# Blood proteins of red deer introduced to Patagonia: genetic origins and variability

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**Abstract.** A small group of European red deer (*Cervus elaphus elaphus*) was introduced into the foothills of the Andes in Patagonia in the early 1920s. This species adapted well to the habitat and climatic conditions in the area and presently may number over 100 000 animals. Several indices commonly used to evaluate the fitness of a species in its environment indicate that red deer thrive under very favourable conditions in Patagonia; for example, body size, antler development, reproductive rates, herd health, and longevity are near the maximum described for the species. Furthermore, some local populations occur at densities much higher than encountered in their native ranges. The objective was to examine several biological enzyme systems to test for variance in protein polymorphism in comparison to populations of red deer in other parts of the world. The protein systems examined by electrophoresis in the plasma included: post-transferrin, transferrin, vitamin D binding protein, plasminogen, and complement component; and in the erythrocytes: hemoglobin, superoxide dismutase, glucose phosphate isomerase, and diaphorase I. Variation in plasminogen was lower than is typical for red deer, and glucose phosphate isomerase showed no variation. Furthermore, some occurrences of alleles typical for North American wapiti (*Cervus elaphus canadensis*) indicate that the introduced deer originated from English or European deer parks which have had a history of introductions of wapiti in the past. In New Zealand, the superoxide dismutase allele typical for wapiti was found in 1% of red deer, whereas it occurred in 11% of animals in the present study. Polymorphism measured across the nine examined protein systems was 2.0 alleles per locus with an overall heterozygosity of 0.30. The low variations are likely the result of the introduction based on few individuals. However, the outstanding performance of the present population contradicts the existence of any overt impact from this founder effect. The observed large body sizes may not only be due to good environmental conditions, but also due to previous hybridisation with wapiti. Several specimens were heterozygous and one specimen was homozygous for wapiti hemoglobin.

**Additional keywords:** *Cervus elaphus*, founder effect, electrophoresis, invasion, wapiti.

## Introduction

European red deer (*Cervus elaphus elaphus*), 14 females and 6 males, were initially introduced to La Pampa province, Argentina, between 1902 and 1906 (reviewed in Flueck and Smith-Flueck[1]). A small group of those red deer was then taken to the foothills of the Andes in Patagonia in the 1920s and released in 1922. That population also provided deer for a second foothill enclosure in 1924 with releases occurring in 1926. These two foothill releases resulted in the population sampled in this study. This species had adapted well to habitat and climatic conditions in the area, was already widespread by the 1940s, estimated at 8000 animals by 1951,[2] when control culling began due to competition with livestock. Red deer were declared a pest species in the province of Neuquén in 1959,[1] and through continued expansion their numbers were later estimated at 100 000 or more (Fig. 1).[3] Several indices commonly used to evaluate the fitness of a species in its environment indicate that red deer thrive under very favourable conditions in Patagonia. For instance, body size, antler development, reproductive rates,

herd health, and longevity in low density populations are near the maximum described for the species.[4–6] Furthermore, some local populations occur at densities much higher than encountered in their native ranges.[3,4,7] The introduction was based on 20 deer and raises the question how the genetic background relates to the apparent success of the population. The objective was to examine several biological systems to test for variance in protein polymorphism in comparison to disjunct populations of red deer in other parts of the world.

## Materials and methods

### Study area

The study site is located in the province of Neuquén (40°58′S, 71°12′W), Argentina (Fig. 1). The topography is primarily mountainous with most features formed by glacial and volcanic processes. The dominant climate is temperate with main precipitation occurring between April and September. There is an abrupt precipitation gradient from west to east due
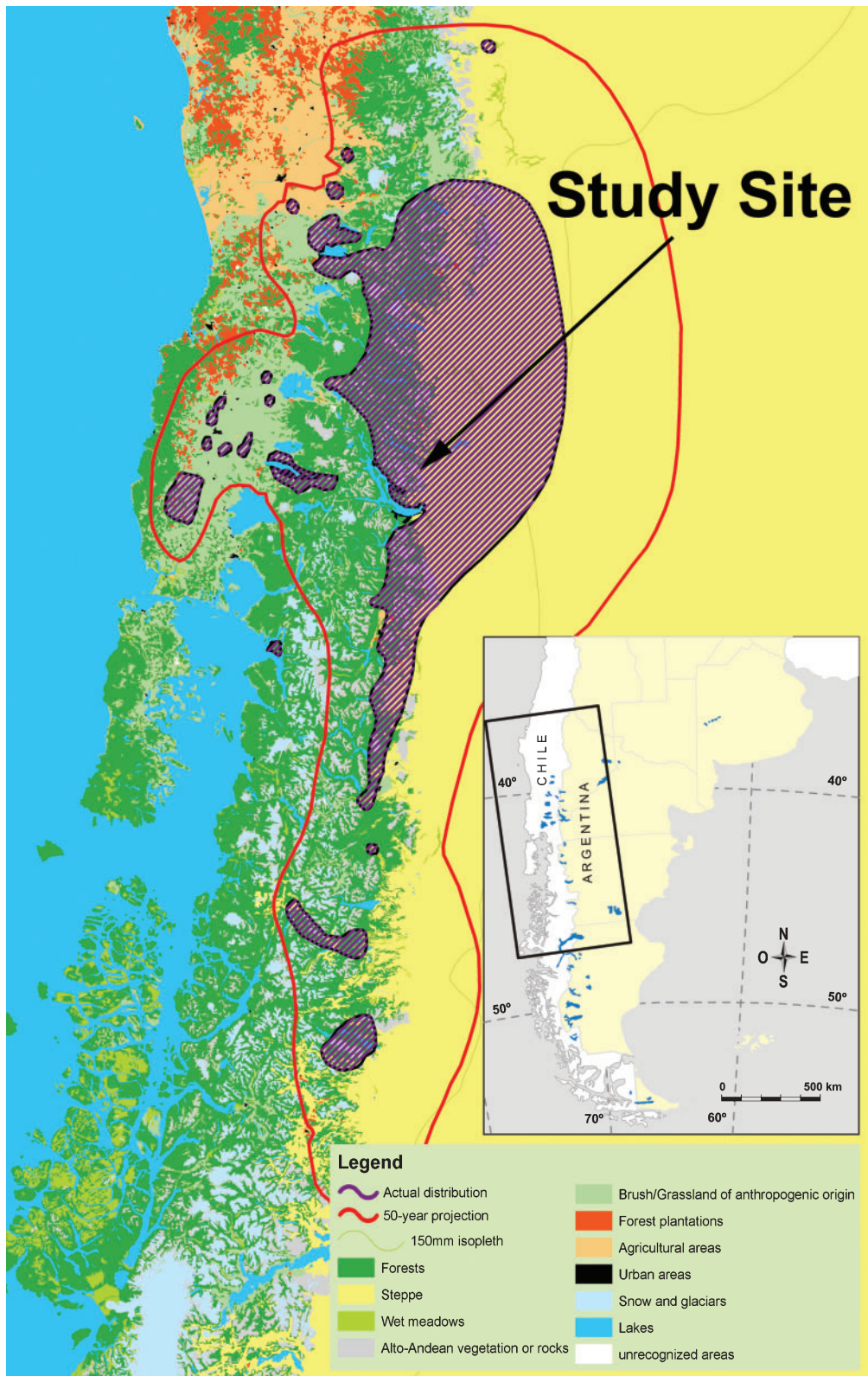
**Fig. 1.** The extent of the red deer distribution (ca. 2000) and location of the study site.

to the orography of the Andes which results in a strongly defined vegetation structure and floristic composition. The study site is between 900 and 1200 m elevation and represents the ecotone between forests and steppe. Patches of forests are characterised by ñire (*Nothofagus antarctica*) and cypress (*Austrocedrus chilensis*) at lower elevations and are replaced by lenga (*Nothofagus pumilio*) at higher elevations. Forest patches at lower elevations alternate with wet grasslands (mallines) with abundant growth of herbaceous plants whereas at high elevation they are replaced by grass-dominated steppe. Riparian areas also contain galleries of tree like *Lomatia hirsuta*, *Maytenus boaria* and *Schinus patagonicus*.

### Red deer population

The area used for collecting deer was ~10 km$^2$ with a population density estimated at 50 deer/km$^2$.[7] Females older than 1 year were approached by stalking and collected at first sight using a rifle without regard for age or size, between late autumn and early spring. All females were part of social groups, the smallest groups consisting of a female with a yearling female and possibly a calf, but typically, group size was between 5 and 20 deer. The average age of collected deer was of 8.3 (s.d. 4.6) years as judged by tooth replacement and wear, using a reference jaw set based on cementum annuli analysis.

### Collection of blood samples and measurements

Blood was collected in heparinised tubes through cardiocentesis from free-ranging adult females immediately after being neck shot. Plasma was separated by centrifugation (400$g$/15 min) and erythrocytes were washed twice in physiological saline and centrifuged. All samples were kept frozen in liquid nitrogen until processed in the laboratory. Alkaline horizontal PAGE was used to separate deer plasma proteins following Gahne *et al.*[8] and as modified by Tate *et al.*[9] and Tate and McEwan.[10] The protein systems examined by electrophoresis in plasma included: post-transferrin (PTF), transferrin (TRF), vitamin D binding protein (GC), plasminogen (PLG), and complement component $C_3$ (C3); and in erythrocytes: hemoglobin (HB), superoxide dismutase (SOD), glucose phosphate isomerase (GPI), and diaphorase I (DIA). Allele frequencies, heterozygosity and the deviation from the Hardy–Weinberg (H–W) equilibrium were calculated. To evaluate population differentiation we calculated the $G_{st}$[11] (expected heterozygosity H for the total population minus H for a subpopulation divided by H for the total population) based on the different subpopulations with allele frequencies reported in several studies, as well as by combining data from other red deer populations to contrast these averages with the present study.

## Results

TRF is polymorphic with 2–4 alleles in this species.[12] However, in the present study, and several populations from New Zealand, only 2 alleles are described.[9] The allele distribution in this study indicates no significant deviation from the expectations of H–W equilibrium ($\chi^2 = 0.011$, $P = 0.92$), with frequencies of allele A and B of 0.61 and 0.39, respectively (Table 1), and an observed heterozygosity of 0.49.

**Table 1. Transferrin (TRF), vitamin D binding protein (GC) and plasminogen (PLG) allele frequencies in four New Zealand sites (A–D) and Patagonia[9]**

|  | Site A | Site B | Site C | Site D | This study |
|---|---|---|---|---|---|
| *TRF allele frequency* | | | | | |
| *n* | 48 | 82 | 20 | 55 | 41 |
| Allele A | 0.72 | 0.56 | 0.70 | 0.49 | 0.61 |
| Allele B | 0.28 | 0.44 | 0.30 | 0.51 | 0.39 |
| *GC allele frequency* | | | | | |
| *n* | 48 | 82 | 20 | 55 | 41 |
| Allele A | 0 | 0.13 | 0.25 | 0.39 | 0.05 |
| Allele B | 0.27 | 0.54 | 0.73 | 0.36 | 0.41 |
| Allele C | 0.73 | 0.33 | 0.02 | 0.25 | 0.54 |
| *PLG allele frequency* | | | | | |
| *n* | 48 | 82 | 20 | 55 | 41 |
| Allele A | 0 | 0.33 | 0 | 0.01 | 0 |
| Allele B | 0.57 | 0.52 | 0.75 | 0.89 | 0.62 |
| Allele C | 0.02 | 0.07 | 0.20 | 0.02 | 0 |
| Allele D | 0.13 | 0.08 | 0.05 | 0.08 | 0.33 |
| Allele E | 0.28 | 0 | 0 | 0 | 0.05 |

GC is polymorphic with 3 alleles in this species, with the frequency distribution in this study falling within the variance found among several populations from New Zealand (Table 1).[9] The heterozygosity observed here was 0.51.

PLG is polymorphic with up to 7 alleles in this species, with frequencies found to vary among several populations from New Zealand (Table 1).[9] The present results indicate reduced variability as only 3 alleles were present, with an observed heterozygosity of 0.51. The populations surveyed in New Zealand revealed 5 of the known alleles.[9]

PTF is polymorphic with 2 alleles in this species. However, 1 allele is found typically only in North American wapiti (*Cervus elaphus canadensis*), while the other is found only in European red deer.[13] Our samples ($n = 41$) were all monomorphic and typically European red deer (Table 2).

C3 is polymorphic with 3 alleles in this species.[13] The distribution of the 2 alleles found in this study indicates no

**Table 2. Complement component $C_3$ (C3), post-transferrin (PTF) and glucose phosphate isomerase (GPI) alleles in wapiti, red deer and hybrids in New Zealand,[13,14] and red deer in Patagonia**

|  | *n* | Allele A | Allele B | Allele C |
|---|---|---|---|---|
| *C3 allele frequency* | | | | |
| Red deer from New Zealand | 5 | 0.50 | 0.50 | 0.00 |
| Wapiti | 5 | 0.60 | 0.00 | 0.40 |
| This study | 41 | 0.76 | 0.24 | 0.00 |
| *PTF allele frequency* | | | | |
| Red deer from New Zealand | 5 | 1.00 | 0.00 | |
| Wapiti | 5 | 0.00 | 1.00 | |
| This study | 41 | 1.00 | 0.00 | |
| *GPI allele frequency* | | | | |
| Red deer from New Zealand | 5 | 0.90 | 0.10 | |
| Wapiti | 5 | 1.00 | 0.00 | |
| This study | 41 | 1.00 | 0.00 | |

significant deviation from the expectations of H–W equilibrium ($\chi^2 = 0.79$, $P = 0.37$), with frequencies of allele A and B of 0.76 and 0.24, respectively, and an observed heterozygosity of 0.42 (Table 2). In New Zealand, red deer had 50% of each allele, whereas wapiti had frequencies of alleles A and C at 0.60 and 0.40, respectively.[13]

HB is polymorphic with 2 alleles in this species. However, 1 allele is found typically only in North American wapiti, while the other is found only in European red deer and thus readily allows to differentiate the two groups.[14] Whereas all wapiti in New Zealand were homozygous,[14] we found a frequency of 0.12 of the allele typical in wapiti, and one animal was homozygous for the wapiti allele (Table 3). The allele distribution indicates no significant deviation from the expectations of H–W equilibrium ($\chi^2 = 0.32$, $P = 0.57$), and the observed heterozygosity was 0.20.

SOD is polymorphic with 2 alleles in this species. One allele is found predominantly in wapiti, while the other is found predominantly in red deer, with a discrimination efficiency of 95%.[14] The allele distribution indicates no significant deviation from the expectations of H–W equilibrium ($\chi^2 = 0.003$, $P = 0.96$), with frequencies of allele S and F of 0.95 and 0.05, respectively (Table 3), and an observed heterozygosity of 0.10.

GPI is polymorphic with 2 alleles in this species.[13] There was only 1 allele present in our samples (Table 2).

DIA is polymorphic with 2 alleles in this species, but only the allele F had been found in wapiti.[10] The allele distribution in this study indicates no significant deviation from the expectations of H–W equilibrium ($\chi^2 = 0.37$, $P = 0.54$), with an observed heterozygosity of 0.53. The frequencies of the allele F was 0.4 (Table 4), compared with 0.49–0.99 in New Zealand populations.[10]

Polymorphism measured across the nine examined protein systems (PTF, TRF, GC, PLG, C3, HB, SOD, GPI, DIA) was 2.0 alleles per locus with an overall heterozygosity of 0.30. Heterozygosity from the total population averaged 27.4% (range 3–63%), whereas the average heterozygosity of the subpopulations averaged 4.7% (range 0.006–12%) (Table 5).

## Discussion

Genetic variability is expected to be reduced when introductions of animals were based on only a few individuals. The present results are consistent with reports that the red deer population in the study area was founded by few individuals. For instance, GPI was represented by only 1 of 2 alleles, and PLG by 3 out of 7 known alleles. Studies of several populations in New Zealand, each based on a separate introduction of less than 20 individuals, revealed significant differences in allele frequencies among the populations, and our results on GC and PLG are likely representatives of another such localised introduction.

The genetic variability detected in the present study (average H = 0.3, polymorphism of 2 alleles/locus) cannot be compared directly to other studies due to the dependence on selected protein systems and sample sizes.[15] Comparisons however can be made on specific proteins measured in different populations. Whereas observed heterozygosity of HB in red deer and wapiti was zero ($n = 186$),[14] it was 20% in our sample. The observed heterozygosity reported in red deer for SOD ($n = 718$)[14,16,17] ranged from 1 to 38% as compared with 10% in our sample; and 5% for GPI ($n = 365$)[16] versus 0% in this study. Heterozygosities were substantially more depressed when considering all subpopulations (including wapiti) versus only red deer (27.4 versus 4.7%) due to the effect from several protein systems being specific for either red deer or wapiti. Thus, heterozygosity depressions in DIA, HB and SOD were several

**Table 4. Diaphorase I allele frequencies in wapiti, four New Zealand sites,[10] and Patagonia**

|          | Site A | Site B | Site C | Site D | Wapiti | This study |
|----------|--------|--------|--------|--------|--------|------------|
| $n$      | 48     | 56     | 51     | 33     | 42     | 30         |
| Allele F | 0.72   | 0.49   | 0.77   | 0.99   | 1.00   | 0.40       |
| Allele S | 0.28   | 0.51   | 0.23   | 0.01   | 0      | 0.60       |

**Table 3. Superoxide dismutase (SOD) and hemoglobin (HB) alleles in wapiti, red deer and hybrids in New Zealand,[13,14] and red deer in Patagonia**

|                           | $n$ | SS  | SF  | FF  | Frequency of allele S |
|---------------------------|-----|-----|-----|-----|-----------------------|
| *SOD allele distribution and frequency* |     |     |     |     |                       |
| Red deer from New Zealand | 157 | 155 | 2   | 0   | 0.99                  |
| Hybrids                   | 37  | 0   | 37  | 0   | 0.50                  |
| Wapiti                    | 26  | 1   | 4   | 21  | 0.12                  |
| This study                | 41  | 37  | 4   | 0   | 0.95                  |

|                           | $n$ | AA  | AB  | BB  | Frequency of allele A |
|---------------------------|-----|-----|-----|-----|-----------------------|
| *HB allele distribution and frequency* |     |     |     |     |                       |
| Red deer from New Zealand | 159 | 159 | 0   | 0   | 1.00                  |
| Hybrids                   | 38  | 0   | 38  | 0   | 0.50                  |
| Wapiti                    | 27  | 0   | 0   | 27  | 0.00                  |
| This study                | 41  | 32  | 8   | 1   | 0.88                  |

**Table 5. Heterozygosity as average of the subpopulations ($H_s$), for the total population ($H_t$) and the corresponding percentage decline of heterozygosity ($G_{st}$) due to population subdivision**

Data are from this study and others[9,10,13,14]. C3, complement component $C_3$; DIA, diaphorase I; GC, vitamin D binding protein; HB, hemoglobin; PLG, plasminogen; SOD, superoxide dismutase; TRF, transferrin

|      | All individual subpopulations | | | Combined New Zealand red deer and this study | | |
|------|-------|-------|----------|-------|-------|----------|
|      | $H_s$ | $H_t$ | $G_{st}$ | $H_s$ | $H_t$ | $G_{st}$ |
| DIA  | 0.29  | 0.4   | 0.26     | 0.43  | 0.49  | 0.12     |
| SOD  | 0.2   | 0.44  | 0.54     | 0.06  | 0.06  | 0.01     |
| HB   | 0.18  | 0.48  | 0.63     | 0.11  | 0.11  | 0.06     |
| GC   | 0.52  | 0.62  | 0.17     | 0.58  | 0.6   | 0.03     |
| PLG  | 0.46  | 0.52  | 0.12     | 0.51  | 0.53  | 0.04     |
| C3   | 0.45  | 0.54  | 0.17     | 0.43  | 0.47  | 0.07     |
| TRF  | 0.46  | 0.47  | 0.03     | 0.47  | 0.47  | 6E–05    |

fold higher (2.2, 10.5, and 54, respectively), if the wapiti samples were included.

Although the analysis of PTF shows only the marker for red deer, results on HB may indicate an influence of wapiti blood lines. Moreover, whereas in New Zealand the SOD allele typical for wapiti was found in 1% of phenotypic red deer (*n* = 157), it occurred in 11% of animals in the present study. Since an introduction of one or a few individuals of wapiti into a large free-ranging population of red deer cannot result in the observed allele distribution, we interpret these results to be related to the origin of the founder animals. Furthermore, there are no records of wapiti having been introduced to Patagonia.[1] Historical records from the company importing the initial red deer into Argentina showed them all to be of Austrian/Hungarian stock from the Alps and Carpathian mountains.[18] However, there are many records of introductions of wapiti into European parks and surrounding areas over a period of more than 180 years. Lever[19] mentioned several hundred wapiti being brought to Austria, and Niethammer[20] listed many occurrences of wapiti introductions since the 19th century. Furthermore, red deer and hybrids have been relocated

frequently all through Eurasia for over 250 years.[20–22] It is therefore likely that the founding stock of red deer brought to Argentina had been affected previously by crossing with wapiti. The existence of biochemical markers that show distinct differences between wapiti and red deer has been corroborated by an mtDNA study, which concluded that wapiti and red deer should be differentiated at the species level.[23] Finally, a recent analysis of vocalisations found higher fundamental frequencies in our population as compared with Scottish deer which was suggested to relate to wapiti genes in this populations.[24]

Body and antler size are a 'proxy' for general performance; in the present case, the adaption of red deer to the Patagonian environment but constrained by their genetics. Liveweights of free-ranging females living in national parks were up to 145 kg, whereas males were 300 kg and more, corroborated by antlered heads frequently weighing 11–12 kg, and up to 13.6 kg (Fig. 2).[25] Although the low genetic variation encountered is likely the result of the introduction based on a few individuals, the excellent performance of the present population contradicts the existence of any overt impact from this founder effect. The rapid population growth after initial releases likely contributed in overcoming



**Fig. 2.** Antler quality as a proxy for founder effects and the capacity of introduced red deer to adapt to the Patagonian environment in Argentina. (*a*) 13.6 kg, Lanin national park; (*b*) 12.5 kg, Neuquén province; (*c*) 9.5 kg, Nahuel Huapi national park; (*d*) 9.5 kg, Nahuel Huapi national reserve.

impediments resulting from founder effects, if there were any. The observed large body and antler sizes may not only be due to favourable environmental conditions, but also due to previous hybridisation with wapiti. Reproductive rates and density are high, and there are no indications of health problems.

## Acknowledgements

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