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Development of a “Wapiti Score” visual assessment tool for determining introgression of wapiti genes in young red deer.

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ABSTRACT

Red deer are the predominant farmed breed type in New Zealand, but crossbreeding red females with wapiti (or elk) males in a terminal sire system is common. Introgression of wapiti genes into the national red deer breeding hind herd has been shown to have a negative impact on the pregnancy rate of 16-18 month old hinds. We devised a four point visual scoring system (‘Wapiti Score’), based on wapiti phenotype characteristics, for application to weaner (6-10 month old) red deer, to allow rapid detection of wapiti introgression. This scoring system was trialled and refined using 449 rising-one-year-old red deer of mixed genotype and sex on a commercial breeding and finishing farm, during three pelage phases. For a subset of 186 animals ‘Wapiti Score’ was compared to the DNA based *GenomnzTM GenometerTM G3 Hybrid Test* ‘Estimated Elk’ (EstElk), where EstElk; is the estimated proportion of wapiti (elk) genes in an individual animal predicted by the *GenometerTM*. A significant positive correlation ($P < 0.05$) was found between EstElk and ‘Wapiti Score’ for two independent observers, with an increase of one point in ‘Wapiti Score’ corresponding to an increase of approximately 0.10 in EstElk. The strongest correlation between Wapiti Score and EstElk were from the winter and spring scores. The effectiveness of Wapiti Score as a tool for classifying young deer by breed-type (red deer or wapiti) was assessed using 0.2 EstElk as the breed-type threshold and comparing that against different selection Wapiti Scores (1-3).

Keywords: Wapiti; red deer; introgression; wapiti score; crossbreed.

INTRODUCTION

Introgression of wapiti (*Cervus elaphus nelsoni*, *manitobensis*, *roosevelti*) genes into the base New Zealand red deer (*Cervus elaphus scoticus*, *hippelaphus*) has been shown by Asher *et al.*, (2005), to be implicated in puberty failure in yearling (16-18 month) ‘red deer’ hinds. Hinds with >20% wapiti parentage often fail to reach predicted threshold live weight for puberty attainment, in herds managed as ‘red deer’. A 5 point scoring system to visually assess wapiti parentage in an individual was used in that study. It was based on general body conformation and pelage characteristics. This system proved a very useful indicator of wapiti introgression in 16-18 month females; with scores ≥ 3 generally identifying animals >20% wapiti genes. However, only one scorer was used by Asher *et al.* (2005) throughout the study, and there were within farm scoring biases noted. The present study aimed to simplify and qualify this scoring system for application to ‘weaners’ (6-10 month old deer); enabling early identification of animals by genotype and facilitating earlier management decisions and animal retention or culling.

MATERIALS AND METHODS

Animals

Two mobs of weaners were assessed on one commercial farm in Otago. These were replacement breeding females of red deer parentage (n=161), and a mixed sex mob of males (n=247) and females (n=41) known to be of varying degrees of wapiti crossbreeding parentage (by siring with wapiti crossbred sires). The crossbred mob was selected by weight as a cohort that would not attain 100kg live weight before the final measurement; which meant they were smaller (winter mean 63kg) than average crossbreds, and that all of the deer in the study were of a similar size and live weight. The deer were run under standard commercial management.

Measurements

All measurements were taken in the farm’s deer yards, with the animals yarded off pasture for no more than 4 hours. Animals were scored and measured under fluorescent lighting. Immediately prior to the first scoring/measurement, a hair sample was taken from each animal and stored in individual paper envelopes for later DNA analysis. Three measurement periods were selected to coincide with pelage phases; winter (early Aug. 2004) 8-9 months of age, spring (mid Oct. 2004)

10-11 months and summer (mid Jan. 2005) 13-14 months. Preliminary observations indicated that autumn (pre-rut weaning) would not be a suitable phase, as pelage would be in various states of: shedding calf coats, and growing winter ones. We also considered that the maximum number of animals would be available for selection post-autumn, as many farmers post-rut wean. The animals were drafted in to a small pen of 5-6 animals for measurement; where they were first independently scored for wapiti characteristics (Wapiti scored) by two observers (the same two observers were used throughout the trial). Wapiti Score (WS) was a four point system for ranking animals according to phenotypic wapiti features using pre-defined sets of seasonal and pelage phase criteria for; stature, body pelage colouration, head and neck characteristics and pelage, and rump patch size and colour (Table 1 illustrates winter characteristics). Wapiti score classes were defined as: WS 1 = no obvious wapiti features (or obvious red parentage), WS 2 = some wapiti features (or unusual red deer), WS 3 = distinct wapiti features and WS 4 = obvious in their wapiti parentage. Wapiti score is not a simple additive process, as preliminary observations also showed that an 'additive' approach (e.g. 2 wapiti characteristics = WS 2) to scoring was not practical or informative. Rather than summing individual characteristics; it is more of a whole animal overview of the combination of characteristics and the strength or obviousness of those characteristics. Following scoring, animals were measured for shoulder height and back length to the nearest cm as described in Asher *et. al.* (2005). Finally they were individually weighed in an electronic weigh crate with a resolution of 0.5kg.

DNA Analysis

DNA analyses were conducted at the *GenomnzTM* laboratory at AgResearch Invermay. A subset of samples comprising a random selection of females (n=41) from the red parentage mob, and all of the females (n=41) and 106 random males from the known wapiti parentage mob, were DNA tested from hair samples. These samples were DNA-profiled using a set of 14 microsatellite markers, then analysed for their estimated proportion of wapiti genes (EstElk) using the *GenometerTM* G3 Hybrid Test (or Elkmeter) as described in Asher *et. al.* (2005). The Estimated Elk proportion (EstElk) is the mean value from a box and whisker plot (used to estimate genetic admixture as described in Dodds & Tate (2006) and Tate *et. al.* (1998)) that represents the posterior distribution for that individual, based on the prior assumption that animals are being sampled from a uniform distribution of between 0 and 100% wapiti and given the genotypes.

Statistical Analysis

EstElk was regressed on WS averaged over both observers (MWS), as both a categorical and numerical variable. This was done separately for males and females at each measurement. Morphometric variables (shoulder height, body length, weight, weight gain (spring and summer only), "shape" (= shoulder height / body length) and "body mass index" (BMI = shoulder height x body length / weight)) were then collectively and sequentially added to the models in each case.

TABLE 1: Phenotypic characteristics for wapiti scoring Red vs. Wapiti weaners in winter pelage

Red (Wapiti Score=1)	Wapiti (Wapiti Score=4)
Stature	
Finer, shorter more proportionate body and limbs	Tall, thicker, blockier, more rectangular body Legs appear more gangly and awkward; less proportionate to body
Colour	
Red and grey mostly. Grey face and underside, red body Legs same colour as underside	Chocolate-black and cream. Head and neck (throat mane) darker, body lighter brownish grey to cream or straw coloured Legs same colour as body
Rump	
Smaller, less apparent rump patch, any colour from fully red to fully white often red top and white bottom. Tail different colour Shaped like an upside down pear	Large obvious creamy-white rump patch. Tail same colour as rump Heart shaped and quite rounded looking
Head	
More elongated muzzle, grey face, and red to brown chevron (forehead) Pointed ears	Muzzle more stout/shorter, brown face and chevron (forehead) Rounded fuzzy ears

Animals were designated as genetic 'wapiti' or 'red deer' using a threshold for EstElk of 0.20 (or 20% wapiti genes), with animals for which EstElk > 0.20 designated 'wapiti', and those with EstElk ≤ 0.20 designated 'red deer'. The value of 0.20 was chosen because it is the value at which Asher *et. al.* (2005) found 'red deer' yearling pregnancy rate was compromised by wapiti gene introgression. To evaluate the subjective wapiti scoring procedure against the genetic procedure, a calculation was made of the percentage of genetic 'red deer' with a 'wapiti-type' WS (i.e. false positives), and the percentage of genetic 'wapiti' with a 'red deer-type' WS (i.e. false negatives), for WS thresholds of ≤1, ≤2, and ≤3, for each observer (as shown in Table 2. for females for each season).

TABLE 2: Percentage of false positives and false negatives for females using the Wapiti score at given selection thresholds against a threshold proportion of wapiti genes of 0.20, for the two observers in the Wapiti Scoring study.

WS cut-off	False positives ¹		False negatives ²	
	Obs. 1	Obs. 2	Obs. 1	Obs. 2
(a) Winter				
1	20	20	0	9
2	4	1	27	36
3	0	0	91	82
(b) Spring				
1	24	23	0	9
2	3	1	27	46
3	0	0	91	82
(c) Summer				
1	27	22	0	27
2	1	3	36	73
3	0	0	100	100

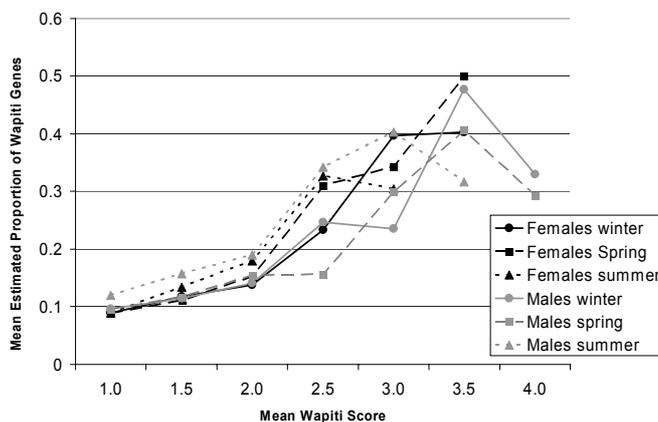
¹ % of 'red deer' (EstElk ≤0.2) with individual WS > selection WS
² % of 'wapiti' (EstElk >0.2) with individual WS ≤ selection WS

RESULTS

A strong positive regression relationship was found between EstElk and MWS, with an increase of approximately 1 in MWS corresponding to an increase of 0.1 in EstElk (Figure 1). Considering MWS as a categorical variable, the relationship was somewhat stronger for females than males; with residual standard deviations of 0.072 and 0.095 for females and males, respectively, for winter data with very similar values obtained for spring data. The association between MWS and EstElk was weaker in summer (RSD = 0.091 for females, 0.097 for males), showing a tendency for WS to underestimate the amount of wapiti genes in individual animals. While the main discrimination

between genotypes occurred for MWS values greater than 2, MWS values 1.0-2.0 could also detect varying proportions of wapiti genes below 0.2 at the 5% significance level (Figure 1). There was generally good agreement between observers with correlations between the wapiti scores of the two observers of about 0.75 for all seasons. The errors likely to occur when making selection decisions based on WS are shown for females (which are the main focus of the scoring tool), in each season; in Table 2. As an example; a policy of selecting 'red deer' only replacements by taking WS = 1 as a selection threshold would exclude 20% of 'red deer' and include 0-10% of 'wapiti', in this herd. For females scored in winter, using a selection threshold of WS = 2 to create separate red and wapiti herds would result in only 1-4% of genetic red deer being put in the wapiti herd, while about a third of the genetic wapiti would be included in the red herd. Some individual scoring bias was noted here, where observer 2 was more conservative (more likely to score in the red part of the scale) than observer 1. None of the morphometric variables showed a strong association with EstElk or WS (P>0.05). In all cases WS alone was the best indicator of true genotype.

FIGURE 1: Mean estimated proportion of wapiti genes vs. mean wapiti score, for R1 male and female red deer across 3 seasons, wapiti score mean of 2 observers.



DISCUSSION

This study has shown that by visual assessment of a combination of wapiti-type phenotypic traits, an observer can predict fairly accurately genotype for lower-order (10-50%) wapiti x red crossbred animals. When used individually, these traits would probably prove to be poor indicators because of natural phenotypic variation, particularly amongst 'red deer' genotypes. For example there was a marked

variation in rump patch size within ‘red’ animals studied; making rump one of the less informative phenotypical characteristics, when observing animals in yards.

The wapiti scoring technique provided a better prediction of genotype for females than males and also while animals were in the winter pelage phase as opposed to summer. However, it has to be accepted that there will always be a certain degree of inaccuracy due to random allele segregation and phenotype not always reflecting the true genotype of an individual; as such, farmers will have to decide which WS value they chose to make their selection cut off. WS provided better differentiation of animals with less than 20% wapiti genes (‘red deer’) than we had expected; we had hypothesised that it would be very difficult to predict levels of wapiti introgression below 20% wapiti. However for MWS 1.0 – 2.0 there was a definite trend of increasing EstElk with increasing MWS.

We believe the ‘wapiti parentage group’ animals in this data set represent a difficult, extreme example of animals that farmers or breeders would have to score; as they were selected across a narrow band of weight and size, compared with the expected range in a population. This made the visual appraisal potentially more difficult, as the obvious large wapiti-type animals were not present; instead the group consisted of well grown red type animals and poorly grown wapiti-type animals. For this reason we would expect farmers familiar with their animals to do at least as well or better than we did in classifying animals to the correct genetic group. This study provides a tangible tool to allow farmers to make use of the findings of the study by Asher *et. al.*, (2005) and has been made freely available to deer farmers and researchers on the DEERResearch website (Ward 2005). We hope that adoption of the WS technique will allow farmers to make objective decisions on genetic management at an early age; and that this will be reflected in improved pregnancy rates for the New Zealand yearling (‘first calver’) herd. If

animals are selected and well managed on the basis of wapiti genes we have conservatively estimated, using 2005 figures (174,000 breeding yearling hinds), a 1% increase in yearling’s calves to weaning is worth an added \$420,000 p.a., and that a 2% per year increase for 5 years, from a base 70% weaning percentage, would be worth >\$14,000,000 over those 5 years to the New Zealand Deer Industry. At a time when deer farmers are searching for productivity gains this is one simple tool, along with appropriate management, that can be taken immediately to improve yearling pregnancy rates and hence productivity.

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