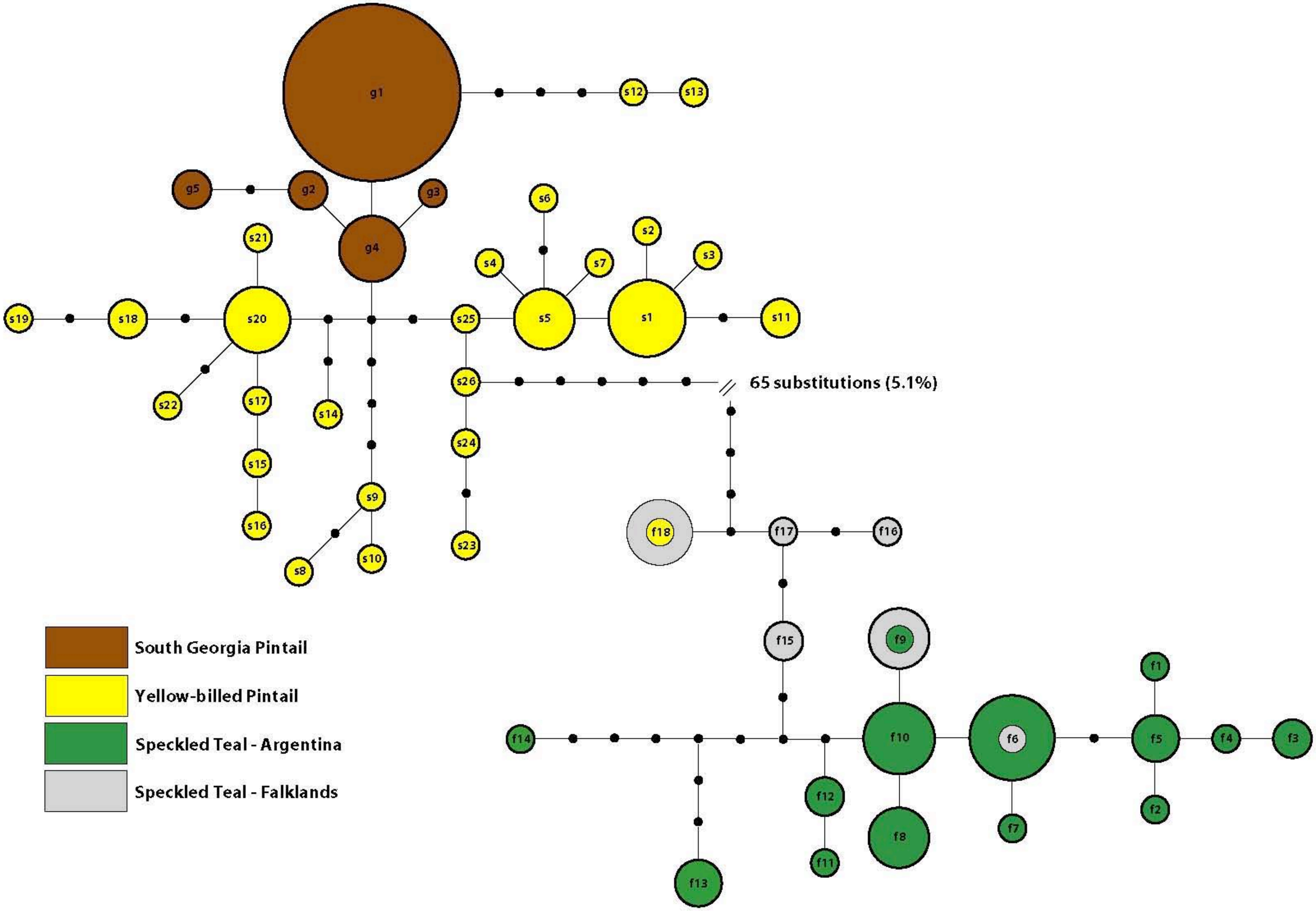


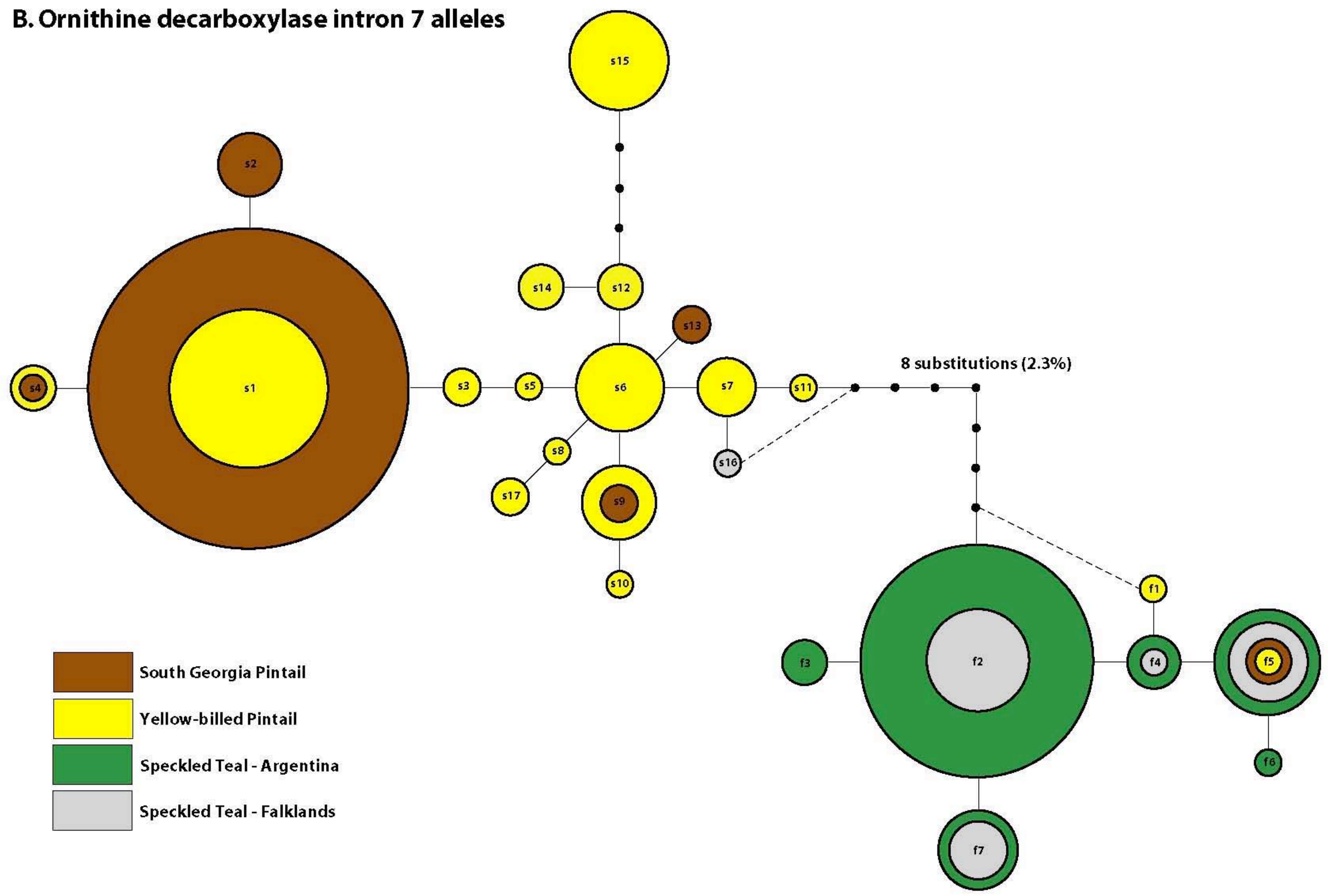
Fig. 2B. Yellow-billed Pintail

Unweighted most parsimonious tree illustrating 30 mtDNA control region haplotypes for two subspecies of Yellow-billed Pintails (length = 51, CI = 0.64).



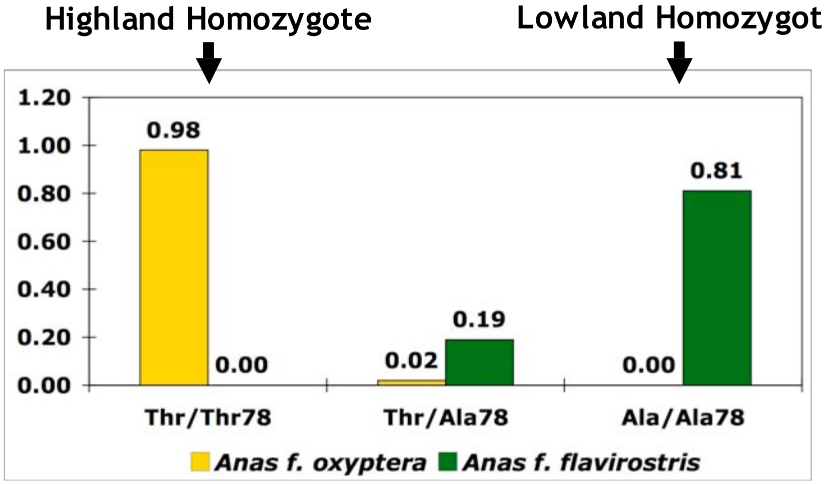


A. mtDNA control region haplotypes



B. Ornithine decarboxylase intron 7 alleles

Highland hemoglobin alleles in the lowlands (and vice versa)

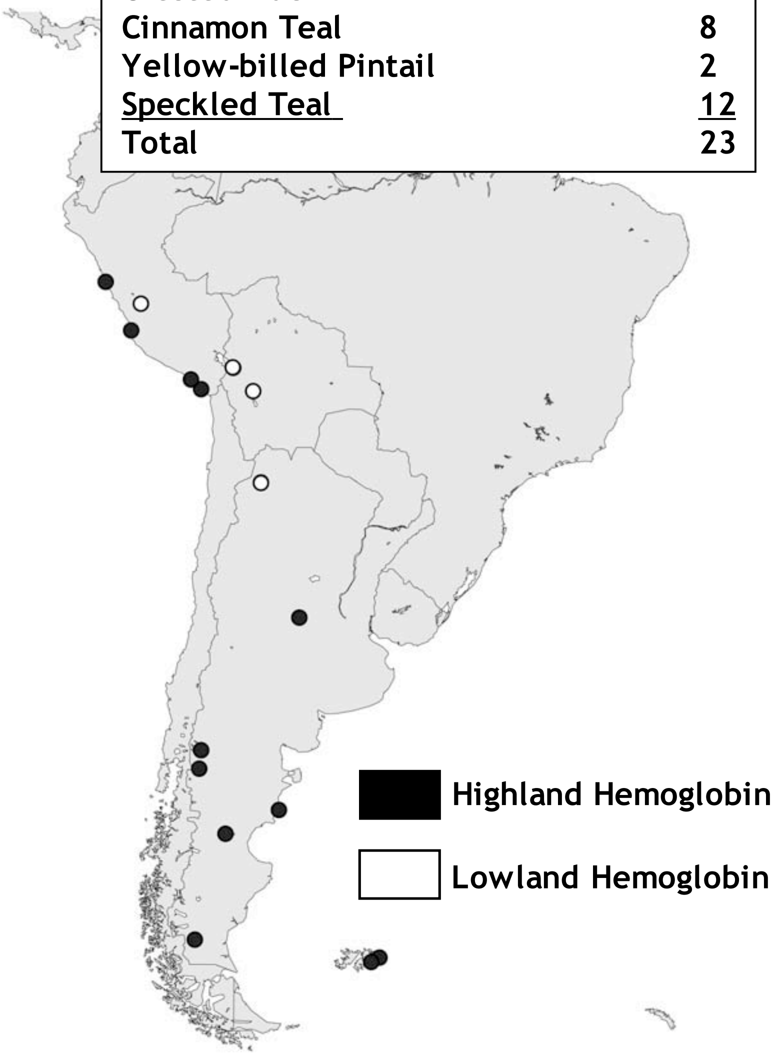


Speckled Teal
□78 Allele Frequencies
Fst = 0.89

<u>H-W equilibrium tests:</u>

Anas f. oxyptera	Yes
(p^2 = 0.0001)	
Anas f. flavirostris	Yes
(q^2 = 0.0081)	
Total population	No ($P < 0.0001$)

Combined Data for:	#s
Crested Duck	1
Cinnamon Teal	8
Yellow-billed Pintail	2
Speckled Teal	12
Total	23



	Crested Duck	**Cinnamon Teal**	**Puna/Silver Teal**	**Yellow-billed**	**Speckled Teal**
Strength of selection on alpha-hemoglobin AAs (Fst)	**MOD/WEAK**	**STRONG**	**MOD/WEAK**	**---**	**STRONG**
Strength of selection on beta-hemoglobin AAs (Fst)	**STRONG**	**---**	**STRONG**	**STRONG**	**STRONG**
Selective sweep - less hemoglobin variation in highlands	**NO**	**YES**	**YES**	**YES**	**YES**
Highland hemoglobin alleles at low frequency in lowlands	**YES**	**YES**	**NO**	**YES**	**YES**
Highland/Lowland - reciprocally monophyletic mtDNA	**YES**	**NO**	**YES**	**NO**	**YES**
Highland/Lowland gene flow detected in mtDNA	**NO**	**(YES)**	**NO**	**(YES)**	**YES**
Interspecidic hybridization observed (mtDNA/nuDNA)	**NO/?**	**NO/?**	**NO/?**	**YES/YES***	**YES/YES***

(YES) = Shared haplotypes possiby due to incomplete lineage sorting

How did the ‘Andean’ hemoglobin alleles originate?

Two (or three) possibilities:

a. Random mutation - colonization of highlands followed by local adaptation and selective sweep for beneficial alleles that increase oxygen binding.

   - ➢ Highland alleles should be rare in non-Andean populations (unless they are redistributed by gene flow between highlands and lowlands).

b. Standing variation (alleles occurring at low frequency) in non-Andean ‘lowland’ populations gives rise to ‘Andean’ alleles.

   - ➢ Highland alleles should occur at low frequency in lowland populations that maintain no connectivity to highland populations.

c. Inter-specific hybridization

   - ➢ Two or more ‘Andean’ species possess identical alleles.

So how did the ‘Andean’ hemoglobin alleles originate?

Three crucial pieces of information:

➢ **Highland alleles occur in the lowlands (and vice versa), but typically at low frequency and for 4 of the 5 species, only in populations that exhibit lowland □ highland gene flow detected in the mtDNA.**

➢ **Highland alleles are completely absent from lowland populations of ducks that are geographically isolated from the Andes (e.g., 70 Cinnamon Teal from North America + 129 □- and □-hemoglobins from non-Andean ducks sampled worldwide).**

➢ **Interspecific hybridization between Yellow-billed Pintail x Speckled Teal was observed on South Georiga and the Falkland Islands, but is uncommon on the mainland and their hemoglobin alleles are distinctly different.**

✓ **‘Andean’ hemoglobin alleles probably originated by random mutation, not standing variation in the lowland populations, or recent inter-specific hybridization.**

Summary/conclusions:

- Seven clades of Andean ducks have evolved amino acid substitutions in the □-A and □-A-hemoglobin subunits, presumably as adaptations to overcome alpine hypoxia.

- Six clades show parallel changes involving 2 to 4 independent acquisitions of the exact same amino acid residue.

- All of these amino acid substitutions resulted from substitution of identical codons (nucleotides).

- Different clades of ducks use different amino acids, but several substitutions occur in the same region of the □/□-tetramer, with ‘complimentary’ changes occurring the □- and □-subunits.

Summary/conclusions continued:

- Genotypic frequencies and Fst/Hardy-Weinberg calculations are consistent with divergent selection and local adaptation to both the highlands and the lowlands.

- The -A subunit shows evidence of varying levels of selection on different amino acid positions. Some sites are under strong divergent selection with ↑Fst, whereas others are under weak selection with Fst.

- Most -A subunit alleles show evidence of strong divergent selection with ↑Fst.

- Highland alleles occur at low frequency in the lowlands (and vice versa), and this pattern results from gene flow between highland and lowland populations.

- ‘Andean’ hemoglobin alleles probably originated by random mutation, not standing variation in the lowland populations, or recent inter-specific hybridization.

Some future directions:

- How do different amino acid substitutions modify hemoglobin oxygen binding properties? Can population genetic data be used to predict which amino acids are of large or small effect?

- What are the selection coefficients on different genotypes?

- How are the different hemoglobin subunits (□-A, □-D, □-A) expressed and combined in different species and highland and lowland environments to create a single functional protein molecule?

- Have gene duplications occurred in Andean ducks?

- How do convergent and parallel evolution work at different hierarchical levels (e.g., among families of birds vs. between birds and mammals)?

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